

BRONCHIECTASIS AS A PHENOTYPE OF ALPHA-1 ANTITRYPSIN DEFICIENCY

by

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Abstract

Alpha-1 Antitrypsin Deficiency (AATD) is a rare, inherited condition characterized by reduced or dysfunctional alpha-1 antitrypsin (AAT), a protease inhibitor that protects lung tissue from inflammation-mediated damage. AATD is a recognised genetic cause of chronic obstructive pulmonary disease (COPD), but its relationship with bronchiectasis, a condition involving permanent bronchial dilatation, recurrent infections, and impaired mucociliary clearance, is less well defined. This thesis investigates the hypothesis that bronchiectasis in AATD represents a distinct clinical phenotype, rather than a secondary complication of COPD, and examines its implications for diagnosis, prognosis, and management.

Using the Birmingham AATD registry, this research systematically examines radiological, microbiological, biochemical, and clinical data to characterise the prevalence, progression, and severity of bronchiectasis in AATD. Throughout the thesis, clinical outcomes of exacerbation rate, baseline FEV₁, lung function decline, symptoms, and mortality are used. A systematic review highlights the scarcity of high-quality data addressing the characteristics of AATD-bronchiectasis. The analysis of registry data demonstrates that unclassified bronchiectasis is not associated with COPD diagnosis, and is associated with serum AAT level, suggesting a direct link between bronchiectasis and AATD, though bronchiectasis without further subclassification has no impact on clinical outcomes.

Sputum samples were obtained and combined with historic data to reveal the association of *Pseudomonas aeruginosa* with higher exacerbation rate and accelerated lung function decline, in common with other causes of bronchiectasis. Biomarker studies highlight the role of systemic inflammation in disease pathogenesis,

with abnormal eosinophils and CRP correlating with poorer clinical outcomes in AATD-bronchiectasis, as did platelets when the whole cohort of AATD was included. Further study of a greater number of inflammatory biomarkers in a proteomics context could reveal therapeutic targets.

Analyses of CT scans assess severity of bronchiectasis using established visual and algorithmic markers, and revealed that bronchiectasis is usually mild, though with a subset of patients with more extensive disease, despite efforts to exclude those with alternative causes. Visual and algorithmic analyses correlated well with each other. In common with other causes of bronchiectasis, cystic bronchiectasis was the only subtype found to associate with clinical outcomes, in this case exacerbation rate and gas transfer decline. This assessment also allowed validation of the bronchiectasis severity index (BSI) in AATD for the first time, demonstrating increasing mortality risk between mild, moderate and severe scores.

In conclusion, this thesis establishes bronchiectasis as a distinct phenotype in AATD, being unrelated to COPD. However, being mostly mild, it is of limited clinical significance unless cystic morphology, *P. aeruginosa* colonisation, or eosinophilia develops, in common with other bronchiectasis aetiologies. This thesis therefore does not demonstrate a sufficiently strong or causal link to suggest widespread screening for AATD in idiopathic bronchiectasis. Avenues for future research include comparison of this data with other causes of bronchiectasis, study of proteomics to assess a broader range of inflammatory biomarkers, and the study of whether a combined prognostic tool can be developed for the bronchiectasis-COPD overlap syndrome, as frequently occurs in AATD. Ultimately, this work advances our understanding of AATD and reinforces the need for personalised approaches to care in this complex patient population.

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List of Abbreviations

AAT - Alpha-1 Antitrypsin

AATD - Alpha-1 Antitrypsin Deficiency

ABPA - Allergic Bronchopulmonary Aspergillosis

AUC - Area Under the Curve

BAL - Bronchoalveolar Lavage

BCOS - Bronchiectasis-COPD Overlap Syndrome

BEI - Bronchiectasis Index Lung (a YACTA derived measure of tapering)

BMI - Body Mass Index

BSI - Bronchiectasis Severity Index

COPD - Chronic Obstructive Pulmonary Disease

CRP - C-Reactive Protein

CT - Computed Tomography

DNA - Deoxyribonucleic Acid

ELF - Epithelial Lining Fluid

FEV1 - Forced Expiratory Volume in 1 Second

FVC - Forced Vital Capacity

GOLD - Global Initiative for Chronic Obstructive Lung Disease

HRCT - High-Resolution Computed Tomography

ICS - Inhaled Corticosteroids

IL - Interleukin

KCO - Transfer Coefficient for Carbon Monoxide

LNP - Lipid Nanoparticle

mMRC - Modified Medical Research Council dyspnoea scale

NE - Neutrophil Elastase

NETT - National Emphysema Treatment Trial

Pi - Protease Inhibitor (e.g., PiZZ, PiSZ)

RNA - Ribonucleic Acid

ROC - Receiver Operating Characteristic

RCL - Reactive Centre Loop

SGRQ - St. George's Respiratory Questionnaire

TLCO - Transfer Factor for Carbon Monoxide

TNF - Tumour Necrosis Factor

Va - Alveolar Volume

VATS - Video-Assisted Thoracoscopic Surgery

YACTA - Yet Another CT Analysis Tool

1 Introduction

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1.1 Alpha-1 Antitrypsin Deficiency

1.1.1 Pathogenesis

1.1.1.1 Serpinopathy

Alpha-1 antitrypsin deficiency (AATD) is a rare inherited disorder of absent or dysfunctional alpha-1 antitrypsin. In health, alpha-1 antitrypsin (AAT) is the principal among several serum protease inhibitors, abbreviated to “serpins” by convention. All serpins have a common basic structure of 3 β -sheets, several α -helices, and a reactive centre loop (RCL), which forms the active surface of the protein with which to bind the protease^{2,3}. The catalytic action of the protease enzyme on the AAT molecule changes its shape without unbinding the AAT-protease complex; this formation change crushes and inactivates the protease^{4,5}.

Mutations in serpin production can give rise to protein structures which mimic the reactive target protease⁶. In this case, the RCL of one serpin molecule will bind to either its own structure, or to another serpin⁷. This gives rise to long unbranched polymer chains, which has two effects: firstly, the serpin molecules are inactivated, meaning the target proteases can act unopposed; secondly, the tissues in which such

polymerisation occurs cannot secrete such long protein chains, and cell stress and death occurs⁸.

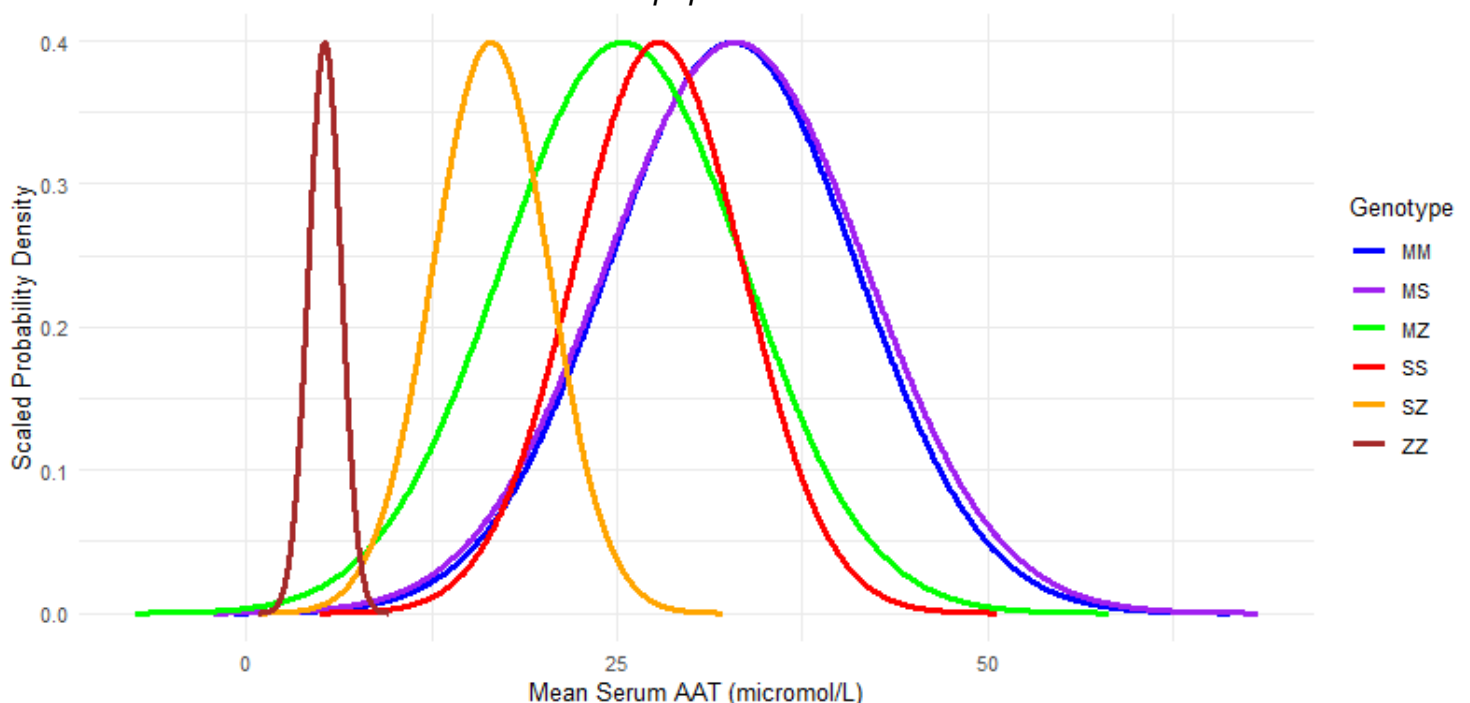
1.1.1.2 Genetics

In AATD, AAT is the serpin of interest, and is encoded by the *SERPINA 1* gene on the long arm of chromosome 14⁹. Multiple point mutations have been identified, the most common of which are ranked A to Z according to the speed of their migration during isoelectric focusing, with the “normal” and most common variant being “M”. “S” and “Z” variants are the most common pathogenic alleles encountered in clinical practice, with “I” and “F” variants encountered more rarely¹⁰. Other rare variants have eponyms based on the discoverer or place of discovery, such as “M-Malton” and “Plowell”^{11,12}. Null variants are also possible, where truncations in the *SERPINA 1* gene lead to no protein, or a very short non-functional protein chain, being produced. The S allele is most common in the Iberian peninsula, where it has a frequency of 28%¹³; the Z allele is most common in northern European populations. Other alleles and null variants are much rarer, which leads to AATD primarily affecting those with Caucasian ancestry; however, spontaneous mutations can occur in any ethnic group^{14,15}. Functionally, the “M” allele encodes normal AAT, “S” alleles encode AAT with around 50% the activity of “M”, and “Z” alleles encode AAT with only around 15% normal activity. Null alleles naturally lead to absent AAT and therefore no AAT function, but polymerisation cannot occur. Rarer alleles can cause different patterns of protease inhibition and polymerisation.

1.1.1.3 Inheritance and epidemiology

Unusually for genetic conditions, AATD cannot be described as either autosomal dominant or autosomal recessive. Instead, both inherited alleles contribute to the phenotype, a pattern described as “autosomal co-dominant”. The combination of alleles is expressed as the two alleles prefixed with “Pi”, an abbreviation of “protease inhibitor”. This combination determines the level of functional serum alpha-1 antitrypsin. The generally accepted hierarchy of the most common phenotypes, in order of lowest to highest levels, is PiZZ, PiSZ, PiSS, PiMZ, and PiMM. Each genotype has its own mean and distribution of AAT levels, meaning that AAT is not normally distributed among patients with AATD (Figure 1.1), and consequently AAT is not a reliable variable in AATD research. Instead, the genotype is used as a categorical variable.

Figure 1.1: Distribution of AAT levels for common genotypes. As per Brantly et al¹: AAT levels do not follow a normal distribution in the population as a whole.



Severity of disease presentation is associated with the genotype in combination with environmental factors. The prevalence of AATD is difficult to estimate: screening

studies have found asymptomatic PiZZ patients¹⁶, diagnosis is often delayed¹⁷, and clinical severity does not always correlate with genotype¹⁸. Among pathogenic phenotypes, PiSZ is more common, affecting around 1.5 million people worldwide¹⁹, whereas the more severe PiZZ phenotype affects around 250000²⁰. However, the individual alleles Pi*S and Pi*Z have an estimated prevalence of 45.7 and 14.7 cases per 1000 population in the UK, respectively, meaning that carrier status is not rare¹⁴. Consequently, it can be assumed that there are undiagnosed cases of severe AATD in the community, but study of the disease can only be limited to known cases, and therefore a degree of ascertainment bias is present in all AATD research.

1.1.1.4 Neutrophil elastase

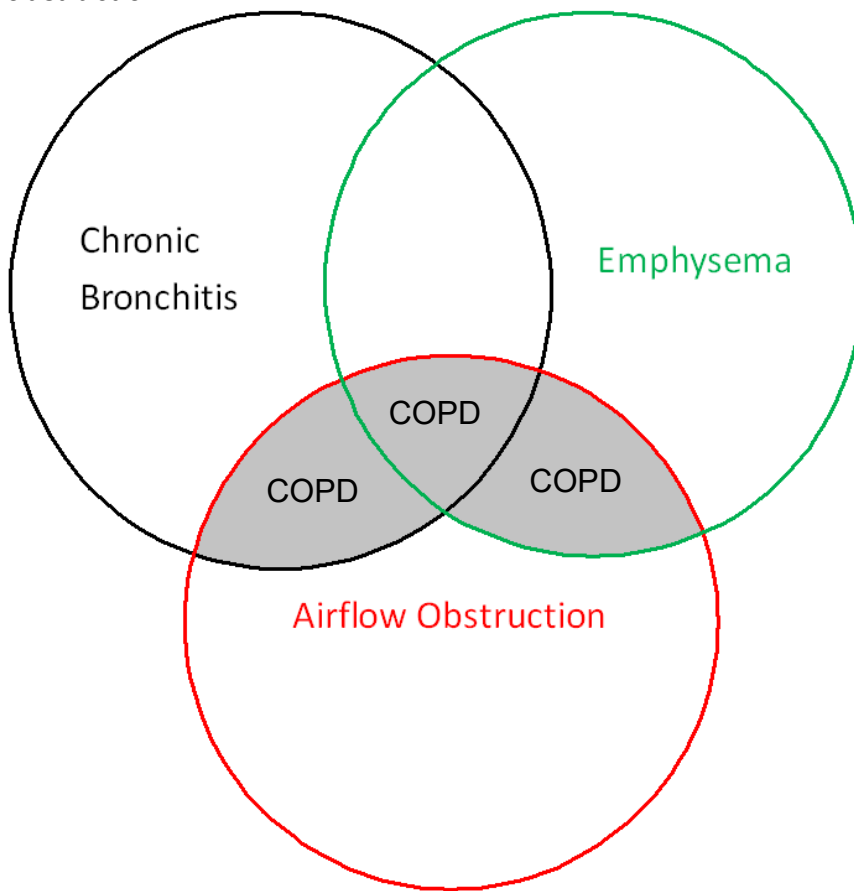
The main target protease for AAT is neutrophil elastase. Excess neutrophil elastase is known from animal studies to cause damage and destruction of alveoli, and consequently emphysema^{21,22}, as well as being associated with elevated inflammatory cell levels in broncho-alveolar lavage (BAL)²³. Murine models of AATD have shown this damage also occurs when neutrophil elastase activity is unopposed by AAT²³. Aside from its inhibition of neutrophil elastase, AAT also binds IL-8, which is associated with neutrophil migration, LTB₄, and α -defensins, which trigger neutrophil degranulation. Other proteinases such as Cathepsin B and G, mannan-binding lectin serine protease-2, proteinase 3, and matrix-metalloproteases are also inhibited. AAT also appears to be secreted in an on-demand manner, in response to the acute phase inflammatory process²⁴. This presents a risk of type II errors when only using serum AAT as a diagnostic test for AATD, as it will be elevated during infections or inflammation²⁵. This effect can be mitigated by measuring other inflammatory markers

simultaneously, such as CRP, or by also assessing genotype, as genes will remain unchanged.

1.1.2 Lung disease in AATD

Through its multiple inhibitory effects on these pro-inflammatory mediators, the net effect of AAT is to reduce inflammation and protect against structural damage. It follows that the main effect of AATD in the lung is chronic inflammation and consequent structural damage in the form of emphysema and bronchiectasis, although the relationship of bronchiectasis in AATD is a controversial one which will be explored in subsequent sections. It is this effect of AATD which caused the identification of the disease in a case series published in 1964 ²⁶. Chronic inflammation of the bronchi is termed chronic bronchitis; it and emphysema often coexist clinically in a syndrome known as chronic obstructive pulmonary disease (COPD, Figure 1.2). Bronchiectasis has historically been treated as a separate clinical syndrome, and will be discussed in more detail in subsequent paragraphs.

Figure 1.2: Phenotypic overlap of chronic bronchitis, emphysema, and airflow obstruction.



1.1.2.1 COPD

COPD refers to chronic obstruction in airflow, as measured by the volume of air which can be forcibly expelled in 1 second (forced expiratory volume in 1 second, FEV₁) relative to the total amount of air which can be expelled (forced vital capacity, FVC). These two measurements are the core components of a set of pulmonary function tests known as spirometry. For a diagnosis of COPD to exist, the FEV₁/FVC ratio must be below the lower limit of normality for the patient's demographics, though a simpler cutoff of FEV₁/FVC <0.7 is often used. This ratio must persist after application of

bronchodilators, such as salbutamol, though a degree of improvement is often seen. Exacerbations are a key feature of the disease, characterised by increased phlegm production, change in colour of phlegm, and acutely worsened shortness of breath, and may be infective or non-infective²⁷. Exacerbation rate is known to be associated with disease progression, as well as inversely associated with quality-of-life²⁸⁻³⁰. The cause of airflow obstruction in COPD is a combination of emphysema, which describes air trapped within the lungs caused by the destruction of alveoli, and chronic bronchitis, which describes chronic inflammation of the airways, manifest clinically as a productive cough of over 3 months duration³¹. The relative contributions of emphysema and chronic bronchitis to the degree of obstruction vary between patients. In the vast majority of cases, COPD is caused by exposure to tobacco or other inhaled toxins, but chronic inflammation caused by unopposed neutrophil elastase, as in the case of AATD, causes a similar picture. AATD is found in up to 5% of patients with COPD³².

Despite the accepted identification of AATD as a genetic cause of COPD, there are some differences between COPD due to smoking (“usual-COPD”) and AATD-COPD. Emphysema is more likely to affect the lower lobes in AATD³³, but distribution is much more variable in smoking-related COPD³⁴. Distribution of emphysema has significance for lung-volume reduction surgery, which has the best evidence for upper-lobe emphysema^{35,36}. Furthermore, the presence of asthma-like features, such as reversibility and raised eosinophils, is higher in usual-COPD than in AATD-COPD³⁷. This has implications for treatment, suggesting bronchodilators and inhaled corticosteroids may be of less benefit³⁸. Exacerbation characteristics also differ, with AATD-COPD exacerbations usually lasting longer at median duration 14 days, compared with the 7 days median duration of usual-COPD³⁹; severe exacerbations requiring hospitalisation are also commoner in AATD-COPD⁴⁰. Rates of ischaemic

heart disease may differ between the two groups, probably related to the lower average age and tobacco exposure in AATD-COPD compared to usual-COPD ⁴¹.

Disease penetrance of COPD in AATD is variable. Subclinical radiological signs are often present from the 3rd decade of life, but some never-smokers may never develop clinical symptoms^{42,43}. Tobacco smoke significantly affects the likelihood of developing COPD in AATD, with sub-clinical emphysema and clinical symptoms of COPD developing earlier in smokers vs non-smokers, due in part to tobacco smoke directly impairing the function of AAT ⁴⁴⁻⁴⁶. Those who are identified by family screening are less likely to develop clinical symptoms than index cases, and may have normal life expectancy ^{37,43,47}. Female patients also appear to present differently, waiting longer for a confirmed diagnosis of AATD, and having less tobacco exposure for a given disease stage when compared to males ⁴⁸. Phenotypically, PiZZ patients have higher rates of COPD, more rapid disease progression, and lower quality-of-life scores than PiSZ patients³¹, but the degree to which heterozygotes PiSZ, PiMZ, and PiSS contribute to disease presentation has been debated in the literature. The prevailing understanding for many years was that PiSZ patients have significantly higher rates of FEV₁ decline than the general population^{49,50}, but that PiMZ patients only have higher rates of FEV₁ decline if they are smokers^{51,52}, though small increases in FEV₁ decline are still seen in non-smokers⁵⁰. However, recent developments suggest PiSZ may only cause clinically significant lung disease in smokers⁵³. This study used a more robust methodology by only including PiSZ patients identified by family screening, excluding index cases, thereby eliminated selection bias resulting from only symptomatic patients presenting to clinic.

1.1.2.2 Differences between AATD and usual-COPD

Objectively, emphysema in AATD appears different to usual COPD, with a lower lobe predominance compared to a more widespread distribution in smoking-related lung disease^{34,54}. Asthma-like features are less prevalent^{37,55}. Patients are younger and less likely to be smokers, with lower rates of cardiac co-morbidities^{41,56}. Exacerbations last longer in AATD-COPD than in usual COPD^{39,57}. Concomitant liver disease affects a significant minority of patients, as described in section 1.1.3.1, and can be independent of lung disease, meaning those without significant emphysema or breathlessness may still require monitoring and treatment.

Furthermore, on a psychosocial level, the earlier age of diagnosis in AATD is likely to bring different considerations, such as impact on careers, physical activity, and family planning^{58,59}. Anxiety related to the hereditary nature of the disease is a burden not shared by usual COPD patients⁶⁰. In those who wish to fund AAT augmentation privately, financial matters will be a concern. AATD specialist services are few and far between, requiring long journeys to attend clinics for some patients; those who cannot commit to such travel may be anxious about the quality of their disease management, however comprehensive their local team may be⁶¹. These major differences warrant management in specialist clinics, but also education for local and secondary care teams.

1.1.3 Multisystem effects of AATD

1.1.3.1 Liver disease

AATD is also a multi-system disorder, with a minority of PiZZ patients having liver disease⁶². Pathogenesis of liver disease in AATD is due to polymerisation of the

protein at the site of its synthesis in the liver. Protein overload of the hepatocytes has been directly linked to inflammatory, fibrosis and hepatocellular carcinoma in mouse models ⁶³⁻⁶⁵. However, disease penetrance is variable, and estimates of prevalence of liver disease vary with definitions. One study of 57 patients found 63% had a history or clinical findings suggestive of liver disease; however, it is not clear from the article exactly how these criteria were defined⁶⁶. In the same study, 17.5% had evidence of severe fibrosis or cirrhosis on biopsy, consistent with a previous smaller study in 1986 ⁶⁷. A systematic review of 36 articles on prognosis found a rate of liver transplantation in adults of 14.7%, and a cirrhosis prevalence of 10.5%, though cirrhosis was not stratified by the conventional Child-Pugh score ⁶⁸. This review also included a mixture of phenotypes including PiMZ, PiSZ and PiZZ, so rates for a cohort of only PiZZ patients may be higher.

Clinically significant liver disease only occurs in around 10% of PiZZ patients, and therefore other factors must play a role in its development. Such factors may include age, male sex, diabetes and concurrent hepatitis from other causes, but current understanding does not allow an accurate prediction of which patients will develop liver disease ⁶⁹. Like in lung disease, evidence for the propensity of heterozygotes to develop chronic liver disease is mixed. However, PiSZ heterozygotes do appear to have an increased risk of chronic liver disease, and PiMZ patients have worse outcomes once chronic liver disease is diagnosed, as measured by decompensation episodes, liver transplantation, or death ⁷⁰⁻⁷².

1.1.3.2 Rare manifestations

Less than 1% of PiZZ suffer panniculitis, a rare dermatological condition consisting of inflammation of the subcutaneous fat, and presenting as painful, palpable bruises.

Previously known as Weber-Christian disease, it is thought to be related to extremely low levels of AAT³¹. A series of case reports described resolution of panniculitis with AAT-augmentation therapy have led to it being the only indication for AAT-augmentation therapy in the UK⁷³⁻⁷⁶. Genetic and clinical associations between with ANCA positive vasculitides have also been reported^{77,78}.

It is therefore important to recognise AATD as a multi-system disorder, with corresponding burdens of frequent healthcare appointments and complex counselling for patients, and to not be reductive by treating AATD as an exclusively respiratory disease. However, given the relatively more common manifestation of lung disease, AATD is managed by respiratory physicians primarily, with input from gastroenterologists, dermatologists, and geneticists as required.

1.1.4 Quantification of progression and severity of AATD

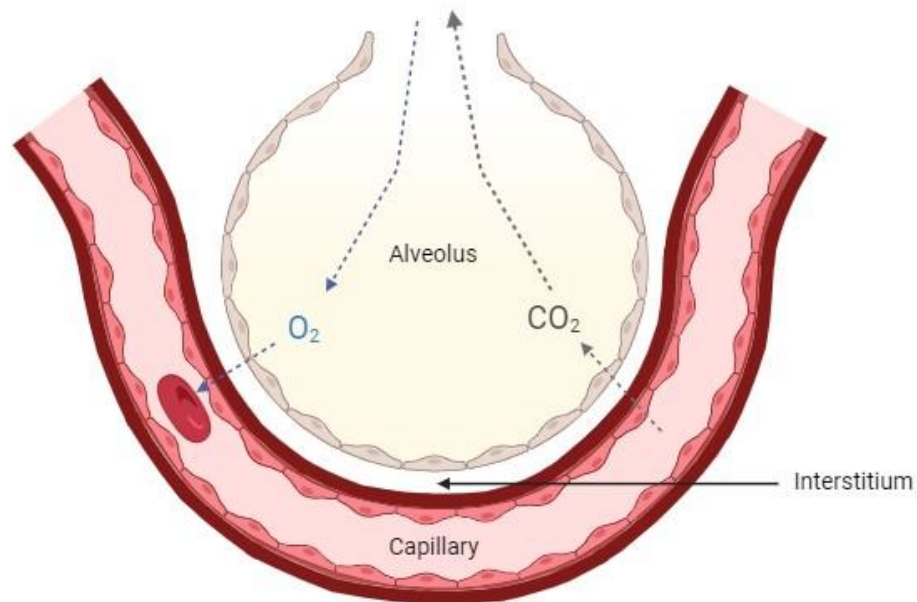
1.1.4.1 *Spirometry*

Like usual-COPD, diagnosis of COPD in AATD relies on a sub-normal FEV_{1.0}/FVC ratio, given the patient's age, gender, ethnicity, and height, which must remain after application of bronchodilators. The severity of airflow obstruction can be assessed by the FEV_{1.0} value, given in litres, and also adjusted for demographics. The Global Initiative for COPD (GOLD) criteria lists 4 stages of COPD, with mild disease having FEV_{1.0} greater than or equal to 80% of the predicted value (pp), moderate disease having FEV_{1.0}pp 50-79, severe disease having FEV_{1.0}pp 30-49, and FEV_{1.0}pp less than 30 represented very severe disease⁷⁹. However, patient's symptoms may not correlate with GOLD severity of FEV_{1.0} impairment, necessitating other methods of quantifying disease progression⁸⁰.

1.1.4.2 Gas transfer

The concept of gas transfer also informs the assessment of severity of lung disease such as COPD. This refers to the ability of oxygen to transfer between the alveoli and the pulmonary capillaries, through an intermediary extracellular layer known as the interstitium (Figure 1.3). Physiologically, this effect is proportional to the surface area available for transfer, the thickness of the interstitium, and a coefficient of gas exchanged which is proportional to the gas' solubility and inversely proportional to its molecular weight. As such, any impairment of surface area (as in emphysema or lung volume loss) or interstitium (as in interstitial lung disease) may lead to a gas transfer impairment. In pulmonary function testing, gas transfer is measured by inhaling a small amount of the surrogate marker of carbon monoxide (CO). Since the capillary CO level is known to be negligible in health, and CO binds to haemoglobin as oxygen does, the discrepancy between inhaled and exhaled CO divided by the time taken between breaths, and corrected for the pressure of the inspired gas mixture, gives a coefficient in mmol/min/kPa which can be compared against predicted values. This coefficient is expressed as the transfer coefficient for carbon monoxide (TLCO), and can be adjusted for lung volume (KCO, measured in mmol/min/kPa/ V_a , where V_a is the alveolar volume). Gas transfer is an important metric in AATD-COPD due to the fact that gas transfer measurements are somewhat independent of FEV₁, with severe impairment of gas transfer often co-existing with relatively milder FEV₁ impairment and vice-versa⁸¹.

Figure 1.3: Schematic of gas exchange in the lung.



1.1.4.3 Exacerbation frequency

Exacerbation frequency is also used as a marker of disease severity, although studies differ in the way in which they measure a clinically important difference in exacerbation status. One retrospective study of 142 AATD patients did not show a relationship between those with 0, 1 or ≥ 2 exacerbations per year and FEV_1 decline, but did show poorer quality of life and a weak association with TLCO decline in those with exacerbations⁵⁷. Other studies have shown an association of exacerbation rate with FEV_1 decline, and with quality-of-life scores, when comparing means^{28,82-84}. Hiller et al. used the median exacerbation rate of 0.66 in their cohort to divide into frequent and non-frequent exacerbators, and found a mean extra 30ml/year decline in FEV_1 , but this raises the question as to whether clinical episodes happening on a less than

annual basis can truly be called frequent⁴⁰. In Dawkin's et al's logistic regression analysis, FEV₁ decline associated with an increased odds ratio for exacerbation rate per year; however this paper took the unusual approach of reducing FEV₁ to binary status, as fast vs slow decline based on tertiles of the cohort as a whole, thus excluding a third of the cohort from the statistical analysis and limiting comparison to the rest of the literature in this section ⁸⁵.

1.1.4.4 Exacerbation frequency in usual-COPD

Articles from the usual-COPD literature also vary in their stratification of exacerbation status. One group found a significant difference in FEV₁ ml/year decline in frequent vs non-frequent exacerbators, divided respectively into those higher and lower than the median exacerbation rate of the whole cohort; however, this median of 2.92 differed markedly from Hiller's AATD study, which hinders comparison between the two groups ^{30,40}. The TORCH study (Towards a Revolution in COPD Health) divided exacerbators into 3 groups based on mean number of exacerbations per year. It found a difference in FEV₁ decline of an extra -15.2 ml/year between those who exacerbate 1 or more times per year vs those who do not, and an extra 17.5ml/year between those who exacerbate at all and those who do not, but no tests of statistical significance of these changes were applied and it was not the primary outcome ⁸⁶. Another study did not use exacerbation rate, but expressed their findings as FEV₁ decline per exacerbation episode, with each episode being associated with an 8-87ml/year decline, dependent on baseline COPD severity and severity of the exacerbation ⁸⁷. The standard used by current GOLD guidance is to stratify based on "frequent" exacerbators having 2 or more exacerbations per year, vs "non-frequent" for less than this ⁸⁸. Frequent exacerbators also experience higher mortality and poorer quality of life ^{29,89}. However,

exacerbation status may not be stable, though there is discordance in the literature as precisely how it varies. It has long been known that a significant proportion of patients change exacerbation status in both directions without clear impetus⁹⁰. More recently, a study of the ECLIPSE cohort found frequent exacerbation to be a stable phenotype and the status of those with less than 2 exacerbations per year to be uncertain⁹¹, but analysis of the SPIROMICS cohort found the opposite: that frequent exacerbator status changes more frequently than non-exacerbator status⁹², as did a study of 465 Bulgarian patients⁹³. Non-exacerbator status is now thought to be the more stable phenotype⁹⁴.

Furthermore, COPD often overlaps with other pulmonary syndromes⁹⁵, and exacerbations in other lung conditions may be quantified differently, including in bronchiectasis, as detailed in section 1.3.

1.1.4.5 *Radiology*

Monitoring of AATD can be supported by high resolution CT (HRCT) scanning, which is sensitive at detecting early signs of emphysema before it is apparent on lung function tests or symptomatically⁹⁶. A key advantage of cross-sectional imaging such as HRCT is that it can also identify other pathologies, such as bronchiectasis, for which it is the gold standard of diagnosis, as discussed in section 1.2⁹⁷. Emphysema will manifest on CT scanning as low-density voxels, a unit of volume in radiology. Visual assessment of this by a radiologist usually suffices clinically, but densitometry can also be objectively calculated as the density of the lowest 15% of voxels, and has been associated with GOLD severity staging and TLCO⁹⁸. Densitometry may even be more predictive of disease progression than lung function tests²⁸. It is also the only clinical

outcome yet shown to be improved by AAT-augmentation therapy, of which more is discussed in section 1.1.7.1.

1.1.4.6 Z polymers as a biomarker of disease severity

The polymerisation of Z-AAT has been suggested to have clinical relevance. In liver disease, increased levels of serum Z polymers are associated with liver-related hospitalisation, liver transplant and all-cause mortality⁹⁹. However, several biologically plausible mechanisms implicate them in the development of lung disease as well. They are seen in broncho-alveolar lavage, reportedly even after liver transplant, suggesting local synthesis and inactivation of Z-AAT^{100,101}. They are known to be pro-inflammatory, and attract neutrophils^{102,103}. As discussed in this section and below in section 1.2, neutrophil elastase is heavily implicated in the development of emphysema in AATD, but also in bronchiectasis in general, thus providing a hypothetical link between Z-polymerisation and bronchiectasis. Consequently, it is of interest whether Z-polymers correlate with disease severity, and therefore whether they could be used clinically as a circulatory surrogate marker. There is conflicting evidence for such a relationship in the literature: one study of 40 patients compared Z polymers with gas transfer and FEV1% predicted, with no correlation observed¹⁰⁴; a different study found a negative relationship between FEV1% predicted and circulating polymer levels in 70 patients of mixed genotype¹⁰⁵. One further study found differences in N2 washout between 9 PiZZ patients and 10 PiMM controls¹⁰⁶. All these studies are small, and given the ambiguity between their results, similar analyses of larger patient cohorts are warranted.

In summary, no single clinical marker can be used to estimate disease progression or severity in AATD, and prognostics must consider spirometry, gas transfer, HRCT, and history of exacerbations.

1.1.5 Challenges of research in AATD

Many challenges of research in AATD are common to all rare diseases. There is a limited pool of patients, amongst whom only few will wish to enrol in clinical research, which may lead to compromises in study design¹⁰⁷. They are distributed across geographic regions, presenting logistical challenges, and introducing selection bias whereby only the most mobile patients travel to clinic appointments¹⁰⁸. Although higher sample sizes may be gained by international collaboration, reimbursement approval varies between different healthcare systems, which leads to asymmetric treatment strategies across the globe, and may hinder international research collaboration. Investigators may also compete for the same pool of patients, meaning fewer studies can be published, or introducing statistical multiplicity¹⁰⁹. Research may also be impacted by fewer experts in the disease¹¹⁰. Additionally, since AATD disease progression occurs over many decades, long trials are necessary to demonstrate significant differences in treatment groups, which adds cost.

1.1.6 The AATD service in Birmingham

To address these challenges, in 1996 an AATD service was established in Birmingham, covering the majority of the UK population. This service now has close to 2000 patient records, around 400 of whom are under routine review and have consented to inclusion in a database of many clinical characteristics. Over recent years this service has evolved to include tertiary NHS care for patients, in addition to its research purposes. This is one of the largest AATD databases in Europe, and allows

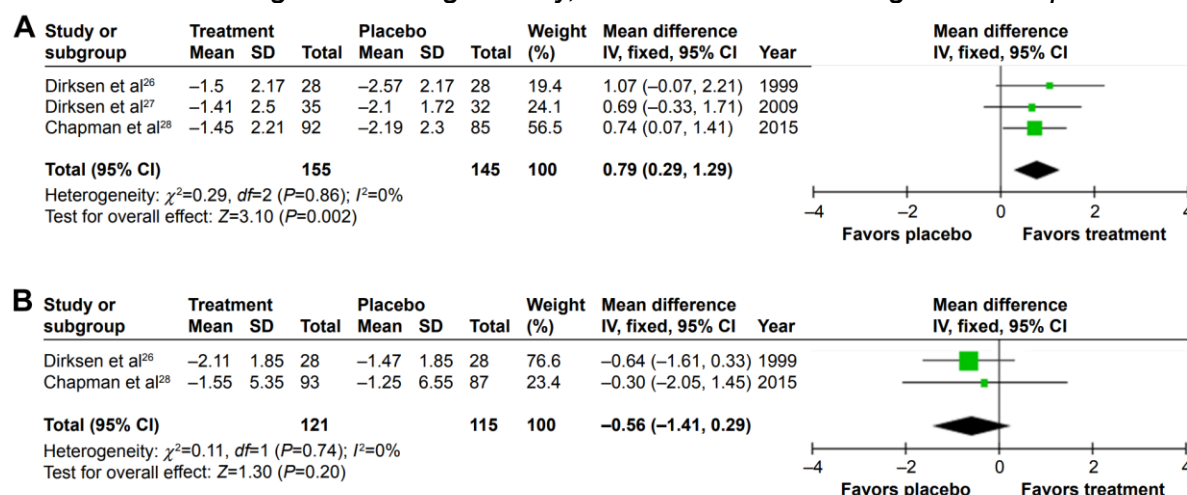
for higher powered statistical analysis, and consequently greater validity of results. The historical data in this doctoral thesis will be taken mainly from this database.

1.1.7 Treatment of AATD

1.1.7.1 *Augmentation therapy*

With technological improvement, replacement of deficient AAT became possible in the early 1980s¹¹¹, and US Food and Drug Administration (FDA) approval was granted in 1987¹¹². Augmentation is licensed in some countries for patients who have emphysema and the PiZZ genotype, at a dose of 60mg/kg/week, though various regimens are used worldwide¹¹³⁻¹¹⁵. Subsequent studies associated AAT replacement therapy with a reduction in progression of emphysema as measured by CT density, but not by FEV1¹¹⁶⁻¹¹⁸(Figure 1.4). However, CT density has been associated with disease severity and mortality in both smoking-related COPD and in AATD related COPD^{119,120}. This indirect link is sufficient for the therapy to be recommended in guidelines in North America and in Europe, but publicly funded health agencies in many countries in these regions do not reimburse treatment, including the UK^{38,121,122}. Internationally, drug regulators also differ on which outcomes to use in trials of therapies targeting emphysema or COPD: the European Medicines Agency (EMA) accepts CT lung density as a suitable outcome, but the FDA does not.

Figure 1.4: Meta-analyses of AAT augmentation vs placebo. As per Edgar et al¹²³: A: mean annual change in CT lung density, B: mean annual change in FEV₁ predicted.

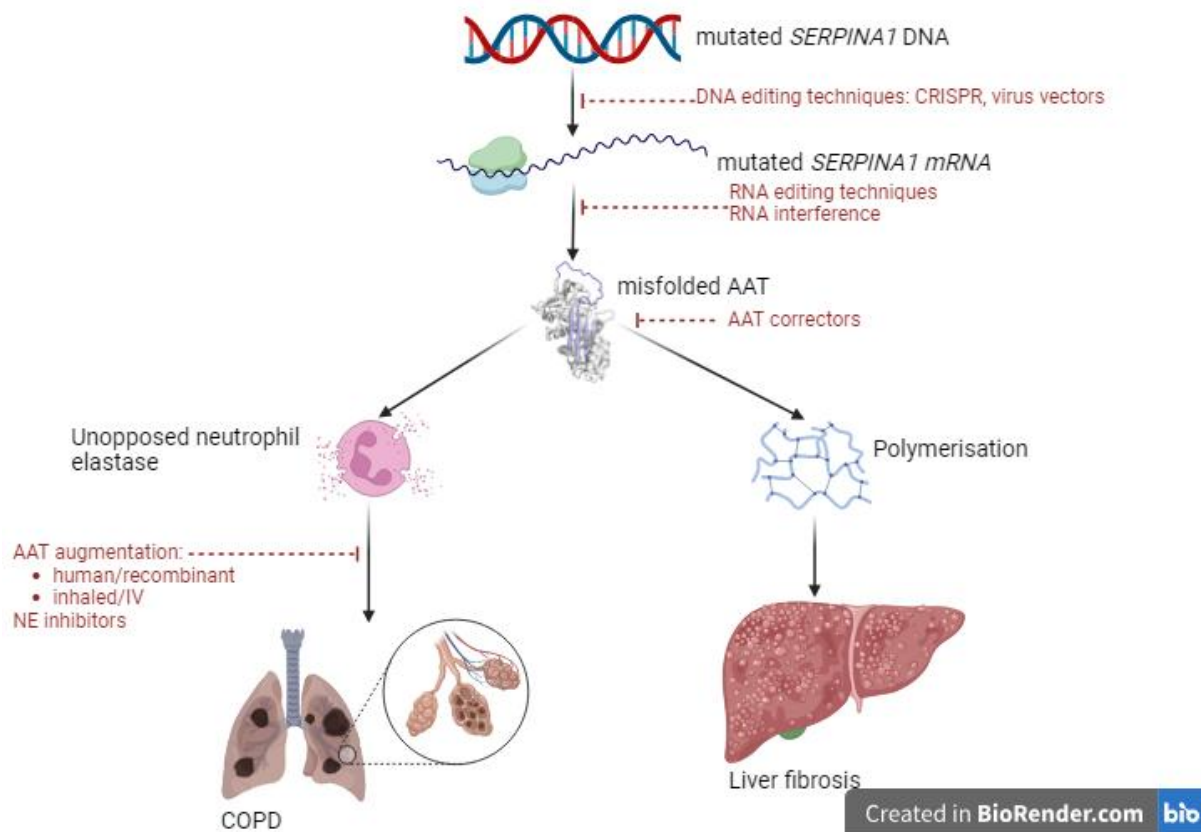


Even in countries which licence AAT replacement therapy, research into alternatives is necessary. AAT augmentation relies on derivation from human plasma donations, requiring many donated units to generate one dose, which in most dosing regimens requires weekly infusions^{111,124}. This is both time-consuming and costly, and relies on the finite resource of plasma donations which are shared with emergency, haematological, and surgical services¹²⁵. Synthetic production of AAT or NE inhibitors could reduce this limitation. Additionally, treatment so far has been intravenous, but only a minority of the AAT infused reaches the lung¹²⁶; an inhaled route may improve efficiency, whilst being less invasive to administer. Furthermore, augmentation dosing strategies vary in the literature, with equivocal evidence between regimens^{115,124,127}. Finally, augmentation therapy only addresses lung disease, such that liver directed approaches are also required if all patients are to be managed adequately. Table 1.1 summarises the current state of AATD drug development, and Figure 1.5 places these in a schematic of AATD pathogenesis.

Table 1.1 Competitive environment: AATD therapies currently in development. AAT: Alpha-1 Antitrypsin.

Drug	Pharmacology	Current Phase	Company	National Clinical Trials Number(s)
Kamada-AAT	Inhaled human AAT	III	Kamada	02001688 04204252
Fazirsiran	RNA interference therapy	III	Takeda	05899673 05677971
Alvelestat	Neutrophil Elastase Inhibitor	II/III	Mereo	03636347 03679598
Inbrx-101	Recombinant AAT	II	Inhibrx	05856331
VX-864	AAT corrector	II	Vertex	05643495
Belcesiran	RNA interference therapy	II	Novo Nordisk	04764448
ADVM-043	Gene editing	I/II	Adverum	02168686
BEAM-302	DNA editing	I/II	Beam	06389877
NTLA-3001	DNA editing	I/II	Intellia	06622668
OsrhAAT	Recombinant AAT	I	Oryzogen	05315921
PHP-303	Neutrophil Elastase Inhibitor	I	pH Pharma	03627845 03804021
WVE-006	mRNA editing	I	Wave Life Sciences/GSK	06186492

Figure 1.5: Pathogenesis of AATD



1.1.7.2 Recombinant AAT (rAAT)

Addressing the limited availability of hAAT, research is underway to synthesise functional AAT, called recombinant AAT (rAAT). This also raises the potential for creating a maximally efficient AAT protein. INBRX-101® (Inhixrx) is a rAAT Fc fusion protein which has completed phase I trials showing normal AAT serum levels in 24 AATD patients, with an acceptable safety profile. A phase 2 study is currently recruiting at 2 doses vs Zemaira® (CSL Behring), a hAAT medicine, with the primary outcome being change in serum AAT levels ¹²⁸. A phase 1 study of another rAAT molecule, OsrhAAT® (Oryzogen), at several IV doses has also begun ¹²⁹.

1.1.7.3 Inhaled AAT

Inhaled AAT offers many advantages over intravenous administration. It negates the need for IV access, and allows administration directly at the site of interest. Since a higher proportion of the treatment is distributed to lung tissue, less of the drug is needed to achieve the same lung tissue concentration, thus reducing dependence on limited plasma pools ¹³⁰. KAMADA-AAT®(Kamada), an inhaled AAT product, has completed phase 2 trials ¹³¹. Results included significantly higher functional AAT levels in epithelial lining fluid (ELF) at both 80mg and 160mg doses; increased serum plasma AAT; and increased ELF AAT-NE complexes. Phase 3 trials are now underway in centres across Europe¹³².

1.1.7.4 Neutrophil elastase inhibitors

NE inhibitors offer an alternative to AAT augmentation which does not rely on IV or nebulised routes, but still achieves NE inhibition. One such drug is Alvelestat® (Mereo), which has recently completed a phase 2/3 trial, known as ASTRAEUS, of reduction in desmosine and Aα-val360 biomarkers, and the St George's Respiratory Questionnaire activity score, over 12 weeks ¹³³. The conference abstract describes an association between circulating biomarker levels and SGRQ score, but does not mention any differences between Alvelestat® and placebo ¹³³. Another Phase 2 placebo-controlled trial of Alvelestat®, known as ATALANTa, is underway at the University of Alabama, with results expected in 2023¹³⁴. A further NE inhibitor, PHP-303® (pH Pharma) has completed phase 1 trials, with a promising safety profile ^{135,136}.

1.1.7.5 AAT correctors

These drugs aim to change the shape of the misfolded dysfunctional AAT protein, thereby improving its function, but also preventing the polymerisation of AAT in the

liver, which is the main cause of AATD liver disease. Trials of these drugs are in the very early stages ¹³⁷⁻¹³⁹, but some useful information is available. A study of VX-814® (Vertex) at several doses was terminated early due to raised liver transaminases ALT and AST at all doses vs placebo ¹⁴⁰. However, VX-864® (Vertex) showed significant improvement in functional AAT levels vs placebo (mean difference +2.2 to +2.3 µmol/L across 3 doses, p <0.0001 in all 3 cases) in a phase 2 trial of 44 participants ¹⁴¹.

1.1.7.6 Gene therapy

Gene therapy has the potential to act even earlier in the disease pathway than AAT correctors, by altering DNA or RNA to produce functional AAT instead of misfolded proteins. The ADVANCE Study is a phase 1/2 trial of ADVM-043® (Adverum), using a Virus Gene Transfer Vector to deliver a functional gene to the liver of 6 patients, to express functional human AAT ¹⁴². The safety profile at present is mixed, with 3/6 patients having severe adverse events including *Clostridium difficile* colitis, flu, and COPD. Results of a long-term follow-up study are expected imminently¹⁴³.

The development of nucleotide editing therapies is a very active area. BEAM-302® (Beam) is described as a “liver-targeting lipid-nanoparticle (LNP) formulation of base editing reagents”, and has reportedly been able to correct some *SERPINA1* DNA in mouse models, with increases in serum AAT and reduced liver polymers ¹⁴⁴. Similarly, Intellia have developed NTLA-3001®, a CRISPR-mediated targeted gene editing therapy, and have begun phase I trials¹⁴⁵. Wave Life Sciences and GSK have collaborated to develop WVE-006®, an RNA editing oligonucleotide which offers to correct the single nucleotide mutation at the mRNA stage, and applied for phase I trial registration¹⁴⁶.

Fazirsiran® (Takeda) is described as an RNA interference therapeutic, causing degradation of AAT RNA and therefore reduction of hepatocyte AAT production, polymerisation, and consequent liver disease¹⁴⁷. In phase 2 trials, liver biopsies of 15 patients with PiZZ AATD liver disease were taken at baseline and at 12 weeks, with median -83.3% reduction of liver AAT, and reduced histological globule burden¹⁴⁷. Serum liver enzymes also reduced in all patients, and fibrosis regression was observed in the majority. The safety profile was also reassuring. Phase 3 trials in larger samples are ongoing. Another RNA interference therapeutic, Belcirsiran® (Dicerna), was also in phase 2 trials but then abandoned¹⁴⁸.

1.1.7.7 Non-specific treatment of AATD: COPD pharmacotherapeutics

The airflow obstruction and inflammation associated with COPD can be treated with a number of pharmacological mechanisms³⁸. Beta agonists are drugs which activate the beta-adrenergic receptors, leading to bronchodilation via the same mechanism as an adrenaline response, and can be applied to the lung via inhaled devices in short- or long-acting forms. Muscarinic antagonists inhibit the muscarinic receptor of the parasympathetic nervous system, and in doing so prevent constriction of the airways. Like beta agonists, they come in short- or long-acting forms. Inhaled corticosteroids (ICS) are of variable benefit – in those with a degree of reversibility or diurnal variation of obstruction raised eosinophils, or concurrent asthma, they may have a benefit. However, in some cases ICS can be associated with pneumonia¹⁴⁹, and are therefore best avoided in those without these qualifying factors. Mucolytics such as carbocysteine are often used to enable expectoration of thick sputum and reduce exacerbation frequency¹⁵⁰. Mucolytics and preventative macrolides are effective in reducing exacerbations in usual-COPD¹⁵⁰⁻¹⁵², and there is no reason to doubt an equivalent

effect in AATD-COPD. At the upper echelons of treatment, theophylline can also be used as a bronchodilator, though its mechanism of action is not well understood and only has a modest effect on spirometry results¹⁵³. Trials are underway to study the use of monoclonal antibodies in COPD, but none are yet licensed¹⁵⁴.

1.1.7.8 Non-specific AATD treatment: non-pharmacological treatment

Vaccinations are an effective way of preventing exacerbations in usual COPD^{155,156}, with annual influenzae vaccination also of proven benefit in AATD¹⁵⁷, and are therefore recommended to all patients with AATD lung disease³¹. Smoking cessation is of vital importance, due to the accelerated lung function decline seen in smokers with AATD, even by comparison to smokers with usual-COPD^{82,158}. Pulmonary rehabilitation, a weekly or biweekly course consisting of breathing techniques, regular aerobic and chest strength exercise, has been shown to work as effectively in increased 6-minute walking distance in AATD-COPD as in usual-COPD¹⁵⁹, and is therefore also recommended³¹.

Surgery is also used as a treatment option in severe AATD-COPD. Both lung volume reduction techniques and lung transplant may be considered. Lung volume reduction can take the form of endoscopic valve insertion, video-assisted thoracoscopy (VATS) techniques, or open surgery. Endoscopic valves have no proven evidence and remain experimental. Lung volume reduction has been shown to improve outcomes which in usual-COPD suffers with heterogenous, primarily upper lobe, emphysema and intact lung fissures^{35,36}. Evidence to support lung volume reduction surgery in AATD has hitherto been drawn from that of usual-COPD, as adequately powered studies in AATD have not been possible. However, a post-hoc analysis of AATD patients included in the National Emphysema Treatment Trial (NETT) found no benefit, and an increased

mortality, in those who underwent surgical procedures. However, techniques have improved since this early trial, and more research is necessary to assess whether surgical options may now benefit AATD patients, particularly as AATD emphysema predominantly affects the lower lobe, whereas upper lobe disease was the only subset found to benefit in the NETT trial ³⁶. Conversely, for endoscopic valve insertion, improvements in lung function, symptoms and quality of life have been documented in AATD-COPD specifically^{160,161}.

Lung transplant is considered for patients with severe disease who are not suitable for lung volume reduction techniques, and is the 4th most common reason for referral to lung transplant services¹⁶². Across all indications, lung transplant is associated with a 56.2% 5 year survival rate¹⁶³, but the limited research in AATD lung transplant shows conflicting evidence on mortality, with one study showing significant improvements in physiology and symptoms but not mortality¹⁶⁴, and another showing median survival increasing by 6 years¹⁶⁵. It can therefore be considered effective at least as a palliative measure.

1.1.7.9 Summary of ongoing AATD therapeutic research

The breadth of creativity and commitment involved in the innovation, study design and implementation of emerging therapies for AATD give cause for optimism. Recombinant AAT has the potential to reduce financial costs and reliance on plasma donations. Nebulised AAT promises similar benefits, by way of increasing the proportion of AAT delivered to the site of interest. AAT “correctors” and RNA interference therapeutics go even further, offering to change the shape of misfolded proteins to prevent liver disease, and in some cases also reduce lung function decline. Gene therapy, as it emerges from the realm of science-fiction to clinical research, even offers to cure the

disease, by aiming to allow production of healthy AAT in the native liver. Ensuring that trial outcomes have clinical relevance, beyond pure biochemical correction, will be vital to ensure meaningful improvement in patients' lives and translation into clinical practice, with reimbursement by health systems to ensure equity of access. Although few of these new therapies have yet produced concrete results, publications of these trials are expected in the near future. Consequently, the next few years will constitute an important, and possibly transformative, period in AATD research, with benefits of interest to patients, physicians, and healthcare systems alike.

1.1.8 Research priorities in AATD

Given the large number of emerging treatments in AATD, research is required to assess which specific subtypes of AATD patients are at risk of disease progression, and therefore which may benefit from these various treatments. Accurate selection of these patients is important, in order to not expose patients to unnecessary treatment risks, and to have maximally efficient use of treatments which are likely to be expensive when first coming to market. The work presented in this thesis centres on establishing whether bronchiectasis in AATD constitutes such a phenotype of AATD.

1.2 Bronchiectasis

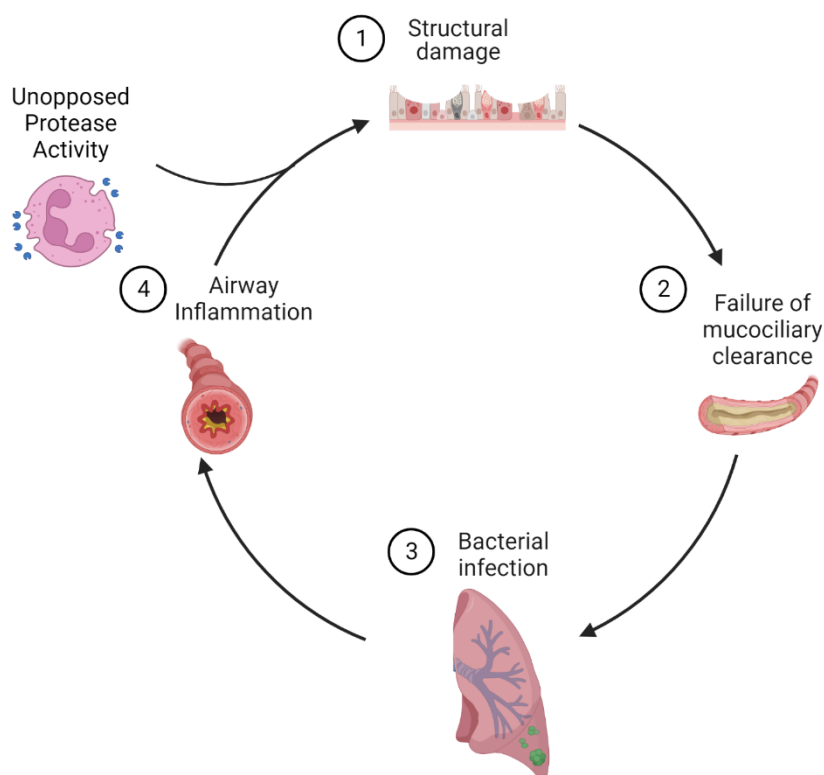
Bronchiectasis is known to occur in AATD, but it is as yet unclear from the literature whether it is directly associated with AATD or simply occurs due to COPD of any cause.

1.2.1 Pathogenesis of bronchiectasis

Bronchiectasis is a disease of the airways, etymologically meaning bronchial dilatation (*-ectasia*). It has multiple causes, including primary genetic diseases, connective tissue diseases, immunodeficiencies, foreign bodies, and post-infectious

syndromes¹⁶⁶; however, heterogeneity of the disease is not exclusively due to differing causes¹⁶⁷. The academic consensus is that bronchiectasis is a result of a vicious cycle consisting of airway inflammation, followed by airway structural damage and dilatation, which leads to failure of mucociliary clearance, which predisposes to bacterial infection¹⁶⁸. Bacterial infection causes further airway inflammation, and the cycle continues. More recently, the cycle has been described as a “vortex”, with all steps of the cycle contributing to all others¹⁶⁹, but the first analogy is intuitive and sufficiently encompasses the known causes. All known causes of bronchiectasis lead to this vicious cycle in one of these four ways; in the case of AATD, airway structural damage by unopposed neutrophil elastase could theoretically be an impetus (Figure 1.6).

Figure 1.6: The vicious circle theory of bronchiectasis pathogenesis



Airway inflammation is apparent from histological examination of bronchiectatic airways, showing an abundance of neutrophils, lymphocytes, and eosinophils and dense chronic inflammatory cell infiltrate¹⁷⁰. Neutrophil activity is mediated by a

complex series of cytokines including interleukins, chemokines, and tumour necrosis factors. Neutrophil elastase, the key protease implicated in development of AATD-emphysema, is known to be elevated in the sputum and BAL of all-cause bronchiectasis¹⁷¹. Interestingly, although neutrophil elastase is known to cause direct parenchymal damage²³, it may cause collateral damage in another way: its unopposed activity has also been shown to inhibit neutrophil phagocytosis¹⁷², thereby impairing clearance of bacteria, and leading to still more inflammation. Neutrophil elastase is therefore of key clinical relevance in bronchiectasis, being associated with severity, lung function, symptoms, and radiological appearance^{171,173}. Other cell types, including macrophages, lymphocytes, and eosinophils are also implicated, but are relatively less studied¹⁷⁰. Eosinophils in particular have been estimated to be elevated in 20% of bronchiectasis patients, even without an alternative cause such as ABPA or asthma¹⁷⁴. Eosinophilia forms a clinically relevant endotype of COPD⁷⁹, and increasing evidence shows a similar endotype may exist in bronchiectasis, where high eosinophils have been associated with more frequent exacerbations¹⁶⁴, and have been identified for treatment with IL-5 modulating agents in some cases, with improvement in lung function and symptoms^{175,176}.

1.2.2 Diagnosis of bronchiectasis

In contrast to the genetic definition of AATD, bronchiectasis is a syndrome defined clinically and radiologically. Clinical symptoms are often non-specific, but include shortness of breath, productive cough, and recurrent chest infections, all of which overlap with COPD, particularly the chronic bronchitis element of COPD. However, due to its radiological definition, many cases are diagnosed in asymptomatic patients¹⁷⁷. Radiological findings centre on establishing abnormally dilated airways, by identifying a lack of tapering or airway visible <1cm from the pleura^{178,179}. Examples

are given in Figures 1.7 and 1.8¹⁷⁹. Less specific findings on CT include bronchial wall thickening, mosaic attenuation, and mucous impaction; however, these findings are not specific to bronchiectasis and therefore, in isolation, are not sufficient for a diagnosis¹⁷⁸.

Figure 1.7: Signet ring sign: the airway diameter significantly exceeds the arterial diameter in transverse view. Case courtesy of The Radswiki, Radiopaedia.org, rID: 11258

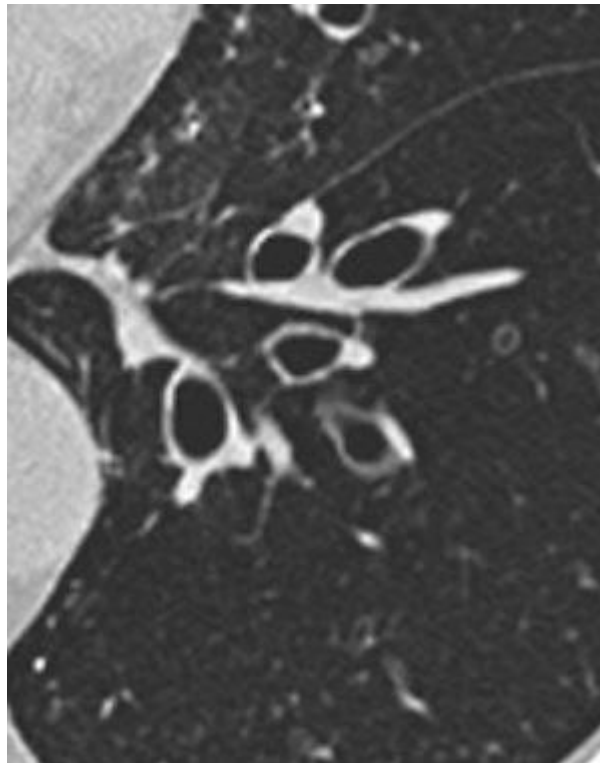
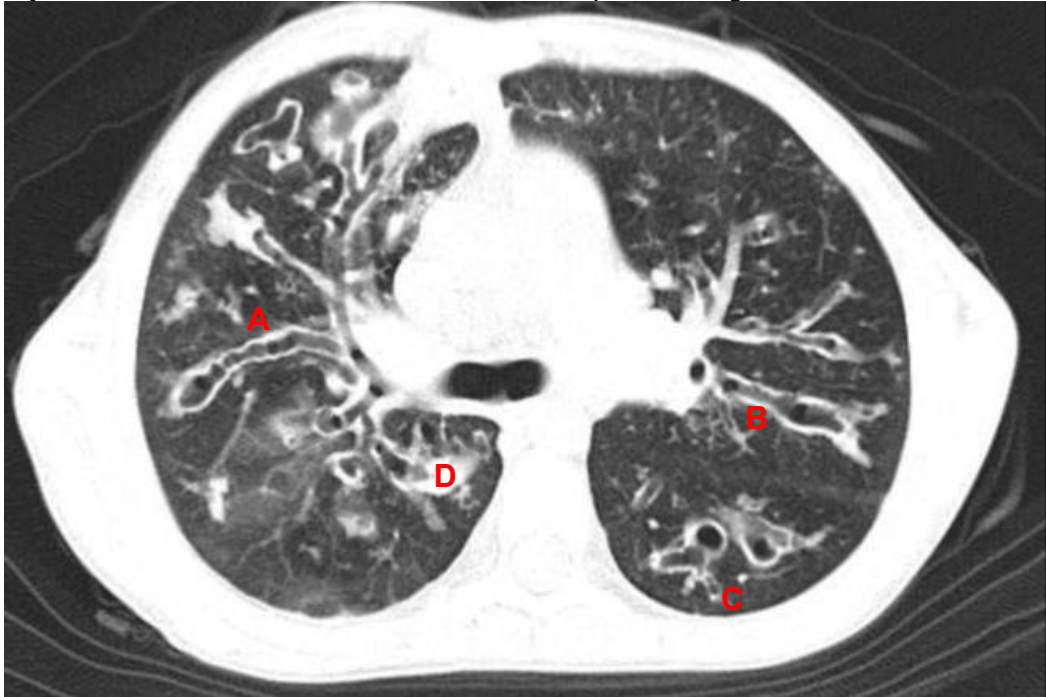


Figure 1.8: Bronchiectasis CT image exhibiting lack of tapering (A), bronchial wall thickening (B), and airway visible <1cm from the pleura (C). Mosaic attenuation is also visible in the right lower lobe, and mucous plugging is also visible (D). Case courtesy of Mohammad Ashkan Moslehi, Radiopaedia.org, rID: 19174



Radiological changes can be present without the classical symptoms of purulent phlegm, recurrent chest infections and shortness of breath, and the availability and quality of CT scanning has now developed to the extent that many cases of bronchiectasis are diagnosed incidentally on CT scans done for other indications¹⁷⁷. Additionally, the precise radiological appearance of bronchiectasis does not always correlate with disease severity as perceived by patients or their physicians¹⁸⁰. Along with the need to give meaningful prognoses to patients, this has led to attempts to develop a standardised method of describing bronchiectasis severity, as described in section 1.2.4.1.

1.2.3 Identifying underlying aetiologies of bronchiectasis

Identifying the underlying cause of bronchiectasis has been found to change management in 18% of cases¹⁸¹, and is therefore of considerable importance. Whilst

the majority of cases are classified as idiopathic or post-infectious, the most important underlying causes are antibody-deficiency syndromes, found in 5% of patients, and allergic-bronchopulmonary aspergillosis (ABPA), found in 2.6% of patients¹⁸¹, both of which have prognostically beneficial managements if identified. By comparison, AATD is estimated to be found in less than 1% of bronchiectasis patients¹⁸². BTS guidelines therefore recommend serum IgG, IgA, IgM, IgE, and specific *Aspergillus* IgE be tested in all patients with bronchiectasis. Sputum microbiology should also be tested in all cases of bronchiectasis, and non-tuberculous *Mycobacteriae* (NTM) are particularly strongly associated¹⁸³, but it remains unclear whether NTM is causative or simply opportunistically colonises affected airways¹⁸⁴. Further tests are warranted depending on clinical suspicion: in young, male, infertile patients with a history of malabsorption or pancreatitis, cystic fibrosis should be tested for; in young patients with recurrent sinusitis or otitis, primary ciliary dyskinesia (PCD) should be tested for.

1.2.4 Quantification of progression and severity of bronchiectasis

Many clinical characteristics are implicated in the assessment of disease severity in bronchiectasis. Many common comorbidities, such as COPD, cognitive impairment, and liver disease, greatly influence mortality¹⁸⁵. FEV1% predicted values are negatively correlated with degree of bronchial dilatation, and also correlate with mortality¹⁸⁶⁻¹⁸⁸. Exacerbations are often also influence both mortality and quality of life¹⁸⁹, though the clinically relevant “frequent” exacerbation threshold appears to be ≥ 3 per year^{187,188}, rather than ≥ 2 year in COPD⁷⁹, potentially creating confusion in patients with both diseases. Microbiology is also implicated and discussed further in section 1.2.4.2.

Options for quantifying the severity of bronchiectasis are available, but have flaws. The FACED score, originally presented in 2014 ¹⁸⁷, includes 5 criteria as shown in Table 1.2. The FACED score had good predictive power for 5-year all-cause mortality, but did not include a wide range of other pathogens in its analysis, and only included 1 radiological parameter (number of lobes affected) in its derivation study. Furthermore, it did not originally attempt to predict exacerbation rate, a symptom of great importance to patients and healthcare economics, though an extended version was later validated in this regard.

The bronchiectasis severity index (BSI) of 2014 is more extensive, providing 9 clinical parameters which are independently associated with mortality and hospital admission. The score accounts for age, BMI, baseline FEV1pp, previous hospital admissions, exacerbation rate, MRC score, pseudomonas colonisation, isolation of other potentially pathogenic bacteria, but only has one domain for radiological severity, defined as *3 or more lobe involvement or cystic bronchiectasis*¹⁸⁸. In comparative studies, BSI has been the more capable score in predicting exacerbations, hospitalisation, but with a roughly equal capability to predict mortality ^{190,191}.

Table 1.2: Comparison of BSI and FACED scores.

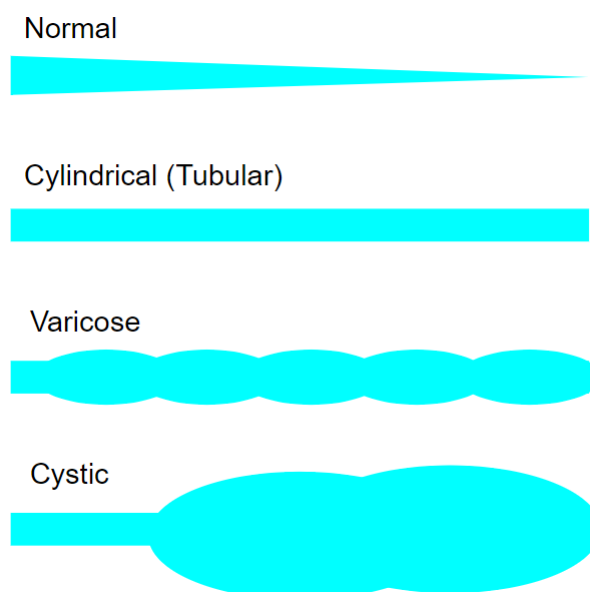
Criterion	Points						
	0	1	2	3	4	5	6
Bronchiectasis Severity Index							
Age	<50		50-69		70-79		80
BMI	≥18.5		<18.5				
FEV1% predicted	>80	50-80	30-49	<30			
Hospital admission <2 years previously	No					Yes	
Annual exacerbation rate	<3		≥3				
MRC	1-3		4	5			
<i>Pseudomonas</i> colonisation	No			Yes			
Colonisation with other pathogens	No	Yes					
≥3 involvement, or cystic morphology	No	Yes					
FACED							
FEV1% predicted	≥50		<50				
Age	70		≥70				
Chronic <i>Pseudomonas</i> colonisation	No	Yes					
Extension (≥3 lobe involvement)	<3	≥3					
mMRC score [MRC - 1]	0-2	3-4					
[Extended FACED only] Annual severe exacerbation rate	No		Yes				

1.2.4.1 Radiological severity in bronchiectasis

The BSI arrived at their radiological criterion by adapting Reiff's score of 1995¹⁹², where severity is based on a score taken from number of lobes involved and morphology of bronchiectasis according to Reid's 1950 classification system¹⁹³ (cylindrical, varicose, cystic, Figure 1.9). Reid's system has been used in most assessments of morphology in the literature, but was in fact based on pathological specimens, rather than radiology. The Reiff score in turn was developed from Bhalla *et al.*'s scoring system with 9 categories, of which some may describe bronchiectasis, but which was only validated in cystic fibrosis¹⁹⁴. These limitations were the rationale for the publication in 2018 of the Bronchiectasis Radiologically Indexed CT Score (BRICS)¹⁸⁰. This study identified two components of the Bhalla score, *bronchial*

dilatation and number of bronchopulmonary segments with emphysema, as predictive of exacerbation rate and degree of obstruction in bronchiectasis, and combined them for a score between 0 and 5. However, for use in cases of combined COPD and bronchiectasis, this presents a conundrum of classification: emphysema is a constituent part of COPD and its severity should therefore not be used to evaluate bronchiectasis, since this would affect the determination of which syndrome is contributing to outcomes. The circular nature of this score is apparent when we conclude from the BRICS article that degree of emphysema is predictive of severe airflow obstruction, which is an integral part of definitions and guidelines of COPD ⁸⁸.

Figure 1.9: Schematic of Reid's bronchiectasis morphology¹⁹³ and a representation of the normal pattern of airway tapering



A further weakness of all measures of bronchial dilatation is that all current scoring systems measure bronchial dilatation in relation to the adjacent artery. However, in the case of pulmonary hypertension, the pulmonary arteries may themselves be enlarged, meaning that a dilated bronchiole may not appear so in relation to the hypertrophied and dilated adjacent pulmonary artery. Pulmonary hypertension has many causes, including bronchiectasis and COPD, and so can be expected to occur in a minority of

all patients in research on this topic¹⁹⁵⁻¹⁹⁷. The identification of lack of tapering may be superior to comparison with adjacent arteries, but is far more labour-intensive and requires expert radiological skill. The future use of algorithmic radiological analysis may reduce these labour requirements, by analysing vast quantities of data with objectivity. So far, some software packages have been tested and validated in other respiratory disorders, but lack validation in bronchiectasis^{198,199}. One example is the YACTA (Yet Another CT Algorithm) software²⁰⁰, which is rare among such algorithms in being able to construct a true tapering index for the whole lung. Parameters derived from this algorithm have been found to associate with FEV1% predicted in adults with cystic fibrosis¹⁹⁹.

A 2007 study of patients with AATD and bronchiectasis used a bespoke radiological severity score based only on degree of bronchial dilatation²⁰¹. This study also used a bespoke scoring system of Airway Disease Score (Bronchial Wall Thickness) (ADS_{BWT}); however the mean value was outside the stated range of scores, thus compromising the reliability of this bespoke score²⁰¹. It may be that severity, whilst useful for clinical assessment, is simply too subjective a term to use in standardised quantification of CT appearances, and radiological description should therefore focus only on bronchial dilatation, lobar involvement, morphology, and bronchial wall thickening. Conversely, radiology is no longer thought to be sufficient to encompass the heterogeneity of clinical symptoms and prognosis seen: BSI estimated radiological appearances to be 3 times less important than *Pseudomonas* colonisation or severe obstructive spirometry, and only as important as colonisation with other pathogens (Table 1.2)²⁰².

1.2.4.2 Microbiology of bronchiectasis

Respiratory tract dysbiosis plays an important role in the prognosis of patients with bronchiectasis. Less diverse microbiomes, and the presence of Pseudomonadota (or Proteobacteria), both appear to correlate with lung function decline²⁰³⁻²⁰⁵. *P. Aeruginosa* in particular is associated with exacerbation frequency, quality of life, and mortality; strong evidence exists for these links, and therefore eradication therapy should be considered irrespective of symptoms^{97,206-208}. Other common colonising bacteria include *H. influenzae*, *S. pneumoniae*, *M. catarrhalis*, *S. aureus*, *S. maltophilia* and non-tuberculous *Mycobacteria* (Table 1.3). Some of these others have been independently associated with disease outcomes, and *H. influenzae* has been associated with reduced microbiome diversity²⁰⁹. However, the identification of this set of bacteria as common and potentially pathogenic does have a limitation; culture mechanisms are often designed so as to preferentially grow these organisms, potentially excluding other pathogens (notably anaerobes) and so propagating the emphasis on the conventional pathogens in the literature²¹⁰. This is where microbiome data can prove useful, by analysing bacterial DNA rather than relying on culture. In large cohorts, this can also provide supportive evidence of complex bacterial interactions²¹¹. Yet microbiome analysis also comes with its own disadvantages; it cannot identify whether bacteria are alive or dead, and cannot quantify bacterial load. Both techniques are therefore required for a complete study of microbiology of the lung.

Table 1.3: Common colonising microorganisms in bronchiectasis. * denotes non-pseudomonal pathogens which associate with mortality and hospitalisation when treated as a group

Organism	Prevalence among sputum-producing bronchiectasis patients in Europe (%)	Clinical significance in bronchiectasis
<i>Pseudomonas aeruginosa</i>	25.1 ²¹²	Linked to higher exacerbation frequency, hospital admission, lower quality of life, and increased mortality ^{97,206-208}
<i>Haemophilus influenzae</i>	23.6 ²¹²	Inconclusive*; associated with reduced microbiome diversity ²⁰⁹
Non-tuberculous <i>Mycobacteriae</i>	8.3-12 ²¹³⁻²¹⁵	Increased mortality and hospitalisations ²¹⁶ .
<i>Staphylococcus aureus</i>	8.6 ²¹²	Inconclusive*
<i>Streptococcus pneumoniae</i>	8.5 ²¹²	Inconclusive*
<i>Enterobacteriaceae</i>	5.9 ²¹²	Inconclusive*
<i>Moraxella catarrhalis</i>	5.4 ²¹²	Inconclusive*
<i>Aspergillus fumigatus</i>	1.6 ²¹²	Associated with increased exacerbations, severity, and poorer pulmonary function ^{217 218}
<i>Stenotrophomonas maltophilia</i>	1.1 ²¹²	Inconclusive; some data suggests increased hospitalisations and lower FEV ₁ ^{219,220}

1.2.4.3 Biomarkers in bronchiectasis

The study of biochemical markers in bronchiectasis is a rapidly developing field, and has great potential in the categorisation of this widely heterogenous disease, enabling targeted treatments based on underlying phenotypes. Biomarkers may also be useful in terms of monitoring disease progression and prognosis in diseases including AATD. Heterogeneity of individual bronchiectasis phenotype is not wholly dependent on underlying aetiology: two patients with the same underlying aetiology of the same age, gender and smoking history may have wildly different phenotypes; one having perhaps only mildly dilated cylindrical bronchiectasis confined to one or two lobes, and the other having widespread cystic bronchiectasis. Evidence for such variation exists for other underlying aetiologies^{188,221}, and anecdotally is also found in AATD. Such wide

heterogeneity, even within the same aetiologies, illustrates the need for identification of non-invasive, cost-effective biomarkers to identify clinical phenotypes and endotypes.

Sputum biomarkers have been widely studied in bronchiectasis, with neutrophil elastase being the most promising identified, as it is independently associated with worse HRCT score, exacerbation rate and FEV1 decline^{171,222}. Neutrophil elastase is a proinflammatory enzyme, and is the main protease involved in the progression of AATD-associated lung disease. Several neutrophil elastase inhibitor trials have been conducted, with promising results. Some have shown association with exacerbation frequency, lung function decline, and disease severity score^{135,209,223-225}. No neutrophil elastase inhibitors, including AAT replacement, are yet licensed in the UK. Other neutrophil-dependent markers have been identified as predictive of disease severity, including matrix metalloproteinase-8 and -9^{226,227}. In one study, sputum interleukin-8 (IL-8) was associated with frequent exacerbations, and interleukin 13 with bronchodilator reversibility, but neither were associated with radiological severity, whereas sputum neutrophil count was²²⁸. Cathepsin G is another serine protease long known to be associated with more severe bronchiectasis²²⁹. More recently, a study including 48 patients with both bronchiectasis and usual COPD found these patients had higher proteobacteria (including *P. aeruginosa*) infection rates, and higher levels of neutrophil degranulation proteins, compared to patients with COPD alone²³⁰. Compared with serum, sputum has the advantage of being non-invasive to obtain and providing a direct sample from the site of inflammation, but not all patients reliably produce sputum, and samples may be diluted with saliva.

Several serum biomarkers have also been studied. Fibrinogen appears one of the most promising, as it has been associated with increased Bronchiectasis Severity Index score, lower FEV1, *P. aeruginosa* infection and impaired quality of life²³¹. Secondly, eosinophilic inflammation appears to comprise a specific endotype of bronchiectasis, with serum counts of $>0.3 \times 10^{-3}/\text{ml}$ associating with frequency of exacerbations in two studies^{175,232}. Conversely, eosinophil level $<0.1 \times 10^{-3}/\text{ml}$ was also associated with disease severity^{232,233}. Eosinophils are easy to measure within existing clinical services, as are thrombocytes, which have been directly associated with disease severity, exacerbation rate, and mortality in stable bronchiectasis²³⁴. TNF-alpha has also been found to associate with bronchiectasis severity score, but only in a single study which was unclear in its definitions of severity²³⁵. Soluble adhesion molecules such as ICAM-1, E-selectin, and VCAM-1 are involved in the mobilisation of leucocytes to areas of inflammation, and their levels have been associated with increased bacterial load, which correlated with increased exacerbation risk in the same study²³⁶. Desmosine is an amino acid only found in elastin, and free serum desmosine therefore correlates with neutrophil elastase activity; it is associated with risk of severe bronchiectasis exacerbations, an important influence on patients' quality of life, but not with other markers of disease severity¹⁷¹. Likewise, CRP over 0.4mg/L has also been found to associate with a higher risk of severe exacerbation, but not of mild or moderate exacerbations²³⁷. More recently, serum interleukin-6 has been found to be negatively associated with FEV1²³⁸.

It has been suggested that combinations of these biomarkers may be more effective in predicting disease outcomes than any single biomarker alone ²³⁹. However, no studies have been done of biomarkers in AATD-bronchiectasis specifically, and relationships of markers to clinical features require validation in multiple independent cohorts.

1.2.5 Treatment of bronchiectasis

1.2.5.1 *Non-pharmacological treatment*

The aims of treatment are to reduce exacerbation frequency and improve symptoms of cough. Airway clearance techniques taught by a respiratory physiotherapist, including active cycle of breathing techniques, oscillating positive respiratory pressure and gravity assisted positioning, are recommended⁹⁷. There are an efficient and low-risk way of achieving cough, breathlessness and exercise tolerance improvement ^{240,241}, but only a minority of patients are likely to sustain these techniques effectively²⁴². Pulmonary rehabilitation has also been shown to improve exercise tolerance and symptoms ²⁴³. Interestingly, this study also found exacerbation frequency to be improved by rehabilitation, the precise mechanism for which was unclear.

1.2.5.2 *Pharmacological treatment*

Pharmacological treatment, other than that for underlying causes, is limited to mucoactives, such as carbocisteine and nebulised saline, and prophylactic antibiotics⁹⁷. Bronchodilators may also be used, but evidence in bronchiectasis is limited. Inhaled corticosteroids have no proven benefit, and may be actively harmful^{244,245}. Evidence for mucoactive use in bronchiectasis is also limited, but one study has found nebulised saline to have a beneficial effect on spirometry and

symptoms²⁴⁶. A further study comparing saline and carbocisteine has completed recruitment, aiming to publish in 2025²⁴⁷.

The use of prophylactic antibiotics has more evidence in the literature, with regular macrolide use being associated with a reduction in exacerbation (but not hospitalisation) frequency being demonstrated on meta-analysis²⁴⁸. In particularly resistant cases of *pseudomonas* colonisation, nebulised colomycin is recommended
97.

Although neutrophil elastase is recognised as a key inflammatory mediator in bronchiectasis (section 1.2.1), there is insufficient evidence to recommend neutrophil elastase inhibitors, though one small phase II trial (n=16) showed a modest improvement in FEV1²²³. Indirect inhibition of neutrophil elastase, by the DPP-1 inhibitor brensocatib, has emerging evidence in reducing exacerbation frequency, but is not yet licensed²⁴⁹.

Many elements of pathophysiology, clinical features, and corresponding treatments, overlap with those of COPD, and increasingly an overlap syndrome is recognised.

1.3 Bronchiectasis-COPD overlap syndrome

1.3.1 Prevalence

Bronchiectasis has been studied in those with COPD, though estimates of its prevalence vary widely, from a small minority to a majority of cases²⁵⁰⁻²⁵³. Increased identification of a bronchiectasis-COPD overlap syndrome (BCOS) may be connected with the ageing COPD population and its increasing burden of comorbidities. Several studies have found an increased prevalence of bronchiectasis in more severe COPD
250,254,255. Increased identification may also be related to the advancement of CT quality

and availability: high resolution CT (HRCT) scanning in particular is much more available in recent years, providing a more detailed assessment of lung parenchymal disease than a standard CT thorax, and meaning more cases of bronchiectasis are detected.

1.3.2 Common aetiologies

Bronchiectasis and COPD can share aetiologies, in that chronic inflammation, particularly related to neutrophil elastase activity, is implicated in the development of emphysema, and bronchiectasis, as discussed in section 1.2.1. Bronchiectasis appears to be more prevalent in those with emphysema-predominant COPD, giving further credence to a shared aetiology²⁵³. Patients often present with similar symptoms, including shortness of breath, productive cough, and recurrent chest infections/exacerbations. Just as in bronchiectasis, pseudomonas has been identified as predictive of poorer outcomes in COPD, as measured by frequency of exacerbations²⁵⁶, airway obstruction severity²⁵⁷, and hospitalisations²⁵⁸. Furthermore, COPD without bronchiectasis could conceivably cause bronchiectasis via the previously described “vicious cycle”, where recurrent COPD exacerbations and consequent inflammation cause structural damage and airway dilatation. Conversely, anecdotal evidence exists for never-smoking patients with bronchiectasis later developing emphysema, with neutrophil elastase activity suggested as a possible cause²⁵⁹.

1.3.3 Diagnosis

The ROSE criteria (Radiology, Obstruction, Symptoms and Exposure)²⁶⁰ has recently been developed for the diagnosis, rather than severity, of bronchiectasis in COPD and could be useful at least for standardising the diagnosis of bronchiectasis, though the

definition of “exposure” should be widened, since several bronchiectasis causes are not exogenous but related to genetic predisposition. Additionally, the aforementioned (Section 1.3.2.1) reliance of bronchiectasis diagnosis on airway-arterial ratio has been suggested to over-diagnose bronchiectasis, due to narrowed bronchial arteries in COPD²⁶¹ and a correlation of airway-arterial ratio with FEV₁²⁶², though this evidence comes from small studies.

1.3.4 Clinical implications

Several groups have studied how bronchiectasis in COPD might change prognosis or treatment. Bronchiectasis in COPD is associated with increased exacerbation frequency and poorer FEV₁²⁶³, an increased duration of hospitalisations²⁶⁴; requirements for ITU care in those admissions²⁶⁵; poorer functional and quality-of-life scores²⁶⁶; higher rates of colonisation with potentially pathogenic organisms²⁶⁷; and mortality^{263,268}, compared with those with COPD alone. These findings have been corroborated by systematic review²⁶⁷. However, only one paper has aimed to quantify the radiological subtypes of BCOS and relate them to outcomes; this is Martinez-Garcia et al’s 2013 data from 115 bronchiectasis patients, which found a moderate correlation between the Bhalla index and number of exacerbations, and a small correlation with mortality²⁶³.

1.3.5 Challenges in management

Due to such overlap, it is often difficult to establish which combination of the diseases is primarily responsible for the patient’s symptoms at any given moment. This can make treatment difficult, or at least reduce treatment decisions to a “trial and error” approach, particularly since guidelines are only available for the separate diseases^{97,269}. Fortunately, many treatment strategies are the same for both

conditions, including pulmonary rehabilitation, airway clearance techniques, smoking cessation, vaccinations, and mucolytic therapy. Some pharmacological treatments can be applied to bronchiectasis with minimal risk, including the use of short or long-acting bronchodilators, and long-term antibiotics where indicated⁹⁷. However, the use of inhaled corticosteroids, recommended in COPD with asthmatic features, may be ineffective or actively harmful in bronchiectasis by increasing the risk of pneumonia^{149,244,270}. Conversely, the use of inhaled antibiotics is often indicated in bronchiectasis, but not recommended in guidelines for COPD^{79,97}, possibly due to fears of inducing bronchospasm. This presents a challenge to clinicians, and necessitates study of treatment in bronchiectasis-COPD overlap syndrome; however, this niche is understudied at present.

1.3.6 Summary of BCOS

Drawing on the sources mentioned above, the current academic consensus is that there is a clinically meaningful overlap between bronchiectasis and usual COPD exists^{259,271}. This can be justified in an analogous way to Hill's criteria of causation²⁷²: the relationship is "dose dependent", in that bronchiectasis seems to occur more frequently in those with more severe COPD; it is plausible given the inflammation common to both conditions; it is consistent between studies, albeit with a wide variation in prevalences reported; it has somewhat specific features, with most studies reporting a predominance of mild basal and cylindrical disease^{250,255,264}; and lately a temporal relationship has even been described: Lu et al in 2022 followed a large cohort over 13 years and described an incidence of bronchiectasis in COPD patients of 87.83 per 10000 person-years, compared to only 69.80 in those without COPD²⁷³.

Bronchiectasis and COPD therefore undoubtedly overlap, but to it is unclear to precisely what extent, though the phenomenon does appear to be associated with poorer clinical outcomes than COPD or bronchiectasis alone.

1.4 Bronchiectasis in AATD

The evidence for an association between bronchiectasis and AATD is more scarce. Bronchiectasis and AATD are known to occur in the same patients. In one study of 74 patients with PiZZ phenotype, 94% had radiological evidence of bronchiectasis, and 27% had both clinical and radiological evidence, with mild cylindrical bronchiectasis predominating²⁰¹. As in usual COPD, more severe COPD sufferers appeared to have more severe bronchiectasis, strengthening the case for an association. In a US cohort, 9.4% of patients with bronchiectasis had PiZZ AATD phenotype²⁷⁴; however, findings from other cohorts vary widely^{182,275-277}. Conversely, one study's authors concluded that bronchiectasis in AATD was primarily due to emphysema, rather than being a direct consequence, after having reviewed AATD alleles in 202 bronchiectasis and 1030 control patients and finding no significant differences in S, Z, M or I frequencies²⁷⁸. It therefore remains unclear as to whether AATD is a driving factor for development of bronchiectasis²⁷⁴.

Differences between COPD in smoking-related lung disease and AATD are known, as discussed in section 1.1.2.1. Currently there are few studies that have explored differences in microbiology and microbiome, but such distinctions could conceivably lead to different strategies for antibiotic therapy. Likewise, distinctions between bronchiectasis in AATD vs usual COPD could translate to differing clinical treatment strategies.

Despite the overlap, AATD is not one of the conditions screened for in cases of bronchiectasis. British Thoracic Society (BTS) guidelines do not include AATD testing among their recommended screening tests (section 1.2.2), as the available evidence for is considered insufficient⁹⁷. European Respiratory Society (ERS) guidelines acknowledge AATD as a potential cause, but only suggest that testing could be done if concurrent basal emphysema or early onset airway obstruction is present²⁷⁹. There is therefore scope to research unique characteristics of bronchiectasis in AATD, to provide better evidence for a decision on screening tests, which would ensure patients with such disease overlap are treated appropriately. Furthermore, although some details of radiological patterns of AATD-bronchiectasis have been reported once before²⁰¹, no validation of the commonly used radiological or clinical scoring systems has been performed. Likewise, clinically relevant microbiology and serum biomarkers of bronchiectasis as mentioned in section 1.2.4 have not been studied in AATD. This thesis therefore aims to establish whether bronchiectasis is independently related to AATD, and whether its radiological, microbiological, or biochemical characteristics may constitute clinically relevant subtypes of AATD which may therefore be a target of emerging drug treatments.

1.5 Research objectives

The overarching aim of this thesis is to understand the impact of bronchiectasis in AATD. This thesis therefore has the following objectives:

1. To review the prevalence of bronchiectasis in the Birmingham AATD cohort, the demographics of patients involved, and compare the presence or absence of bronchiectasis with disease progression and severity as measured by baseline

and decline in lung function parameters, exacerbation frequency, and symptoms. Results of this work are presented in chapter 4.

2. To subcategorise bronchiectasis according to lobar involvement, morphological category, extent of bronchial dilatation and bronchial wall thickening, with parameters taken from established scoring systems in non-AATD bronchiectasis. These subcategories will also be related to the outcomes listed in objective 1, along with mortality, to enable validation of the Bronchiectasis Severity Index score in AATD. Results of this work are presented in chapter 5.
3. To assess whether an objective algorithmic analysis of bronchiectasis correlates with visual assessment, and whether algorithmic data is associated with the aforementioned clinical outcomes. Results of this work are also presented in Chapter 5.
4. To study the microbiological characteristics of bronchiectasis in our cohort, by primary and quantitative culture. Presence or absence of identified bacteria will be related to the same disease outcomes in aim 1. Results are presented in Chapter 4.
5. To study serum biomarkers of bronchiectasis in our cohort, to assess whether bronchiectasis in AATD is associated with a particular endotype, and compare with the aforementioned clinical outcomes. Results are presented in Chapter 5.
6. To review the outcomes of points 1 to 5 and assess whether this information provides evidence for or against bronchiectasis as a clinically significant phenotype of alpha-1 antitrypsin deficiency.

First, the existing literature was reviewed systematically, to ensure complete coverage of a niche topic.

2 A systematic review of the radiological, microbiological, and biochemical characteristics of bronchiectasis in AATD

Work from this chapter was presented at ERS 2023 and published in abstract form as: J De Soyza, A Pye, DA Spittle, AM Turner. What are the radiological, microbiological and biochemical characteristics of bronchiectasis in alpha-1 antitrypsin deficiency? A systematic review. European Respiratory Journal Sep 2023, 62 (suppl 67) PA394; DOI: 10.1183/13993003.congress-2023.PA394

2.1 Introduction

Bronchiectasis in AATD constitutes a subgroup of an already rare disease. Consequently, relevant research may have been published in lower impact forms or journals. To increase confidence that the entire literature base for subclassification of bronchiectasis in AATD had been reviewed, a systematic review was also conducted.

2.2 Material and methods

2.2.1 Search strategy

Databases searched were MEDLINE and EMBASE (via Ovid), Cochrane Library (Wiley) Central, Health Technology Assessment (HTA), NHS Economic Evaluation Database (EED), and the Database of Abstracts of Reviews of Effect (DARE). The precise search strategy is shown in Table 2.1. Search results were uploaded to

rayyan.ai²⁸⁰ for management of the review process. Duplicates were removed prior to abstract review.

*Table 2.1: Database Search Strategy. Exp: explode, ti: title, ab: abstract. / denotes a Medical Subject Heading (MeSH). * denotes truncation.*

Step	Search term
1	exp bronchiectasis/
2	bronchiect*.ti,ab.
3	bronchoect*.ti,ab.
4	(bronchial* adjacent dilat*).ti,ab.
5	or(1-4)
6	alpha 1-antitrypsin/
7	alpha-1 antitrypsin.ti,ab.
8	alpha 1 antitrypsin.ti,ab.
9	alpha1 antitrypsin.ti,ab.
10	alpha-1-at.ti,ab.
11	alpha-1-antitrypsin.ti,ab.
12	alpha one antitrypsin.ti,ab.
13	alpha one antitrypsin.ti,ab.
14	AAT.ti,ab.
15	A1AT.ti,ab.
16	or(6-15)
17	deficien* or lack*.ti,ab.
18	16 and 17
19	AATD.ti,ab.
20	alpha 1-antitrypsin deficiency/
21	18 or 19 or 20
22	5 and 21
23	limit 22 to humans

2.2.2 Eligibility

Eligible study designs included case-control studies, cohort studies, individual case reports, case series, randomised controlled trials (RCTs), observational controlled studies (OCSs) or observational uncontrolled studies (OUSs). Studies had to have included patients with both AATD and bronchiectasis. Only studies which contained detail of CT scan findings, microbiology or biochemical markers were included; an overview of study of unclassified bronchiectasis in AATD is included in Chapter 1. No restrictions on outcome measures were used, in order to widen the search in what is

a small topic. Non-English abstracts and articles were translated using Google Translate²⁸¹. The intention was to check translations with a native speaker if necessary, but this was not required.

2.2.3 Review process

Three reviewers screened abstracts. Reviewer 1 reviewed every abstract; reviewers 2 and 3 each reviewed 50% of the abstracts sorted alphabetically by author, such that every abstract was independently reviewed by 2 individuals. Conflicting opinions were resolved through discussion and escalated if necessary to the named collaborator. The three reviewers then screened the full texts of the accepted articles against selection criteria. Reviewer 1 read all articles, and reviewers 2 and 3 read the articles corresponding with any abstract which had been included from their original allocations. Again, conflicting opinions were resolved by discussion, and, if necessary, escalation to the named collaborator. Missing full-text articles were sought from available author contact details where possible. Reasons for excluding studies were documented.

The following data were collected and recorded in a spreadsheet in Microsoft Excel: number of AATD patients; age; sex; proportion of AATD patients with bronchiectasis; record of type of bronchiectasis (cystic, varicose, cylindrical); tapering value on CT; airway wall thickness on CT; Bronchiectasis Severity Index or other quantification of disease severity on CT; sputum culture results in stable and/or exacerbated status; smoking status (ex/current/never); degree of smoke exposure (pack years); occupational exposures; FEV₁ (% predicted); DLCO or KCO (% predicted); COPD stage (determined using current GOLD categories⁸⁸); and reversibility of FEV to bronchodilators (defined as >200ml and >12%).

2.2.4 Synthesis

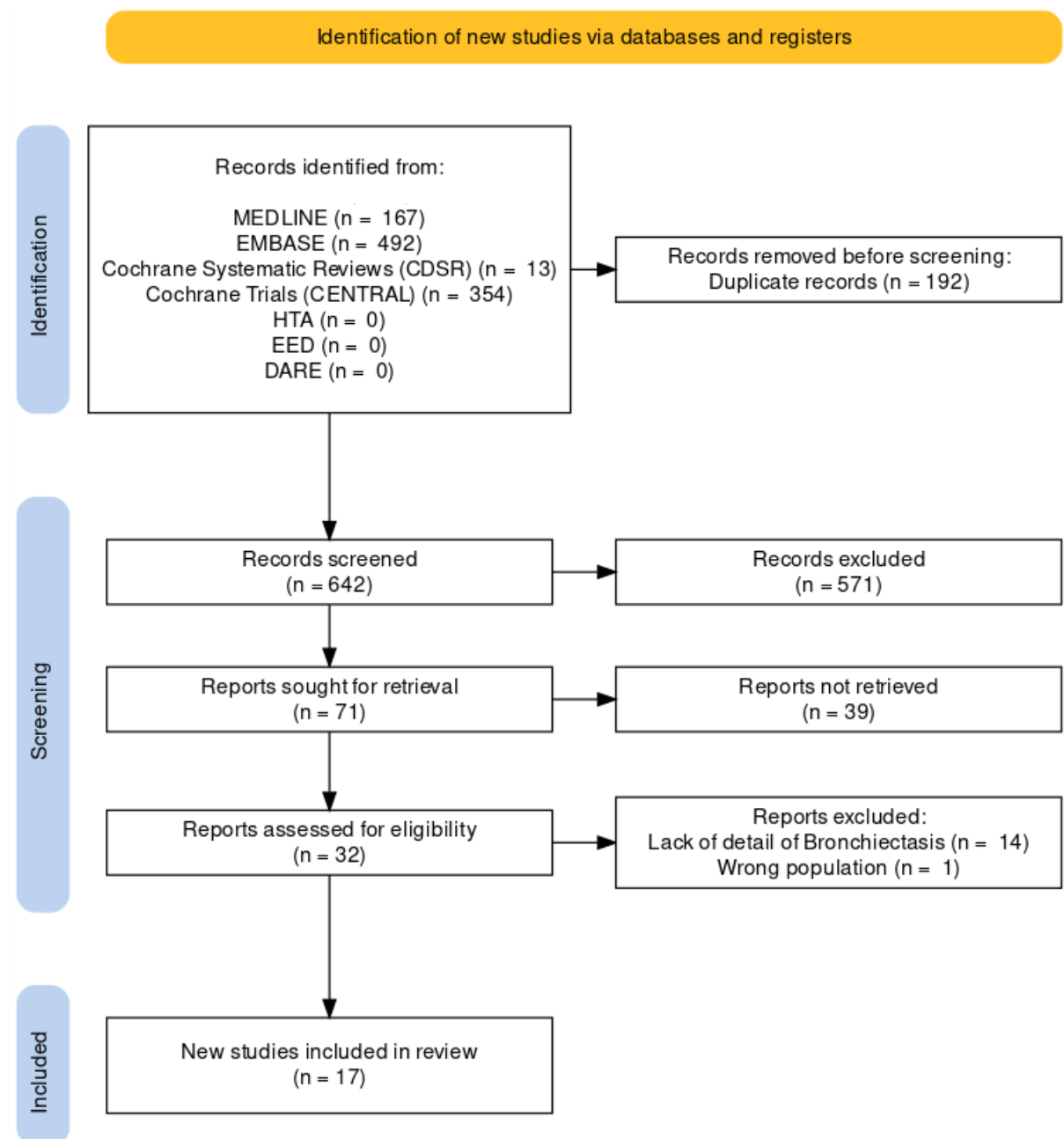
The included studies were too heterogenous to allow for direct comparison or meta-analysis; results were therefore grouped into one of three categories: radiological, microbiological, and biomarkers.

2.2.5 Quality assessment

Quality assessment was performed using the Joanna Briggs Institute tools²⁸² for analysis of case studies/series; case-control studies; and cohort studies.

2.3 Results

Figure 2.1: PRISMA flow diagram detailing study selection process



Initial searches of titles and abstracts identified 834 records. 192 of these were duplicates and were removed. The remaining 642 abstracts were screened, with 571 of these excluded. 71 articles were identified for screening of full texts; for 31 of these articles full texts were either unavailable or not published as a full paper (in the case of conference abstracts). Where contact details for authors of conference abstracts

had been published, efforts were made to contact them for full unpublished manuscripts; however, none responded with such manuscripts. 40 full-text articles were assessed for eligibility. 1 was excluded on the basis of the population not having both AATD and bronchiectasis; 22 were excluded due to lack of detail of bronchiectasis. 17 studies were included in the review (Figure 2.1). The table of characteristics is shown in Table 2.2.

Of the 17 studies, 9 were case reports²⁸³⁻²⁹¹, 3 were case series²⁹²⁻²⁹⁴, 2 were case-control studies^{295,296} and 3 were retrospective cohort studies^{201,274,297}. 12 of these addressed radiological findings (3 case series²⁹²⁻²⁹⁴, 8 case reports^{283,284,286-291}, 1 retrospective cohort study²⁰¹ and 1 case-control study²⁹⁶). 3 case reports^{283,286,287} and 1 retrospective cohort study²⁷⁴ reported microbiological findings. 1 retrospective cohort study²⁹⁷ and 1 case-control study²⁹⁵ focused on biomarkers.

Table 2.2: Characteristics of included articles: Bx = Bronchiectasis

Author	Year	Study type	n AATD	n AATD and Bx	n Male	n Female	Ages	Focus	Exposure measures	Outcome measures	Data
Eden E.	2019	Retrospective Cohort study	58	58	NA	NA	mean 66.9 (10.7)	Microbiology	Culture results	FEV ₁ ; FVC; FEV ₁ /FVC ratio; exacerbation frequency	Mean + SD
Wood AM	2011	Retrospective Cohort study	471	111	281	190	NA	Biomarkers	Genotyping DBP gene (GC); Vit D and DBP levels	None	Mean + SD
Parr D	2007	Retrospective Cohort study	74	70	53	21	50.6 (9.2)	Radiology	Morphology; Severity; ADS(BWT) Score; Lobar involvement; ADS(B) score	None specific to bronchiectasis patients	Mean + SD
O'Brien E	2015	Case-control Study	107	58	61	46	NA	Radiology	Severity; Lobar involvement	None	Mean + SD
Miravittles	1999	Case-control Study	18	9	11	7	NA	Biomarkers	Quantification of specific IgG and its subclasses	FEV ₁ ; FVC; FEV ₁ /FVC ratio; transfer factor	Mean + SD
Hersh CP	2019	Case series	3	1	2	1	49	Radiology	Severity; lobar involvement	FEV ₁ ; Transfer factor	Raw values
Jouhadi Z.	2018	Case series	3	2	0	3	21; 29	Radiology	Morphology; Lobar involvement	FVC	Raw values
Myung	1993	Case series	7	3	3	4	52/21/56	Radiology	Morphology; lobar involvement	None	Raw values
Mosella M	2017	Case report	1	1	1	0	52	Radiology; microbiology	Lobar involvement; culture results	FEV ₁ ; FVC; FEV ₁ /FVC ratio; transfer factor	Raw values
Carpagnano GE	2017	Case report	1	1	0	1	52	Radiology	Lobar involvement	FEV ₁ ; FVC; gas transfer	Raw values
Aggarwal N	2016	Case report	1	1	1	0	18	Radiology; microbiology	Airway thickening; lobar involvement; culture results	FEV ₁	Raw values
Simpson S	2016	Case report	2	2	2	0	70, 72	Radiology	Morphology; severity; lobar involvement	None	Raw values
Milger K	2015	Case report	1	1	0	1	44	Radiology; microbiology	Morphology; severity; Lobar involvement; culture results	FEV ₁ ; FVC	Raw values
A Fahim	2013	Case report	1	1	1	0	NA	Radiology	Lobar involvement	FEV ₁ ; transfer factor	Raw values
Veloso S	2002	Case report	1	1	0	1	47	Radiology	Morphology; lobar involvement	None	Mean + SD
Rodriguez-Cintron	1994	Case report	1	1	1	0	21	Radiology	Morphology; lobar involvement	FEV ₁ ; FVC; FEV ₁ /FVC ratio; transfer factor	Raw values
Scott J	1977	Case report	1	1	1	0	53	Radiology	Morphology	FVC; FEV ₁ /FVC ratio; transfer factor	Raw values

2.3.1 Demographics

Across all studies, 848 patients were included: 418 (49.3%) male, 275 (23.4%) female, and 58 (6.8%) from one cohort study which did not report sex. Only 6 patients were current smokers, 91 were ex-smokers, and 55 had never smoked. Smoking status was unreported in the remainder. Pack year history was only recorded in one case study, and occupational exposure was universally unrecorded. 342 of the total 848 patients had bronchiectasis.

2.3.2 Articles focused on radiology

Three case series, 8 case reports, 1 case-control study and 1 cohort study reported details of radiological bronchiectasis. 1 of the case reports used bronchography; the remainder of articles used computed tomography (CT). 4 case studies, 2 case series, and the cohort study reported subtype of bronchiectasis, for a total of 82 patients. 61 (74.4%) of these patients had cylindrical bronchiectasis, 14 (17.1%) had varicose bronchiectasis, 6 (7.3%) had cystic bronchiectasis, and 1 (1.2%) had traction bronchiectasis. No studies reported tapering value. One case study noted that airway thickening was present in their study; Parr *et al.*'s cohort study reported airway thickening and quantified it with a Bronchial Wall Thickening element of Airway Disease Severity score (ADS_{BWT})²⁰¹. Bronchial Wall Thickening in this score is given one of 3 categories according to thickness relative to the adjacent pulmonary artery: 0 for no thickening, 1 for thickness <50% that of adjacent pulmonary artery; and 2 for thickness >50% that of adjacent pulmonary artery. However, the mean ADS_{BWT} for the whole cohort was reported as 2.02 (SD 0.19); it is not clear from the manuscript how this can be, since the maximum score for wall thickening was apparently 2. The study

also combined subtype, extent and severity of bronchiectasis into a disease severity score called Airway Disease Severity: Bronchiectasis (ADS_B). Mean ADS_B score was 14.8 (SD 0.95).

Disease outcomes in these studies were variably reported. Parr *et al.*'s cohort study calculated correlation coefficients for ADS_{BWT} with the outcomes of St George's Respiratory Questionnaire (SGRQ) Score (correlation coefficient [cc] 0.393 (p<0.001)), FEV₁ (cc 20.531 (p<0.001)), FVC (cc 20.399 (p<0.001)) and DL_{CO}/V_A (cc 20.181 (p=0.062))²⁰¹. ADS_B was also correlated with the same outcomes, although only in combining subtype, extent and severity, rather than separating each variable. ADS_B correlation coefficients (cc) were: SGRQ score cc 0.380 (p<0.001); FEV₁ cc -0.087 (p=0.229); FVC cc -0.086 (p=0.223) and DL_{CO}/V_A cc -0.110 (p=0.176). The paper concluded that the significant results were firstly that ADS_{BWT} correlated positively with increasing symptoms as measured by SGRQ; and negatively with FEV₁ and FVC; and secondly that ADS_B correlated positively with SGRQ, with no significant effects on FEV₁, FVC or DL_{CO}/V_A.

The case-control study, by O'Brien *et al.*, commented on CT findings of bronchiectasis only so far as to state that bronchiectasis was "mild in the majority of cases" and that there was "no lobar preponderance"²⁹⁶. However, unlike Parr *et al.*, the detailed description of bronchiectasis in AATD was not the primary outcome of the study - instead the authors were primarily concerned with the impact of smoke exposure on the clinical phenotype of AATD.

Every case report commented on the lobar distribution of bronchiectasis to some extent, with 3 of the 8 reporting bilateral lower lobe bronchiectasis; one reported right upper and middle lobes; one reporting bilateral upper lobe and lingula; one reporting

left lower lobe only; and one reporting left lower lobe and right middle lobe. Therefore 5 reported lower zone predominant bronchiectasis, and 3 reported upper zone predominant bronchiectasis. All 3 case series reported lobar distribution, with 3 patients from these articles having bilateral lower lobe disease, one further patient also having right middle lobe disease, one having diffuse disease, and one being unrecorded. Parr *et al*'s study did not record exact lobar distribution, but did report that the mean number of lobes involved was 3.7 (SD 1.74)²⁰¹.

2.3.3 Articles focused on microbiology

Three case studies and one cohort study reported findings on culture, with a combined 61 patients. Of the 39 patients who had appropriately sensitive bacterial culture testing, 14 tested positive for *P. aeruginosa* and 8 tested positive for *Staphylococcus aureus*^{274,283,286,287}. Of 31 patients tested for fungal growth, 9 had positive cultures, with only one case report giving the exact species as *Aspergillus Fumigatus*. Only in Eden *et al*'s cohort study was mycobacterial culture recorded. 27 of the 36 patients tested for mycobacteria had at least one positive culture, with 18 of these showing *Mycobacterium avium complex*, and 2 showing *Mycobacterium abscessus*. No studies reported microbiome data, nor did any study report whether patients had concurrent exacerbations or whether samples were taken in a stable state. Eden *et al* reported lung function of FEV₁ (mean 74.8% predicted SD 21.1), FVC (mean 85.3% predicted SD 21.0) and FEV₁/FVC (mean 68.4% SD 12.4) but did not correlate this with microbiology findings. The three case reports all reported FEV₁ (mean 64% predicted), but only two reported both FEV₁ and FVC (FVC mean 60% predicted), and only one reported gas transfer (Mosella *et al*, DL_{CO} 97 K_{CO} 117 % predicted). Exacerbation

frequency was recorded: 61.8% had exacerbated in the last 2 years; 19.3% of patients had had a severe exacerbation.

2.3.4 Articles focused on biochemical markers

One cohort study and one case-control study focused on biochemical markers. Wood *et al.*'s 2011 cohort study measured Vitamin D and Vitamin D Binding Protein (DBP) polymorphisms in 471 patients with AATD, 111 of whom also had bronchiectasis²⁹⁷. DBP variants *GC2*, *GC1F* and *GC1S* were identified as potential markers of COPD disease state. Single-nucleotide polymorphisms (SNP) coding for each DBP variant were identified. Each patient's DBP gene was analysed for these variants. Those with the *GC2* variant were associated with an increased risk of bronchiectasis (Odds Ratio 1.51, 95% CI 1.02 to 2.22, $p=0.034$). One SNP, labelled rs2070741, was associated with bronchiectasis (1.80, 95% CI 1.02 to 3.19). Lung function was not reported.

Miravittles *et al.* measured antibody response to pneumococcal vaccination in 18 patients with AATD, 9 of whom also had bronchiectasis²⁹⁵. In testing for IgA, IgM, and 4 IgG subclasses, patients with AATD and bronchiectasis exhibited higher IgA levels than healthy controls (mean 2.93 gL^{-1} SD 1.56 vs. mean 1.90 gL^{-1} SD 0.88, $p < 0.05$), whereas AATD patients without bronchiectasis did not. Patients with AATD and bronchiectasis also exhibited higher IgG4 levels than AATD patients without bronchiectasis (mean 0.80 gL^{-1} SD 0.55 vs mean 0.17 gL^{-1} SD 0.19, $p < 0.05$). Lung function was measured but was not compared with biomarker levels (FEV₁ mean 58% predicted SD 36, FVC mean 69 SD 31, FEV₁/FVC ratio mean 55 SD 20, DL_{CO} mean 59 SD 18).

2.3.5 Quality assessment

Quality assessment of all articles is presented in Tables 2.3-6. All 12 case reports clearly stated demographics; but only three adequately reported the case history. Of the remaining nine reports, none recorded occupational history, and one did not record smoking history. Two of the 12 case reports did not adequately describe current clinical condition, and one did not adequately describe the diagnostic assessment. Interventions were not documented in any study, so intervention, post-intervention assessment, and adverse events descriptions were not deemed applicable. All 12 reports adequately described their learning points.

Of the two case-control studies, both used comparable groups. One study matched its groups and used the same criteria to identify both cases and controls, the other did not. Both studies used standard, valid, and reliable measures of exposure; measured these exposures in the same way for cases and controls; assessed outcomes in a standard, valid and reliable way; gave a long enough exposure time; and used appropriate statistical analysis. Neither identified confounding factors nor gave strategies for dealing with them.

Of the retrospective cohort studies, all 3 used comparable groups. One out of the 3 used the same criteria for identification of exposed and non-exposed groups. All 3 used standard, valid, and reliable measures of exposure and measured these in the same way for exposed and unexposed groups. Two identified confounding factors. Only Wood *et al.*'s study documented their strategy for dealing with confounding factors²⁹⁷. All 3 assessed outcomes in a standard, valid and reliable way; and used appropriate statistical analysis. None documented whether groups were free of the

outcome at the beginning of exposure. Since all were retrospective, the remaining factors for quality assessment in the tool used²⁸² (sufficient follow up time; completion rates of follow up; strategies to address incomplete follow up) were deemed not applicable. Funnel plot analysis for publication bias was not undertaken due to the heterogeneity in population and outcomes among the studies.

Table 2.3: Quality assessment of Case Studies

Case studies									
First Author	Demographics Clearly Recorded?	History clearly described and presented?	Current clinical condition clearly described?	Diagnostic tests/assessment methods clearly described?	Intervention clearly described?	Post-intervention clinical condition clearly described?	Adverse Events clearly described?	Takeaway lessons stated?	Score
Mosella M	Yes	Yes	Yes	Yes	No intervention	NA	NA	Yes	5/5
Carpagnano G	Yes	No occupational history	Yes	Yes	No intervention	NA	NA	Yes	4/5
Milger K	Yes	No occupational history	Yes	Yes	Yes	No	No	Yes	5/8
Fahim A	Yes	No occupational history	Yes	Yes	Yes	No	No	Yes	5/8
Veloso S	Yes	No occupational history	Yes	Yes	Yes	Yes	No	Yes	6/8
Rodriguez-Cintron W	Yes	No occupational history	Yes	Yes	No intervention	NA	NA	Yes	4/5
Scott J	Yes	No occupational history	Yes	Yes	No intervention	NA	NA	Yes	4/5
Aggarwal N	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	7/8
Simpson S	Yes	No smoking history	Yes	Yes	No	No	No	Yes	4/8

Table 2.4: Quality assessment of Case Series

Case series											
First Author	Clear Inclusion Criteria?	Condition measured in standard, reliable way?	Valid methods of disease identification?	Consecutive inclusion of participants	Complete inclusion of participants?	Clear demographics?	Clear clinical information?	Outcomes clearly reported?	Clear reporting of site demographics?	Appropriate statistical analysis?	Score
Hersh CP	No	Yes	Yes	No	No	Yes	Yes	No intervention	No	NA	4/8
Jouhadi Z	Yes	Yes	Yes	No	No	Yes	No exposure history	Yes	No	NA	5/9
Myung S	No	Yes	Yes	No	No	Yes	No	No intervention	No	NA	3/9

Table 2.5: Quality assessment of Case-Control Studies

Case Control Studies											
First Author	Were groups comparable?	Matched?	Same criteria used for identification of cases/controls?	Exposure measures: standard, valid, reliable?	Exposure measured in same way for cases/controls?	Confounding factors identified?	Strategies for dealing with confounding factors stated?	Outcomes assess in standard, valid and reliable way?	Exposure period long enough?	Appropriate statistical analysis?	Score
O'Brien E	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	8/10
Miravittles M	Yes	No	No	Yes	Yes	No	No	Yes	Yes	Yes	6/10

Table 2.6: Quality assessment of cohort studies

Cohort Studies											
First Author	Were groups similar/recruited from same population?	Were groups free of outcome at start of study?	Exposure measures: standard, valid, reliable?	Exposure measured in same way?	Confounding factors identified?	Strategies for dealing with confounding factors stated?	Outcomes assess in standard, valid and reliable way?	Was follow up time long enough?	Strategies to address incomplete follow-up described?	Appropriate statistical analysis?	Score
Eden E.	Y	NA - retrospective	Yes	Yes	Yes	No	Yes	Yes	NA - retrospective	Yes	7/8
Wood AM	Y	NA - retrospective	Yes	Yes	Yes	Yes	Yes	Yes	NA - retrospective	Yes	8/8
Parr D	Y	NA - retrospective	Yes	Yes	No	No	Yes	Yes	NA - retrospective	Yes	6/8

2.4 Discussion

The available data for the detailed description of bronchiectasis in AATD is relatively low in volume. The majority of articles providing detailed evidence are case series or case reports, which form the lower tiers of the traditional evidence pyramid²⁹⁸. Several of the cohort and case-control studies were not primarily concerned with the detailed description of bronchiectasis, and even if such detail was described, bronchiectasis characteristics were not compared with outcomes, limiting the utility of the data.

Most of the available data centres on the description of radiology, which is expected since by definition bronchiectasis is a description of anatomical abnormalities, which can be captured radiologically. However, there was great heterogeneity across case reports and cohort/case-control studies as to how the bronchiectasis was quantified. Lobar distribution was the most popular method, but this was variably reported, being as precise lobes in the case studies, and average number of lobes affected in Parr *et al.*'s cohort, thus preventing meaningful analysis²⁸³⁻²⁹⁴. Severity was also variably measured with bespoke scores (as in ADS_B and ADS_{BWT} Parr *et al.*'s cohort) and categorisation used; radiological subtype was only variably reported; and no studies commented on airway tapering. Without standardised methods of quantification, it is difficult to draw conclusions. Future research in this field should use a standardised method of quantification; however, the options of ROSE criteria²⁶⁰, BSI¹⁸⁸, FACED¹⁸⁷ and BRICS¹⁸⁰ were not available at the time most papers in this systematic review were published, and each have their own flaws, as discussed in the introduction. Severity in Parr *et al.*'s study was based only on degree of bronchial dilatation²⁰¹, rather than morphology or extent, and also used an objective measure in the form of ADS_{BWT} with a mean value was outside the stated range of scores, thus compromising

the reliability of this result²⁰¹. It may be that severity is simply too subjective a term to use in standardised quantification of CT appearances, and description should always include more detail of bronchial dilatation, lobar involvement, morphology, and bronchial wall thickening.

Studies focused on microbiology were also limited, though do suggest that research in this field should be particularly vigilant for mycobacterial species, as well as *S. aureus* and *P. aeruginosa*. However, study of mycobacterial species presents a challenge in research, as these bacteria require a higher standard of laboratory isolation²⁹⁹.

No studies have yet examined the microbiome for cases of bronchiectasis in AATD, and this could be an area of future research.

Studies focused on biomarkers were very low in number, but did provide 2 further biomarkers for consideration in future research on bronchiectasis in AATD. Anti-pneumococcal antibodies are unlikely to be selected for future research, since Miravittles *et al*'s research was published some time ago, and batch manufacturing of the pneumococcal vaccine may well have changed in that time, and could vary between different countries²⁹⁵. Vitamin D binding protein subtype GC2 is likely to be more reliable, but evidence from Wood *et al*'s article²⁹⁷ would need to be compared with evidence for biomarkers in usual bronchiectasis/COPD overlap before considering which are the most clinically significant.

Quality as a whole was lacking, particularly among case reports, many of which did not report the most basic respiratory history of occupational exposure and smoking exposure. Cohort/case-control studies were largely of better quality, but the majority did not identify confounding factors, or identify strategies for dealing with them.

The quantity and quality of available data for bronchiectasis in AATD is also lacking by comparison to data for bronchiectasis in usual COPD. Though this review has not systematically examined data for usual COPD, CT findings have been described more thoroughly and using more standardised techniques, such as the bronchiectasis severity index in at least 3 articles^{266,300,301}. Other characteristics of bronchiectasis in usual COPD have been better described. In Patel's study of 54 patients, bronchiectasis was most frequently found in the lower lobes, and was associated with higher IL-8 levels and longer symptom resolution times at exacerbation²⁶⁴. Conversely, in a different study of 71 patients, bronchiectasis was most commonly found in the upper lobes and right lower lobe³⁰⁰; such discrepancy is consistent with the heterogeneity in the studies in this review. Gatheral *et al.* found minor bronchiectasis to be the most common level of severity²⁵¹; similarly, Bak *et al.* found mild bronchiectasis to be most common, and found moderate to severe bronchiectasis to be associated with poorer lung function³⁰². However, Sahin *et al.* instead found that severe bronchiectasis was most common,²⁶⁶ as did Tiew *et al.*³⁰¹, though both studies used criteria selecting for more severe COPD. Sahin's study also found that severity of bronchiectasis in COPD was associated with lower TL_{CO}, higher SGRQ score, poorer performance on 6-minute walk testing, and anxiety and depression. Microbiologically, *P. aeruginosa*, *S. aureus* and non-tuberculous mycobacterial infections feature frequently in the data for usual COPD and bronchiectasis, as they did in this review; though in usual COPD *S. aureus*, *H. influenzae*, and *Acinetobacter* species also feature^{251,303,304}. *S. pneumoniae* also features commonly, but may be less frequent than in COPD without bronchiectasis³⁰⁴. It may be that these organisms also appear in AATD-bronchiectasis, but smaller sample sizes and fewer articles limit the likelihood of finding them. Tiew *et al.* also found frequent rates of *Aspergillus*

colonisation in bronchiectasis-COPD overlap syndrome recruited from tertiary centres³⁰¹, which was found in one case report in this review and serves to highlight the value of including fungal organisms in any testing for microbiology or microbiome.

2.5 Conclusion

The available evidence for the detailed description of bronchiectasis in AATD is lacking, especially when compared with the data for bronchiectasis in COPD. This systematic review suggests that future research in this area should use detailed, standardised methods of CT description; should be vigilant for mycobacterial species; and should consider including Vitamin-D-binding protein GC2, IgA and IgG4 when correlating biomarkers with outcomes. Whilst ideas for the subsequent chapters had been conceived prior to the review, the findings confirmed that the studies in the remainder of the thesis were warranted.

3 Methodology

Sections 3.2 is published as the “Methodology” section of De Soyza J, Ellis P, Newnham M, Rickard L, Turner AM. Bronchiectasis Occurs Independently of Chronic Obstructive Pulmonary Disease in Alpha-1 Antitrypsin Deficiency. Chronic Obstr Pulm Dis. 2024;11(5):507-514. doi:10.15326/jcopdf.2024.0526

3.1 Collection of pre-existing clinical data

3.1.1 The Birmingham AATD registry

The Birmingham AATD registry was founded in 1996 and accepts referrals from all UK patients. Originally, as the only tertiary service in the UK, patients attended from across the country; however, as other tertiary centres have been established, the bulk of patients referred in recent years come from the west and east midlands, northern and south-western England, and Wales. Data from the registry was originally stored on paper, which was later transferred to a digital database referred to as “ADAPT”. The tertiary clinic in Birmingham temporarily ceased activity in 2014 due to lack of research funding; however, with NHS approval, it resumed in 2015/16. Digital records were transferred to a different system known as REDCap in 2021. Clinical data was taken from both the ADAPT and Redcap databases, and merged using R version 4.4.2 ³⁰⁵.

3.1.2 Ethics and consent

The study has been ethically approved. Pre-2018 data collection is covered under approval by the NRES Committee West Midlands: South Birmingham on 14th February 1996 (REC reference number 3359a), and data collection from 2018 onwards is covered by approval from the South Central Oxford C Research Ethics Committee on

24th October 2018 (IRAS ID 233675, REC reference number 18/SC/0541). Patients consent to be entered into the registry. Although the exact wording of consent forms has changed over time, patients have always been asked to consent to the sharing of their data for analysis of disease progression, which encompasses this work. Those who do not consent are not entered into the database, and consequently are not included in this work; approximately 99% of clinic attendees consent to data entry.

3.1.3 Choice of clinical characteristics

Data collected included age at baseline, sex, ethnicity, height, weight, smoking history (in the forms of both pack-year history and ever-smoker status), AATD genotype, serum AAT level at baseline, COPD status, asthma status, chronic bronchitis status, bronchiectasis status, and date of lung transplant if relevant. Clinical records were checked for missing data on age, sex, genotype, smoking status, and COPD, chronic bronchitis, and asthma status. For clinical outcomes, lung function raw values for FEV₁, FVC, KCO and TLCO, exacerbation rate, and mMRC and SGRQ scores at each visit were recorded. Data taken from local services are included where primary evidence (e.g. lung function reports) are seen by the clinic. Lung function percent predicted (pp) values were calculated using the GLI method^{306,307}.

For those with CT scans available, date of death or last follow-up was also recorded by searching clinical records, and survival status and time was calculated, to facilitate the survival analyses in Chapter 5.

3.1.4 Categorisation of genotypes

Although PiZZ, PiSZ and PiMZ genotypes are the most prevalent forms of AATD, many other less common genotypes are known, as discussed in section 1.1.1.2. Some produce similarly low levels of functional AAT, or none at all in the case of null

variants³⁰⁸. The inclusion of these rare genotypes in statistical analysis presents a dilemma: by excluding them, more robust validity to the commoner genotypes can be achieved, but this perpetuates the lack of knowledge of the characteristics of these rarer genotypes. By including them, the reverse may be true: applicability to the commoner genotypes may be weakened, to the benefit of increasing validity to the rarer genotypes. Selection of which genotypes to include in the various statistical models is included in the statistical analysis sections below. Genotypes were categorised as severe if they produce functional AAT levels at or below the level of a PiZZ patient. For clarity, the classifications of the various alleles are detailed in Table 3.1, which follows the accepted academic consensus as published by the ERS³⁰⁹.

Table 3.1: Categorisation of allele combinations (SD = severe deficiency, NS = non-severe, N = normal)

Allele	Allele								
	Z	S	M	Null	I	Mmalton	Mprocida	Plowell	F
Z	SD								
S	NS	NS							
M	NS	NS	N						
Null	SD	SD	NS	SD					
I	NS	NS	NS	SD	NS				
Mmalton	SD	NS	NS	SD	NS	SD			
Mprocida	SD	NS	NS	SD	NS	SD	SD		
Plowell	SD	NS	NS	SD	NS	SD	SD	SD	
F	SD	NS	NS	SD	NS	SD	SD	SD	SD

3.1.5 Accuracy of clinical diagnoses

In the Birmingham registry, COPD diagnosis is defined as post-bronchodilator FEV₁/FVC ratio <0.7 on spirometry with compatible symptoms, and bronchiectasis diagnosis is defined as a radiological diagnosis made on a CT thorax report issued by a radiologist at either the referring or tertiary centre, along with compatible symptoms.

COPD diagnosis accuracy was checked by reviewing spirometry for post-bronchodilator FEV₁/FVC ratio <0.7. Bronchiectasis accuracy was checked in the course of reviewing available CT scan images as described in section 4.

3.1.6 Summarising clinical outcomes per patient

For those with 3 or more measurements of spirometry or gas transfer, lung function decline estimates were calculated using linear regression. Median yearly exacerbation rate was calculated for each patient. MRC score was converted to mMRC score where necessary. For the mMRC score, which is an ordinal variable ranging from 0 to 4 depending on severity of breathlessness³¹⁰, the mode was used; this avoided the issue of median values giving multiples of 0.5, which would not be useful in a variable spanning only 5 integers.

3.1.7 Statistical models

Section 3.1.7 describes features common to all statistical models in the thesis. R statistical programming language version 4.2.3 was used for all statistical analysis³⁰⁵. Multivariate regression models were constructed, appropriate to each clinical outcome of interest. Assumptions of linearity, homogeneity of variance, collinearity and normality of residuals were checked in each case, using the “performance” package within R³¹¹. An example of this is given in Appendix section 1. Where data transformation was required, this is described in the relevant results sections of Chapters 4 and 5.

3.1.7.1 *Modelling normally distributed linear outcomes*

When models featured dependent variables of baseline FEV₁; FEV₁, KCO or TLCO yearly decline; or SGRQ score; linear models were used, as these outcomes followed normal distribution patterns.

3.1.7.2 Modelling categorical outcomes

Where mMRC was the dependent variable, multinomial models were constructed using the “Feed-Forward Neural Networks and Multinomial Log-Linear Models” (nnet) package within R³¹². This model gives an estimate for each level of a categorical dependent variable, relative to a reference level. In the case of mMRC, estimates were obtained for each of levels 1-4, relative to the reference level of mMRC 0.

3.1.7.3 Modelling exacerbation rate

Where yearly exacerbation rate was the dependent variable, zero-inflated models were constructed using the Political Science Computation Laboratory (pscl) package within R³¹³. This model is used for distributions with an excess of zeros where the outcome can only be a positive integer (such as exacerbation rate), and gives two incidence risk-ratios, one for the probability of an excess zero, and the using a Poisson distribution to model the count data in the subgroup of patients with non-zero values. This model was selected as exacerbation rates in the dataset did not fit a conventional Poisson distribution, due to an excess of zeros.

3.1.7.4 Modelling mortality

Where mortality was the outcome of interest, two methods were used, using the “survival” package in R³¹⁴. The Kaplan-Meier method was selected since Kaplan-Meier curves are a clear way to visualise differences in mortality between groups. The log-rank test was used to determine statistical significance of differences in survival curves. The Cox proportional hazard method was also used due to the ability to control for other covariates, with covariates eliminated using the backward selection method, and the resultant models subjected to Schoenfeld residuals tests.

3.1.7.5 Covariates common to all statistical models

Covariates were selected based on those values which are known or suspected to influence lung function, symptoms, exacerbations, and mortality, in the context of alpha-1 antitrypsin, bronchiectasis, or COPD.

3.1.7.5.1 Age and sex

Models using baseline FEV₁% predicted values as the dependent variable did not use age and sex as covariates, as these are already included in the GLI method for calculation of predicted values³⁰⁷. However, in all other models age and sex were included as covariates, as they are also known to influence exacerbations^{188,315}, mortality^{188,315}, and symptoms³¹⁶⁻³¹⁸ in the context of COPD, bronchiectasis and AATD. This includes models with the dependent variables of annual lung function change, as the rate of decline in lung function varies with age and sex³¹⁹.

3.1.7.5.2 Smoking status

Smoking status was adjusted for in all models, as it is known to influence lung function decline, symptoms and exacerbation rate in COPD³²⁰ and bronchiectasis³²¹, as well as mortality in the general population³²².

3.1.7.5.3 COPD

COPD diagnosis was adjusted for in all models, since it is defined physiologically and therefore influences baseline lung function and lung function decline. Furthermore, it is characterised by symptoms of shortness of breath, and has a exacerbator phenotype accounting for a large proportion of cases⁸⁸, and therefore can be expected to influence mMRC and SGRQ scores, and exacerbation rate. Furthermore, a relationship with mortality is long established, with COPD estimated to cause 3 million deaths worldwide annually³²³.

3.1.7.6 *The multiple comparisons problem*

As detailed above, many statistical models were constructed on the same patients. This inevitably led to a multiple comparisons problem, whereby conventional limits of statistical significance ($p < 0.05$) could be expected to occur by chance. However, this work is a study of a rare disease, and conventional solutions to this problem were not available: the widest possible retrospective sample size was already planned, and conventional solutions such as Bonferroni adjustments would risk over-correcting and missing true relationships. Therefore, from the outset it was decided to simply consider each statistically significant result cautiously, and with reference to biological plausibility or similar effects in other respiratory diseases.

3.2 The analysis of unclassified bronchiectasis in the cohort

The first analysis undertaken aimed to assess whether unclassified bronchiectasis was related to demographic or clinical characteristics and disease outcomes. Separate analyses were conducted for each phenotype: PiZZ, PiSZ and PiMZ.

3.2.1 Correlation with demographic data

Age, sex, serum AAT, and smoking status were compared according to bronchiectasis diagnosis using standard statistical tests.

3.2.2 Correlation of unclassified bronchiectasis with COPD

Multivariate binomial logistic regression modelling was used to assess whether the odds ratio for bronchiectasis diagnosis was related to COPD diagnosis, with covariates of age, sex, smoking status, and serum AAT level. A subgroup analysis to look for associations of bronchiectasis in patients without a COPD diagnosis was performed;

multivariate binomial logistic regression analysis was used again, with the same co-variates.

3.2.3 Correlation of unclassified bronchiectasis with clinical outcomes

The outcomes of baseline FEV₁, lung function annual change and exacerbation rates were compared between those with bronchiectasis and those without, using linear regression models for lung function models. Model assumptions were assessed for linearity and normality of residuals. Co-variates of age, sex, phenotype, smoking history, and COPD diagnosis were selected for use in all multivariate analysis in this study. Zero-sum inflated Poisson models were used for exacerbation rates, due to the high proportion of patients with no exacerbations, resulting in a heavily skewed distribution with an excess of zeroes, which did not meet assumptions for a standard Poisson model. Multinomial regression models were calculated for mMRC score data. All models were conducted in PiZZ patients first, and repeated in PiSZ and MZ patients if significant results were found.

3.3 Sputum microbiology analysis

3.3.1 Recruitment

Samples of sputum were sought from all patients in the dataset who produced sputum. Such patients were identified from responses to questions in the registry's case report form. Any positive answers to the following questions were treated as eligible:

1. Phlegm? (Yes or No)
2. Chronic Bronchitis (Yes or No)

Other methods of obtaining airway secretions were considered. Bronchoscopy would have enabled sampling from all consenting patients with bronchiectasis, regardless of

whether they had a productive cough, but would subject the patient to including allergic reactions to sedation, infection, bleeding, bronchospasm or laryngospasm, and more rarely pneumothorax or even death. Additionally, bronchoscopy would have required use of limited NHS resources, which would not have been justifiable for a cross-sectional study such as this. Nasopharyngeal swabs can also be done in all consenting patients regardless of cough status, and have demonstrated utility in diagnosing viral pneumonias³²⁴, but their usage in other cohorts unable to expectorate on demand (e.g. children) has not shown any association with lower airway bacterial species³²⁵.

Sample pots, with return envelopes, were posted to all eligible patients. Patients were asked to produce the sample and post it on the same day, preferably at the start of the week to enable the shortest possible transit time in the post, which has previously been established as a valid method of sampling by my colleagues³²⁶.

Patients who had CT scans analysed, but who did not send in sputum samples, had their clinical case notes reviewed for any evidence of historic sputum culture results. These results were recorded in a separate observation in the dataset.

I was trained in the following methodology by an experienced microbiology laboratory technician, DA Spittle. The processing of sputum samples for basic assessment, quantitative culture, and presumptive identification described in this methodology was shared between us. Presumptive identification often required the input of A Pye, also an experienced microbiologist.

3.3.2 Basic assessment

Colour, weight, and whether the sample was taken in a stable state or during an exacerbation, were recorded for all samples. For sputum colour, the Bronkotest®

colour chart was used, as it appears commonly in other trials as an objective measure of colour^{327,328} (Figure 3.1).

Figure 3.1: Bronkotest sputum colour chart



3.3.3 Quantitative culture

Quantitative culture was performed on all incoming samples under sterile conditions. Sputum was mixed with an equal amount of Sputolysin® (dithiothreitol [DTT] in phosphate buffer) at a 1g:1ml ratio, up to a maximum of 1g. This suspension was vortexed for 60 seconds. A primary streak plate on chocolate agar was prepared from this suspension as displayed in Figure 3.2. Serial dilutions in sterile saline (0.9% NaCl) were prepared, using precision pipettes and sterile tips, at 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5} concentrations, as per Figure 3.3. Spread plates were prepared on chocolate agar using 10µl samples from the 10^{-3} , 10^{-4} , and 10^{-5} dilutions, with the addition of a blood agar plate for the 10^{-4} dilution. The additional blood agar plate helps to differentiate bacteria which do not produce haemolytic enzymes: the most relevant such bacterial

genus for respiratory pathology is *Haemophilus*, which accordingly will grow on chocolate agar but not on blood.

Figure 3.2: Four quadrant streak plate method.

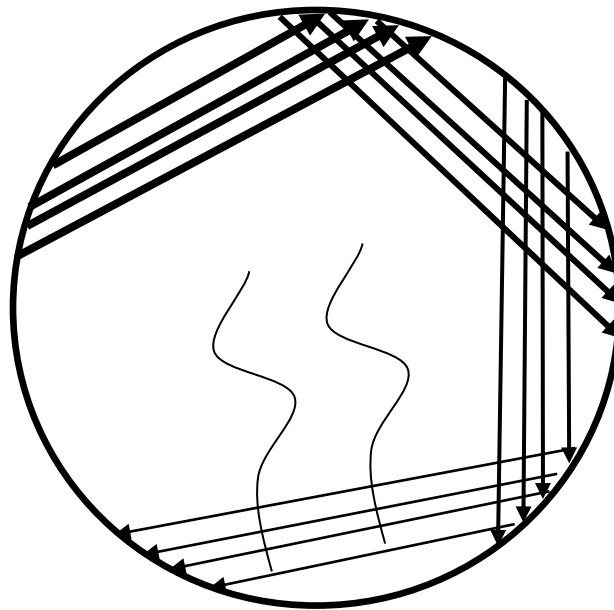
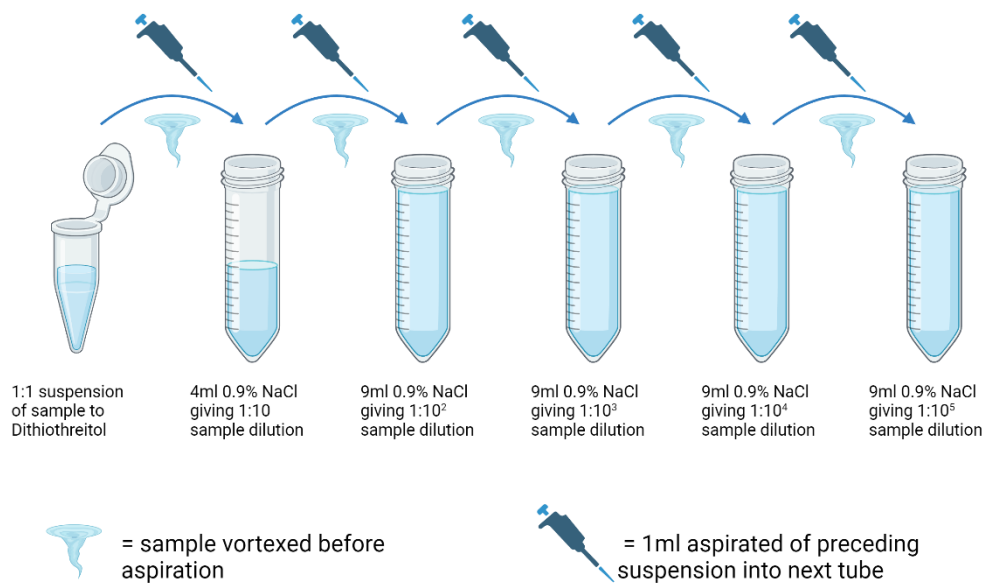


Figure 3.3: Procedure for serial dilution of sputum samples



All plates were incubated for 24 hours at 37°C and 5% CO₂. After 48 hours, colonies were counted on the dilution plate containing the fewest colonies. Plates containing no obvious dominant organism were presumed mixed normal flora and discarded, but those with clear dominant organisms were considered potentially pathogenic. The aid of experienced microbiologists (DA Spittle and A Pye) was sought in making this decision. Such potential pathogens were sub-cultured by sampling with a loop, and creating new streak plates on chocolate agar. These plates were incubated for a further 48 hours.

Immediate presumptive identification of potentially pathogenic bacteria was not always possible due to time constraints; in this case, samples were stored on cryo-preserved MicroBank™ beads at -80°C.

3.3.4 Presumptive identification of potentially pathogenic bacteria

3.3.4.1 *Gram stain*

Gram staining was performed on all samples, using only agar plates which were less than 48 hours old (whether from fresh sputum samples or grown from cryo-preserved beads). This technique was first used in 1884, but has the advantage of being inexpensive and requiring only simple procedural skills, although requiring some experience to interpret. Colonies were swabbed and smeared on a glass slide, which was then fixed with methanol for 60 seconds. The slide was then rinsed and allowed to air dry, before being covered with crystal violet. Crystal violet was rinsed off after 60 seconds. Lugol's Iodine solution was then added, and rinsed off after 60 seconds. The slide was then exposed to no more than 5 seconds of Gram differentiator solution (50% acetone/50% alcohol), and then immediately rinsed with water. Safranin counterstain was then added to the slide for 90 seconds, and rinsed with water. Slides

were then examined with a light microscope under 1000x resolution, using a cedarwood oil immersion, to establish morphology.

3.3.4.2 Catalase test

Catalase testing was performed on all gram-positive cocci. This distinguishes staphylococci from streptococci (and enterococci). Staphylococci produce catalase, which is capable of breaking down hydrogen peroxide into water and oxygen. A positive test is therefore indicated by a bubbling reaction when transferring drops of 3% hydrogen peroxide onto a glass slide containing a bacterial colony.

3.3.4.3 Coagulase test

Catalase-positive gram-positive cocci (i.e. staphylococci) were subject to a coagulase test. This test distinguishes *S. aureus* from other staphylococci, since *S. aureus* produces an enzyme capable of triggering the clotting cascade, by binding with prothrombin and potentiating the cleaving of fibrinogen into fibrin. A suspension of bacterial colonies and rabbit plasma is pipetted onto a slide. Visible clumping of bacterial cells indicates a positive result.

3.3.4.4 Optochin sensitivity test

α -haemolysis capability is indicated by green discolouration of blood agar around bacterial colonies. This is due to the activity of hydrogen peroxide, which oxidises, but does not completely lyse, haemoglobin, forming methaemoglobin, which has a green colour. The most important clinical distinction between hydrogen peroxide-producing streptococci is between *Streptococcus pneumoniae*, which is a common pneumonia-causing pathogen, and other *Streptococci*. A suspension of bacterial colonies is spread on a blood agar plate, and an optochin disc added to the centre. At 24 hours, the plate is reviewed. *S. pneumoniae* is not resistant to optochin, and a ring of several

millimetres containing no bacterial growth will be visible around the disc. Other *Streptococci* will show no such ring, and include *S. viridans*, *S. mutans* and *S. sanguis*, which are commensal organisms of the oral mucosa.

3.3.4.5 Bacitracin test

β -haemolysis refers to complete lysis of haemoglobin by streptolysin, and is indicated by transparent changes in the blood agar. The most important clinical distinction of streptolysin-producing streptococci is between *S. pyogenes*, responsible for most Group A Streptococcal infections, and *S. agalactiae*, responsible for Group B Streptococcal infections, particularly important in neonatal infections. These two can be distinguished by the addition of a bacitracin disc to a blood agar plate, on which a suspension of the colony has been spread. *S. agalactiae* is resistant to bacitracin, a naturally occurring antibiotic product of *Bacillus licheniformis*, whereas *S. pyogenes* is not.

3.3.4.6 Tributyrin test

Gram-negative cocci were subject to a tributyrin test to differentiate the pathogenic *Moraxella Catarrhalis* from the commensal genus *Neisseria*. *M. catarrhalis* produces butyric acid, which cleaves tributyrin, and turns a tributyrin-containing bacterial suspension from pink to yellow.

3.3.4.7 Oxidase test

Gram-negative bacilli were subject to an oxidase test. This differentiates *Pseudomonas* species, which are known to be highly pathogenic even in colonisation⁹⁷, from other species. A bacterial colony is swabbed, and a drop of a redox indicator added to the swab. Organisms containing the cytochrome c oxidase enzyme,

such as *Pseudomonads*, will oxidise the indicator, and cause a change in colour from colourless to blue.

Oxidase-negative gram-negative bacilli were grown on MacConkey agar to distinguish *Klebsiella pneumoniae* from *Escherichia coli*. The lactose fermenting *Klebsiella* turns red or pink; *E. coli* does not change colour.

3.3.4.8 Factor X and V test

Haemophilus species are so small that, even at 1000x magnification, the distinction between cocci and bacilli is unreliable. Such bacteria are termed “coccobacilli”. The most important distinction between these species is between *Haemophilus influenzae*, a common pathogen responsible for many cases of infective COPD exacerbations, and other *Haemophilus* species. *H. influenzae* requires both factors X and V for growth, whereas other species only require factors X or V in isolation. A Colombia agar plate is prepared with 3 discs containing factor X, factor V, and both, respectively. Unlike other species, *H. influenzae* will only grow around the “X and V” disc.

Identification of potential pathogens followed the method shown in Figures 3.4 and 3.5. Interpretation of test results was done only with the supervision of DA Spittle and/or A Pye, in order to ensure accuracy.

Figure 3.4: Presumptive Identification algorithm for Gram positive bacteria.

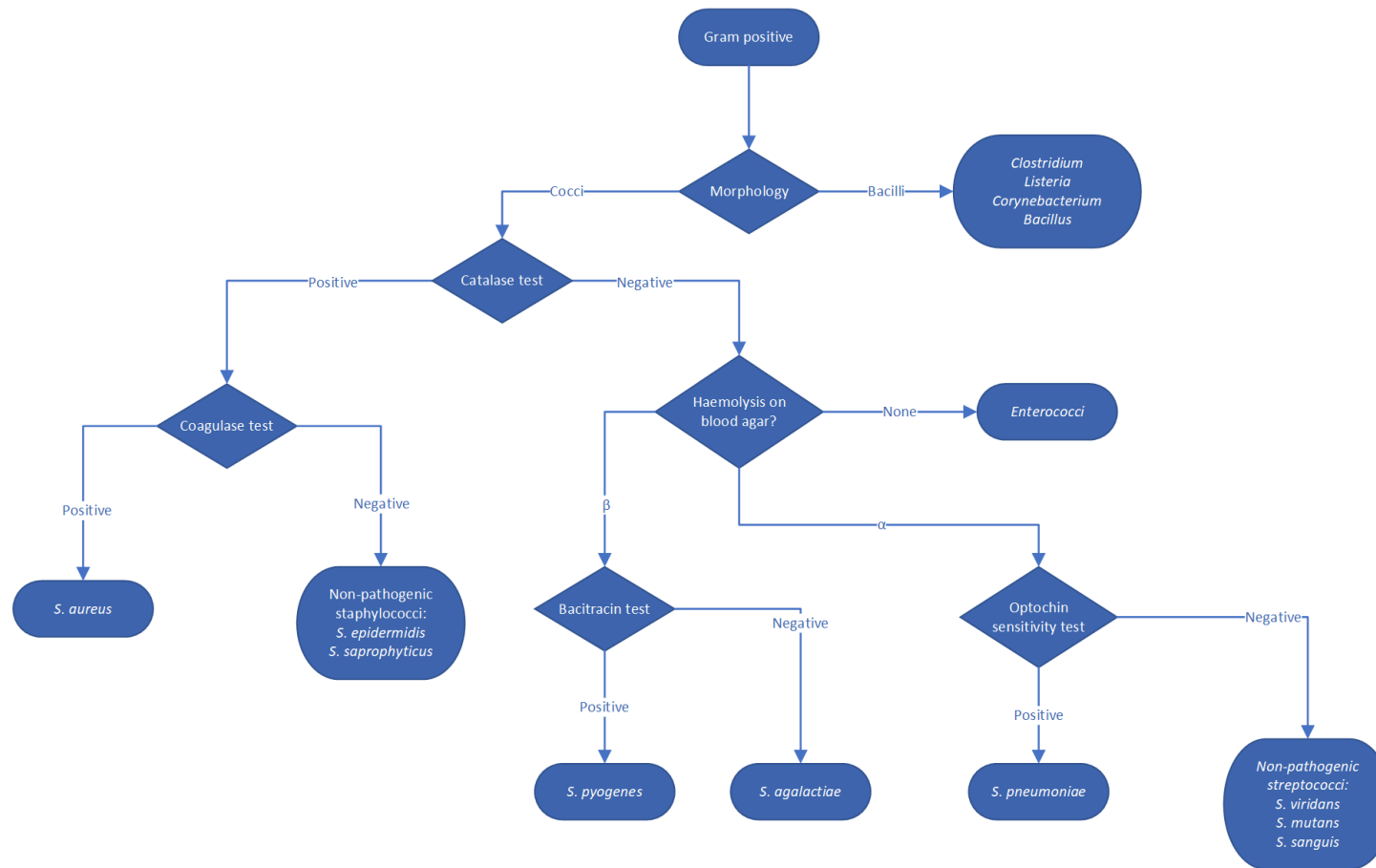
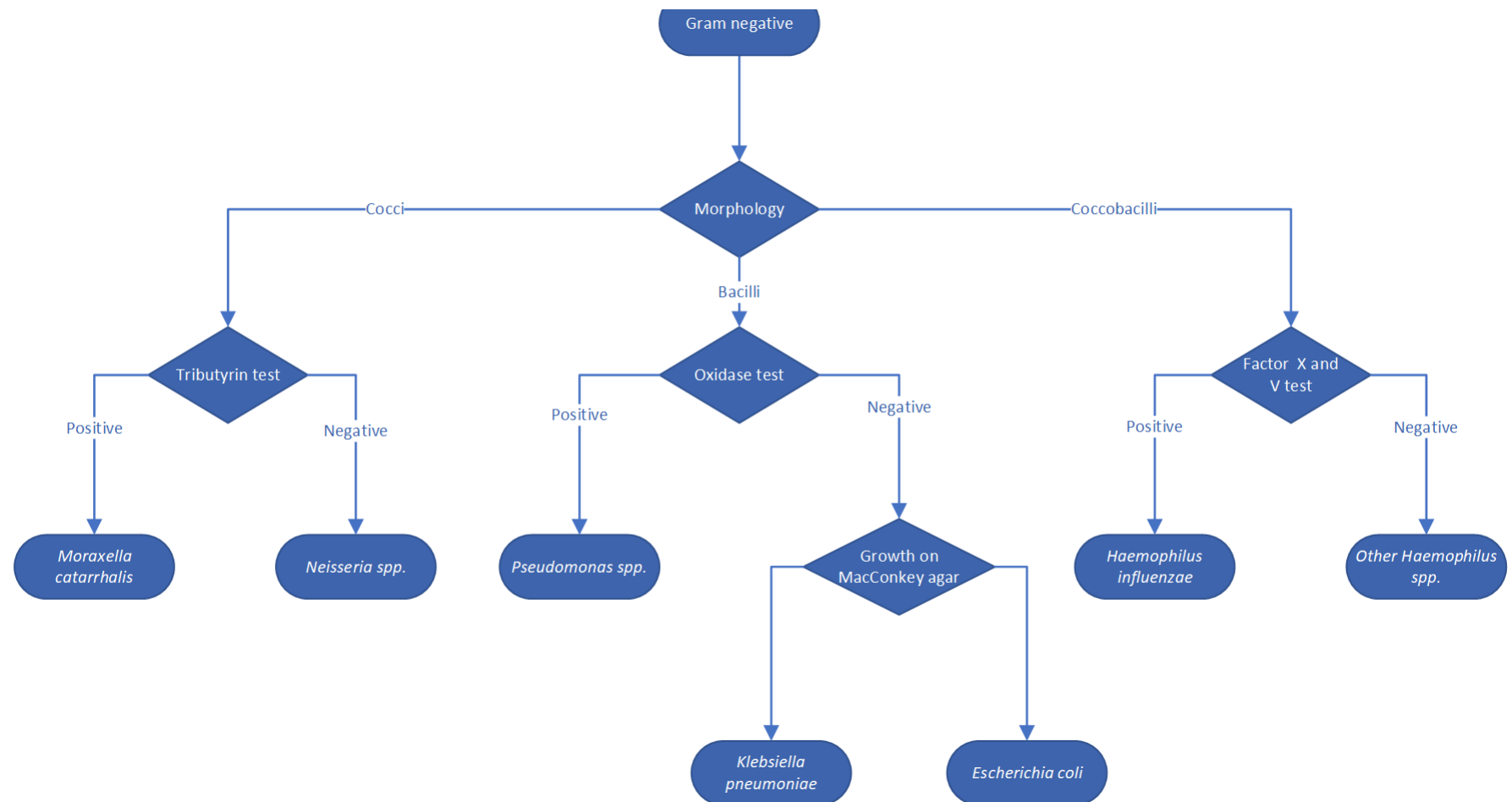


Figure 3.5: Presumptive identification algorithm for Gram negative bacteria.



3.3.5 Historic microbiology data

Some microbiology data was available from the ADAPT and REDCap databases; from these database, microbiology species were recorded. Binary outcomes were also recorded for whether patients had ever grown the pathogens of interest (section 2.6).

For those patients with CT scans available for analysis in Chapter 4, but without microbiology samples or historic data, digital records were searched for a clinical microbiology report, in order to allow more accurate calculation of the bronchiectasis severity index. A further 12 patients with stable sputum cultures were identified in this way.

3.3.6 Pathogens of interest

The pathogens of interest chosen for extraction from the data collected above were: *Pseudomonas* species; *Haemophilus influenzae*; *Moraxella catarrhalis*; *Staphylococcus aureus*; *Streptococcus pneumoniae*; Coliforms including *Enterobacter*, *Klebsiella pneumoniae* and *Escherichia coli*; non-tuberculous *Mycobacteria* (NTM), and the fungal *Aspergillus* species. These were selected as they have been documented as the most important pathogens in bronchiectasis ^{182,188,329}. *Pseudomonas* species are particularly pathogenic, having been associated with increased mortality ^{330,331}, hospitalisation ¹⁸⁸, poorer quality of life scores ³³², more frequent exacerbations¹⁸⁸, and poorer cross-sectional lung function measurements³³³. Data on the influence of *Pseudomonas* on lung function decline is more mixed, but some studies have found increased disease progression rates in those colonised with *Pseudomonas* ^{207,333}. As in the BSI study, the other bacteria listed above were combined in a “other pathogenic organisms” group ¹⁸⁸.

These bacteria are understudied in Alpha-1 Antitrypsin Deficiency, with no published data available comparing bacteriology to disease outcomes at the time of writing. However, the US registry has reported the most commonly found bacteria in their cohort to be *Pseudomonas* species and *S. aureus*. In usual-COPD, more data is available, with colonisation with any of *H. influenzae*, *M. catarrhalis*, *S. aureus*, *K. pneumoniae*, or *P. aeruginosa* being associated with increased lung function decline, and also with only a single *Pseudomonas* culture³³⁴.

“Mixed normal flora” refers to growth on culture without a dominant organism. This growth is assumed to be of organisms which normally colonise the airway. Such organisms include *Neisseria* species, non-haemolytic *Streptococci*, *Bordetella* species, and *Corynebacteria*. These organisms were not recorded individually in the database, as they are accepted to be non-pathogenic unless in extreme cases of immunodeficiency³³⁵.

Colonisation was defined as 2 or more isolations of the same organism, 3 or more months apart. For each respective organism, columns were generated in the database for whether the patient had ever had a positive sputum culture, and whether they had ever been colonised. If the patient had only ever grown mixed normal flora, this was recorded in a separate column.

3.3.7 Statistical analysis of microbiology results

Species names were recorded. Logistic regression models were constructed to assess whether particular species were associated with the disease outcomes of FEV₁, KCO and TLCO decline, exacerbation rate, and mMRC score. Statistical models were limited to PiZZ or other severely deficient genotypes; unlike in the work for the entire cohort, rare phenotypes identified as severely deficient (Table 3.1) were included. This

is because some frequent attenders to the clinic have rare phenotypes, and are therefore well represented in the data available; including them increases the sample size without significantly reducing validity of the results, due to equivalent functional AAT in many rare variants as described in section 3.1.4.

Kaplan-Meier and Cox proportional hazard models were constructed to assess influence of bacterial species on mortality from time of baseline assessment. Because these models assume variables are constant over time, but bronchiectasis develops at an unknown time, these models were repeated in those with bronchiectasis only.

3.4 Blood biomarkers

3.4.1 Haematological biomarkers

Data from full blood counts were readily available from the database, as they are routinely performed at each clinical visit. Platelet and eosinophil counts were recorded. In the case of multiple results per patient, mean averages were calculated, and coefficient of variance was also calculated per patient.

3.4.2 Z-polymers

Samples for Z-polymer analysis were identified from samples stored at -80C, which had been taken from patients who had consented for their blood samples to be stored and used in then-unspecified cross-sectional studies. These samples were sent to a 3rd party (Vertex Pharmaceuticals) for analysis, using a sandwich ELISA. In this method, the capture antibody (mouse monoclonal anti-AAT polymer) is coated onto 96-well plates, which are then blocked with Blocker Casein in PBS and incubated overnight with human M-AAT polymer reference material, standards, quality controls, and samples. After washing, a detection antibody (HRP-conjugated goat anti-human

AAT) is added. Plates are washed, and a substrate solution is applied for colorisation, followed by stop solution (1N sulfuric acid) to halt the reaction. The absorbance is then measured immediately using a Bio-Tek PowerWave plate reader at 450 nm. The calibration curve is fitted with a 4-PL equation, with a range of 75.0–10,000 pg/mL, and a validated lower limit of 50.0 pg/mL. Notably, this assay is unable to determine polymer size.

From these results, mean Z-polymer level was calculated for each patient. For patients with more than 1 measurement, the coefficient of variance was calculated.

3.4.3 Biochemical biomarkers

For the analysis of the remaining biomarkers referred to in the introduction, the intended methodology would have been performed by Vertex Pharmaceuticals, using a grant obtained for a 46-biomarker multi-analyte profile (InflammationMAP® v. 1.1³³⁶), which includes CRP, TNF-alpha, interleukin, ICAM-1, E-selectin, desmosine and VCAM-1 in its panel. Unfortunately, due to operational limitations at Vertex Pharmaceuticals, this analysis was delayed and could not be performed within the timeframe of the present work. Many patients had had CRP measurements at routine clinical visits, and this data was collected from the database. In 2019, some patients had had biomarker analysis on a different multi-analyte panel, including TNF-alpha, interleukins, and CRP, and this data was also collected. In the case of CRP, data was merged with the clinical CRP results before averages and coefficients of variance were calculated.

3.4.4 Statistical analysis of haematological and biochemical biomarkers

3.4.4.1 *Correlation with bronchiectasis*

Linear regression models compared biomarker levels between those with bronchiectasis and those without, with covariates of genotype, age, sex, COPD, and smoking status.

3.4.4.2 *Stability of biomarkers*

For biomarkers where more than one measurement per patient was available, stability over time was assessed using the coefficient of variance method, which gives the ratio of the standard deviation to the mean for each patient. The Kruskal-Wallis test was used to correlate stability of biomarker levels over time with annualised exacerbation rate (which demonstrated a non-normal zero-inflated distribution, as discussed in section 3.1.7.3), to assess whether variability of these inflammatory biomarkers was related to exacerbation, and therefore inflammatory period, frequency.

3.4.4.3 *Correlation of biomarkers levels with clinical outcomes*

The availability of biomarkers form separate groups. In descending order of measurement frequency, these are: firstly, full blood count measurements, encompassing eosinophil and platelet results (section 4.1). Secondly, CRP measurements, which are occasionally taken in clinic, but more rarely than the full blood count (section 4.3). Thirdly, patients with Z polymer levels constitute a specific group (section 4.2). Fourthly, a separate group of patients had had TNF alpha, IL-6 and IL-8 studied in a multianalyte profile (section 4.3). The below models were repeated in each of these groups, except the TNF and interleukin group, in which the most promising models were identified by network analysis as detailed in 3.4.4.

Linear multivariate models assessed correlation of the biomarkers with baseline FEV₁, FEV₁ decline, KCO decline, and mean SGRQ score. Age, sex, COPD and ever-smoker status were inserted as co-variables, and models containing eosinophil level were also controlled for asthma diagnosis, due to the known relationship between asthma and eosinophilia.

Zero-inflated models were used to correlate with annualised exacerbation rate. Multinomial models were used for correlation with mMRC score. Cox proportional hazard models were used to study correlation with mortality, using survival time from baseline.

All models were repeated in a subgroup of patients with bronchiectasis only.

3.4.4.4 Network analysis of biomarkers and numeric clinical outcomes

A correlation matrix was constructed to examine the pairwise relationships between all biomarkers and the numerical clinical outcomes (baseline FEV₁, FEV₁ decline, KCO decline, annualised exacerbation rate, and mean SGRQ score). The Pearson correlation coefficient was used to quantify the linear relationships between all pairs of biomarkers. To visualise the correlation matrix, a heatmap was generated using the `pheatmap()` function in R³³⁷.

To further explore the relationships between biomarkers, a network graph was constructed using the `qgraph()` function in R. The network layout was determined using the spring layout algorithm, which positions nodes (biomarkers) based on the strength of their correlations. Edges were only included if the absolute correlation was greater than or equal to 0.5, allowing for the visualisation of significant relationships. These methods were applied to the whole cohort, and repeated for a subset of patients with bronchiectasis.

Associations between biomarkers and clinical outcomes with Pearson correlation coefficients > 0.5 or < -0.5 were examined with regression models to assess significance. These statistical models were limited to patients with the PiZZ or severely deficient phenotypes (Table 3.1). These rare genotypes were included for the same reason outlined in section 2.7. All models were multivariate, using baseline age, sex, COPD, and smoking status as covariates.

3.5 Visual assessment of bronchiectasis on CT imaging

Due to their establishment in the literature and clinical relevance, as discussed in the introduction, the following parameters were assessed in the present work: lobar involvement, Morphology score, bronchial dilatation, and bronchial wall thickness. Carestream Vue Motion imaging software³³⁸ was used to view the images, using NHS hardware. Lobar involvement was recorded as a binary outcome for each of the 6 lobes: right upper, right middle, right lower, left upper and left upper lobes, and the lingula. The total number of lobes affected was also recorded. Subdividing by bronchopulmonary segmentation was deemed unfeasible for a general physician to identify reliably, and therefore less useful clinically. Morphology was recorded as binary outcomes for each of cylindrical, varicose, and cystic bronchiectasis, depending on their respective presence anywhere within the lung. Additionally, morphology was scored as 0 for no bronchiectasis, 1 for cylindrical without varicose or cystic bronchiectasis, 2 for varicose without cystic bronchiectasis, and 3 for cystic bronchiectasis. Bronchial dilatation was scored as per the modified Reiff score: 0 for no dilatation; 1 for dilatation 1-2x the diameter of the accompanying pulmonary artery; 2 for dilatation 2-3x arterial diameter; and 3 for dilatation $>3x$ arterial diameter.

Bronchial wall thickness was likewise scored as per the modified Reiff score: 0 for no thickening; 1 for thickening $<0.5\times$ the diameter of the accompanying pulmonary artery; 2 for thickening $0.5\text{--}1\times$ arterial diameter; and 3 for thickening wider than the accompanying artery. For both wall thickness and dilatation, the measurements tool from Carestream Vue Motion was used to objectively assess these ratios before categorisation. Mucous plugging was not recorded due to its unreliability between observers, implying unfeasibility for a general physician to identify reliably ³³⁹. Emphysema and lung density parameters were not recorded due to the significant overlap of COPD as a separate diagnosis in the study population; instead, the clinical diagnosis of COPD was used as a covariate in statistical analysis.

Combining radiological, microbiological, and clinical data allowed calculation of the bronchiectasis severity index (BSI) score, as described in section 1.2.4. However, since BSI is a prospective score, and much of the clinical data available was only cross-sectional in nature, some adjustments were made: the most recent values were taken for BMI and FEV₁% predicted; median annual exacerbation rate and mode mMRC were used; *Pseudomonas* or other pathogen colonisation was scored if the patient had ever been colonised.

3.5.1 Obtaining CT scans

All patients with a clinical diagnosis of bronchiectasis were identified from the database. All available CT scans for these patients were included in the study, with CT images being acquired from both digital hospital records and, in the case of patients who had not attended the clinic since digital records became available, physical storage media (CD-ROM). A sample of scans from patients without a clinical diagnosis of bronchiectasis was also included, numbering half of the bronchiectasis scans, to

assess the rate of undiagnosed radiological bronchiectasis in our population. Where patients had more than one CT scan available, the most recent scan was assessed.

3.5.2 Inter-rater reliability

10% of scans were assessed by a radiologist with 5 years' experience and a special interest in thoracic radiology. Inter-rater reliability was calculated for each individual parameter using the Cohen's κ method³⁴⁰.

3.5.3 Statistical analysis

Each visually assessed parameter was correlated with the clinical outcomes of annualised exacerbation rate; mMRC score; SGRQ score; baseline FEV₁pp; and lung function yearly decline, as measured by FEV₁pp, FEV₁ml, KCOpp and TLCOpp. Zero-inflated logistic regression models were used for exacerbation rate; multinomial regression models were used for mMRC score; and linear regression models were used for SGRQ and lung function. Those with alternative bronchiectasis aetiologies were excluded from these models. Age, sex, smoking status (ever vs never), and COPD status were used as covariates. PiZZ and similarly severe phenotypes were analysed as one group, with PiSZ analysed separately. Linear models were checked for normality of residuals and collinearity. Zero-inflated models were assessed for mis-specified dispersion and collinearity. Where high collinearities were found, Lasso regularised models were constructed to penalise high collinearity coefficients and reduce impact of multicollinearity.

3.5.4 Survival analysis

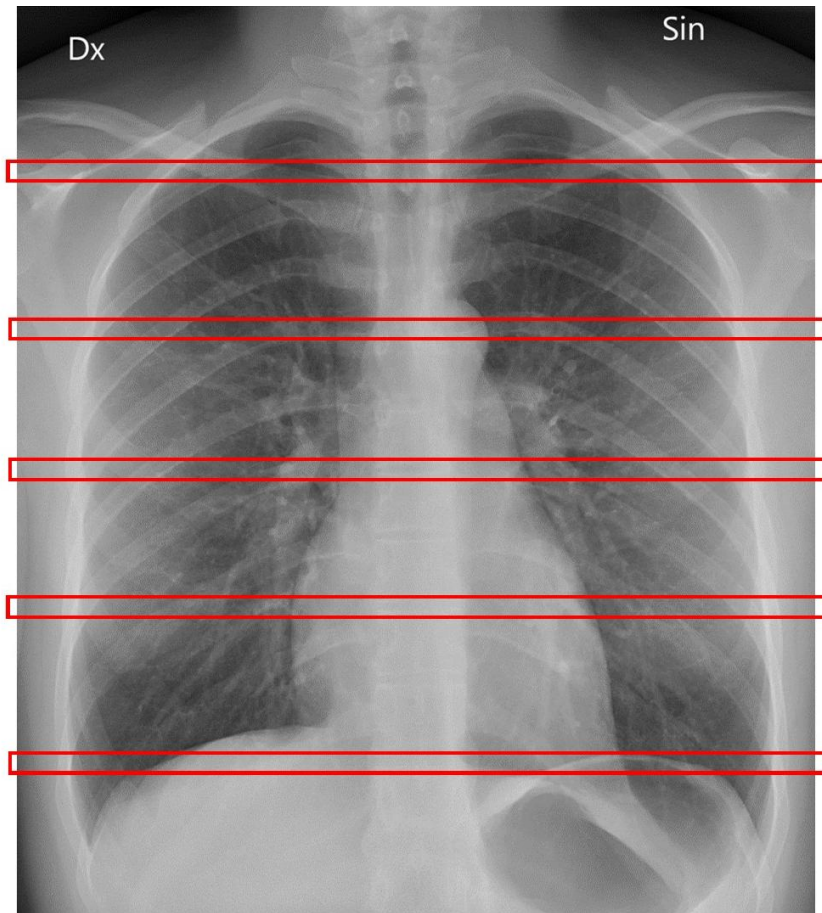
To assess survival from baseline, and survival since birth, Kaplan-Meier survival curves and Cox-proportional hazard models using the backward selection method were constructed to control for the aforementioned covariates.

3.6 Algorithmic analysis of bronchiectasis on CT imaging

3.6.1 Eligibility of CT scans for algorithmic analysis

CT scans which had been analysed visually were downloaded as DICOM files. These files were then assessed with a CT analysis algorithm known as YACTA (Yet Another Computed Tomography Algorithm). Scans with less than 100 images are not able to be effectively analysed by YACTA; in practice this excluded older scans, which were often performed in older generation CT scanners under a historic High-Resolution CT protocol. These historic protocols gave detailed scan images with thin transverse slices, but only of samples of the lungs³⁴¹. A schematic of this process is shown in Figure 3.6. These protocols existed as a compromise between the benefit of image detail and the combined risks of radiation exposure from older generation scanners, and the increased time these scanners took to acquire an image of such detail, which would increase breathing artefacts. With the increased efficiency of CT scanning allowed by multidetector row CT scanning, high resolution CT scanning of the entire lung parenchyma became possible at scale in the 2000s³⁴².

Figure 3.6: Chest X ray image highlighting areas of the chest captured by historic HRCT protocols.



3.6.2 Algorithmic detection of the bronchial tree

YACTA [the algorithm] detects the bronchial tree by the use of landmarks within the chest³⁴³. In transverse CT images, for each voxel, it assesses whether the surrounding voxels are air (i.e. whether the average HU are below -975HU). If not, it assesses whether all voxels are soft tissue (i.e. above -525HU). If this analysis results in an area of air less than 8cm², this area is designated as the trachea. Areas of pure air larger than 8cm² are likely to be outside the chest and therefore irrelevant. Errors are sometimes encountered with large bullae, which will also result in pure air areas >8cm²; for this reason, the algorithm starts at the most superior transverse image and works down. If necessary, a trachea landmark can be set manually. The algorithm continues down the bronchial tree, assessing for areas of pure air or soft tissue. If a

voxel is surrounded in all 6 cardinal directions by pure air, or if it is only adjacent to soft tissue on one side, it is designated as bronchus.

Next, the result of this analysis is reconstructed into a 3D object. This object is skeletonised by deletion of “simple” voxels, which are those whose deletion does not alter the topology of the 3D object. The diameter of the airways is thereby reduced uniformly in each direction, and establishes the midpoint of the airway throughout the bronchial tree. Perpendicular measurements from this midpoint to the edge of the original 3D object can then be calculated, and therefore bronchial dilatation and tapering can be assessed from the transverse plane *of the bronchus*, not the whole body³⁴⁴.

Branching of the airway is used to identify each of the six lobes (right upper, middle and lower; left upper and lower lobes; and the lingula), using a pre-existing automated method³⁴⁵. The algorithm is able to account for the rare anatomical variant of a tracheal bronchus, when a bronchus branches directly from the trachea, proximal to the carina²⁰⁰. This method also assigns bronchial levels to each airway, with G0 for the trachea, G1 for the right and left main bronchi, and so on for each division to G10.

3.6.3 Bronchiectasis Index (BEI)

The algorithm returns a large number of parameters by default, but only some of these were deemed suitable for further analysis.

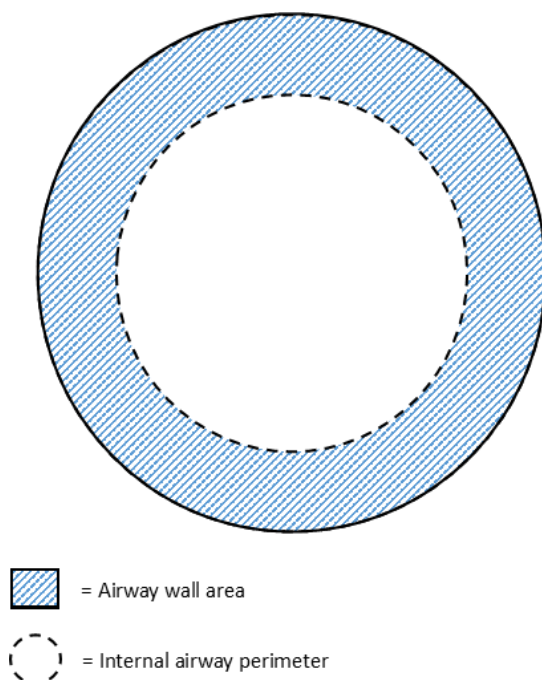
The first parameter selected was the Bronchiectasis Index (BEI, not to be confused with Bronchiectasis Severity Index, BSI, see introduction and chapter 5). This is a measure of airway tapering. Tapering is assessed by establishing the plane of the airway as per the skeletonisation method above, and measuring the transverse diameter of the airway in that plane. Diameter measurements in close proximity to a

branch point are disregarded, as measurements in this area will be anomalously high as the airway diverges. The diameter of each airway at each respective bronchial level can be assessed, and a numerical value assigned to the degree to which this diameter reduces with each airway generation. The reference ratio for this diameter reduction was 0.7 for $G_n:G_{n+1}$; i.e. the airway diameter should reduce by a factor of 0.7 with each generation. Airway generations can be reliably identified up to the 10th generation. BEI can be calculated for each of the 6 lobes of the lung. The BEI parameter was selected as lack of tapering is accepted to be a specific marker of bronchiectasis³⁴⁶; unlike broncho-arterial ratio, it is unaffected by pulmonary hypertension or normal variation in arterial size, which can be significantly smaller than the accompanying bronchus in health³⁴⁷. However, an accurate assessment of tapering by human eye is difficult and time consuming³⁴⁸, creating clear scope for an automated alternative such as BEI.

3.6.4 Quantification of airway wall thickness (AWPi10)

AWPi10 (Figure 3.7) is a standardised measure of airway wall thickness. From each airway assessed in transverse profile, the area of the airway wall is plotted against the internal perimeter of the airway, as per diagram. For each scan, a linear regression is then calculated. From this regression line, AWPi10 is given by the estimated airway wall area for an airway of perimeter 10mm. This variable was selected as it has been used in recent studies as a standardised measure of airway wall thickness, with a weak to moderate correlation with BSI score in one study.³⁴⁹⁻³⁵¹

Figure 3.7: Schematic demonstrating derivation of airway wall area.



3.6.5 Emphysema quantification (PD15)

PD15 is a quantitative measure of density, measured in Hounsfield units. It represents the value below which the 15% of the lung has lower density. It has been established as a reliable measure of lung density in both usual and AATD-COPD since 2004 ³⁵²⁻³⁵⁴.

3.6.6 Statistical analysis of algorithm-derived data

Analysis was focused on 3 areas. Firstly, to assess whether the YACTA parameters correlated with my visual assessment of the CT scans; secondly, whether thresholds of the continuous YACTA data could be identified to diagnose bronchiectasis with an acceptable level of sensitivity and specificity; thirdly, whether the YACTA parameters correlated with the clinical outcomes of interest.

3.6.7 Correlation between YACTA parameters and visual CT assessment

For the assessment of correlation between YACTA and visual CT scan assessment, standard statistical tests were used. Student's T-test or one-way analysis-of-variance for comparison of means between groups, and Wilcoxon rank sum or Kruskal-Wallis tests for comparison of binary groups where data was not normally distributed. The BEI was compared with visual assessment of number of lobes affected, morphology, bronchial dilatation and bronchial wall thickening. The AWPi10 YACTA parameter was compared with visual assessment of bronchial wall thickening only. BEI for each of the 6 lobes was compared with the visual diagnosis of bronchiectasis for each respective lobe.

3.6.8 Identification of YACTA thresholds correlating with visual assessment

For the assessment of thresholds which correlated with bronchiectasis, Receiver Operating Characteristic (ROC) curves were calculated using R. If the 95% confidence interval of the area under the curve (AUC) included 50%, no further analysis was conducted; if the AUC 95% confidence interval range did not include 50%, the optimal threshold was identified using Youden's J method. This analysis was performed firstly for the ability of BEIndexLung to identify the following visual assessment parameters: bronchiectasis of any kind; cystic morphology; varicose or cystic morphology; bronchial wall thickening at each grade; bronchial dilatation at each grade; and number of lobes affected. Secondly, ROC analysis was conducted for the association of the BEIndex with bronchiectasis in each respective lobe. Thirdly, ROC analysis was also conducted for the association of AWPi10 with bronchial wall thickening scores of each grade.

3.6.9 Correlation of YACTA parameters with clinical outcomes

For the assessment of correlation between YACTA parameters and clinical outcomes, regression models were calculated in a similar way to the assessment of visual CT scan parameters described previously. This used linear regression for FEV₁, KCO and TLCO percent predicted yearly decline, FEV₁ baseline percent predicted, and FEV₁ ml yearly decline; zero-inflated logistic regression models were used for exacerbation rate analysis; and multinomial logistic regression models were used for mMRC score. These models were only conducted on patients with either PiZZ genotypes or phenotypes which produce similarly severe Alpha-1 Antitrypsin Deficiency.

3.7 Presentation of results

This chapter has summarised all methods used in the production of this thesis. Results referring to unclassified bronchiectasis, including microbiology and biomarkers, are presented in Chapter 4. Results referring to the radiological classification, both visually and algorithmically, are presented in Chapter 5.

4 Clinical, microbiological and blood biomarker characteristics of unclassified bronchiectasis

N.B: sections 1 and 4.1 are published as part of “De Soyza J, Ellis P, Newnham M, Rickard L, Turner AM. Bronchiectasis Occurs Independently of Chronic Obstructive Pulmonary Disease in Alpha-1 Antitrypsin Deficiency. Chronic Obstr Pulm Dis. 2024 Sep 27;11(5):507-514. doi: 10.15326/jcopdf.2024.0526. PMID: 39213377”.

4.1 Prevalence of bronchiectasis in AATD

4.1.1 Descriptive statistics

The inclusion/exclusion process is shown in Figure 4.1. Four patients were excluded due to having other causes of bronchiectasis: 1 had Allergic Bronchopulmonary Aspergillosis (ABPA), 2 had childhood pertussis, and 1 had traction bronchiectasis secondary to interstitial lung disease (ILD). All 4 had the PiZZ genotype. Among those with ≥ 3 lung function measurements, median follow-up time was 6.4 years (IQR 3.9 - 11.2).

Baseline characteristics of the PiZZ, PiSZ and PiMZ groups, stratified by bronchiectasis diagnosis, are shown in Table 4.1. 967 patients had spirometry available for review of diagnostic accuracy of COPD: type I and II error rates were 0.6% and 1.7% respectively (Table 4.2). 290 patients had CT scans available for review of diagnostic accuracy of bronchiectasis: type I and II errors were 1.6% and 7.5% respectively (Table 4.3).

PiZZ patients with bronchiectasis were older at baseline than those without (54 vs 49 years, $p < 0.001$), and more likely to be never smokers (35 vs 24 %, $p < 0.001$). PiSZ

patients with bronchiectasis were also older on average (63 vs 48 years, $p < 0.001$), but no differences in smoking status were seen. PiMZ numbers were small with little variance between the bronchiectasis and non-bronchiectasis groups. 134 PiZZ patients did not have COPD, 25 (18.6%) of whom had bronchiectasis.

Figure 4.1: Inclusion/exclusion process for regression model of bronchiectasis vs COPD.

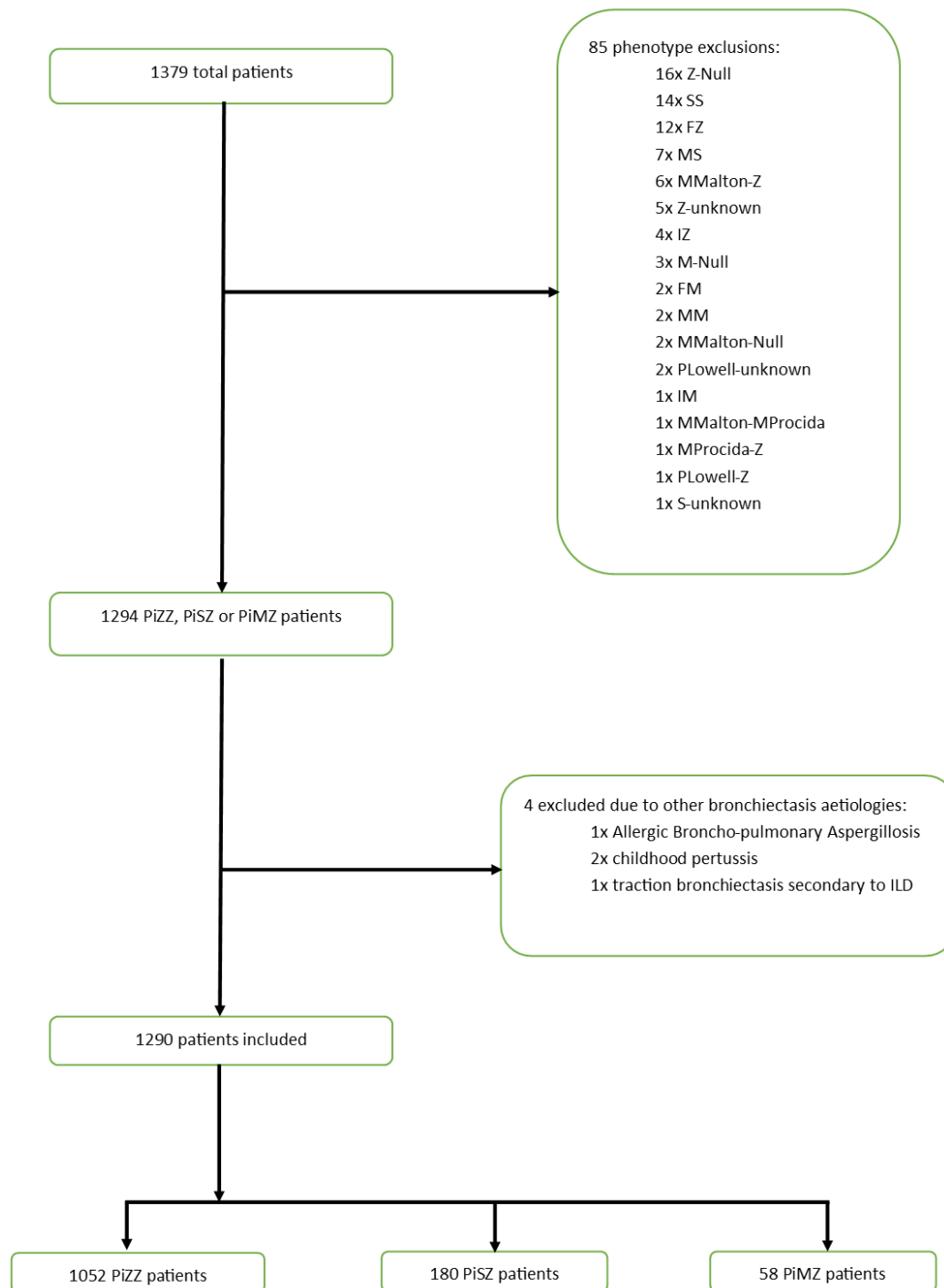


Table 4.1: Characteristics of 1290 AATD patients stratified by phenotype and bronchiectasis diagnosis.

	PiZZ			PiSZ			PiMZ		
	No			No			No		
	Bronchiectasis	Bronchiectasis	p	Bronchiectasis	Bronchiectasis	p	Bronchiectasis	Bronchiectasis	p
n	220	832		15	165		5	53	
Sex: Male (%)	117 (53.2)	468 (56.2)	0.46	10 (66.7)	80 (48.5)	0.281	1 (20.0)	27(50.9)	0.392
Age (years)	54.2 +/- 10.24	49.41 +/-11.71	<0.001	63.00 +/-8.71	48.27 +/-14.64	<0.001	62.75 +/-9.60	49.95+/-14.16	0.085
Body Mass Index	25.77 +/-5.83	26.46 +/- 5.26	0.137	24.60 +/-5.68	27.27 +/-5.19	0.090	Missing data	27.22 +/-5.58	NA
Serum AAT (μmol/L)	4.02 +/-1.53	4.40 +/-2.35	0.030	13.81 +/-3.98	13.70 +/-3.49	0.922	Missing data	12.36 +/-4.68	NA
Ever Smoker (%)	143 (65.0)	632 (76.1)	0.001	10 (66.7)	102 (61.8)	0.926	3 (60.0)	35 (67.3)	1.00
Smoking: pack year history	13.89 +/-15.72	15.45 +/-15.50	0.193	26.57 +/-25.04	17.30 +/-23.72	0.164	13.2 +/-15.35	19.33 +/-20.76	0.524
COPD (%)	192 (88.5)	655 (85.7)	0.354	13 (86.7)	69 (54.3)	0.034	2 (50.0)	19 (40.4)	1.00

AATD: Alpha-1 Antitrypsin Deficiency; +/- standard deviation. Continuous variables compared with t-tests; categorical variables compared with Fisher's Exact or Chi-squared tests.

Table 4.2: Diagnostic accuracy of COPD

<i>n</i> = 967	FEV ₁ /FVC ≤0.7	FEV ₁ /FVC >0.7
COPD diagnosis (%)	766 (79.2)	6 (0.6)
No COPD diagnosis (%)	16 (1.7)	179 (19.6)

Table 4.3: Diagnostic accuracy of Bronchiectasis

<i>n</i> = 290	CT evidence	No CT evidence
Bronchiectasis diagnosis	193 (66.5%)	0 (0.0%)
No Bronchiectasis diagnosis	22 (7.5%)	75 (25.8%)

4.1.2 Correlation of bronchiectasis with COPD in AATD

The clinical diagnosis of bronchiectasis was unrelated to a concurrent diagnosis of COPD on multivariate binomial logistic regression modelling in all phenotypes (Table 4.4, Figures 4.2 and 4.3), instead having a small but significant association with older age in both phenotypes (OR 1.03, *p* = 0.001), and with smoking status in PiZZ patients, being more likely in those who have never smoked (OR 0.59, *p* = 0.031). There was also an inverse relationship between serum AAT and bronchiectasis diagnosis in PiZZ patients (OR 0.88, *p* = 0.018).

Figure 4.2: Forest plot of multivariate binomial logistic regression for the association of COPD with bronchiectasis in 1052 patients with PiZZ AATD: bronchiectasis diagnosis is associated with serum alpha-1 antitrypsin level, but not with COPD diagnosis.

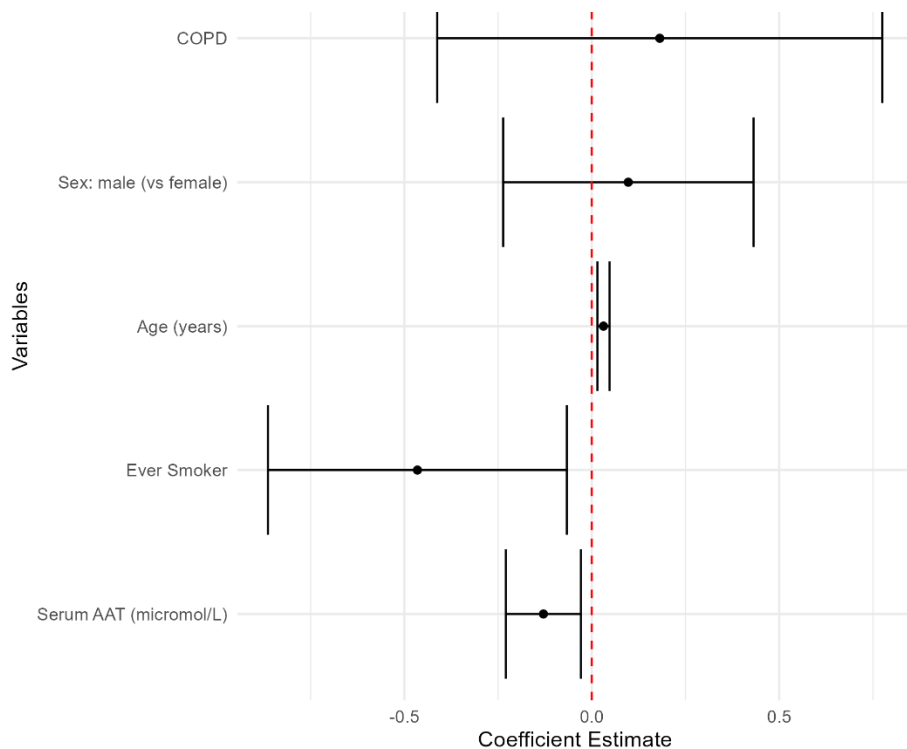


Figure 4.3: Forest plot of multivariate binomial logistic regression for the association of COPD with bronchiectasis in 180 patients with PiSZ alpha-1 antitrypsin deficiency: bronchiectasis is not associated with COPD diagnosis.

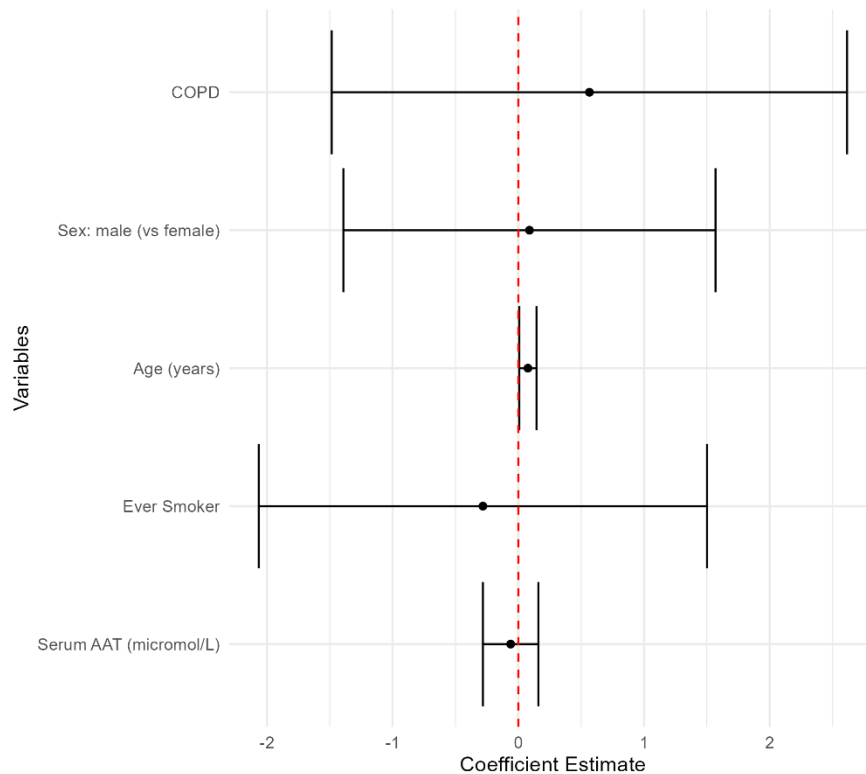


Table 4.4: Associations of Bronchiectasis in patients with AATD, stratified by phenotype.

Variables	Bronchiectasis: PiZZ patients			Bronchiectasis: PiSZ patients			Bronchiectasis: PiMZ patients		
	Odds	CI	p	Odds	CI	p	Odds	CI	P
	Ratios			Ratios			Ratios		
COPD	1.2	0.67 – 2.21	0.550	1.76	0.26 – 17.44	0.589	1.69	0.13 – 41.02	0.693
Age (years)	1.03	1.02 – 1.05	<0.001	1.08	1.01 – 1.17	0.030	1.07	0.97 – 1.24	0.208
Sex (Male)	0.91	0.65– 1.27	0.568	0.92	0.20 – 4.27	0.907	NA*	-	-
Ever smoker (vs never)	0.63	0.42 – 0.94	0.022	0.75	0.13 – 5.00	0.757	NA	-	-
Serum AAT (µm/L)	0.88	0.79 – 0.97	0.012	0.94	0.75 – 1.18	0.588	NA	-	-
Observations	851			102			39		
R ² Tjur	0.039			0.086			0.061		

AATD: Alpha-1 Antitrypsin Deficiency; COPD: Chronic Obstructive Pulmonary Disease. NA refers to missing covariate data; NA* refers to insufficient variance for regression modelling

Baseline FEV₁, as well as FEV₁, KCO and TLCO pp/year decline did not differ significantly between patients with and without bronchiectasis in multivariate linear regression analysis (Table 4.5). Exacerbation rate was also unrelated to bronchiectasis diagnosis (Table 4.5).

Table 4.5: Summary of multiple multivariate regression model estimates, comparing lung function and exacerbation rate between bronchiectasis and non-bronchiectasis patients with AATD, stratified by phenotype.

Outcome	PiZZ				PiSZ			
	n	Estimate (bronchiectasis vs non- bronchiectasis)	CI	p	n	Estimate (bronchiectasis vs non- bronchiectasis)	CI	p
FEV ₁ pp baseline	973	-0.49	-3.78 – 2.79	0.768	140	-10.25	-23.22 – 2.72	0.121
FEV ₁ pp/year decline	687	-0.02	-0.33 – 0.29	0.903	85	-0.44	-1.78 – 0.90	0.518
KCOpp/year decline	525	-0.23	-0.70 – -0.24	0.338	58	-1.59	-3.95 – 0.77	0.181
TLCOpp/year decline	498	-0.12	-0.57 – 0.32	0.577	60	1.54	-0.85 – 3.94	0.202
Exacerbation yearly rate	979	0.99	0.61- 1.59	0.963	141	0.63	0.05 – 7.89	0.721

n = number of patients included in model; FEV₁ = Forced Expiratory Volume in 1 second; KCO = transfer coefficient of carbon monoxide; TLCO = transfer factor of the lung for carbon monoxide; pp = % predicted; multivariate estimates adjust for age, sex, phenotype, smoking history, and concurrent COPD diagnosis. Linear regression models used for FEV₁pp, KCOpp and TLCOpp; zero-inflated model used for exacerbation rate.

Bronchiectasis was associated with higher mMRC scores in PiZZ patients on multivariate multinomial logistic regression modelling (Table 4.6); however, this pattern did not persist in PiSZ and PiMZ patients.

Table 4.6: Bronchiectasis is associated with mMRC scores of 2 or higher on multivariate multinomial regression analysis in PiZZ AATD patients.

Variables	mMRC	PiZZ			PiSZ			PiMZ		
		Odds Ratios	CI	p	Odds Ratios	CI	p	Odds Ratios	CI	p
Bronchiectasis	1	1.72	0.99 – 2.98	0.054	4.13	1.01 – 16.86	0.048	1.93	0.14 – 26.15	0.614
	2	1.97	1.20 – 3.25	0.008	4.52	1.10 – 18.57	0.036	NA	NA	0.940
	3	2.58	1.59 – 4.19	<0.001	NA	NA	NA	2.25	0.16 – 31.08	0.537
	4	2.2	1.23 – 3.92	0.008	2.11	0.21 – 21.40	0.525	13.54	0.55 – 333.18	0.108
	Observations	991			161			56		

AATD: Alpha-1 Antitrypsin Deficiency; COPD: Chronic Obstructive Pulmonary Disease; mMRC: Modified Medical Research Council breathlessness score 0-4, Odds ratios are relative to mMRC 0; Multivariate analysis accounts for age, sex, ever smoker status, and serum Alpha-1 Antitrypsin – estimates for co-variates not displayed for ease of presentation.

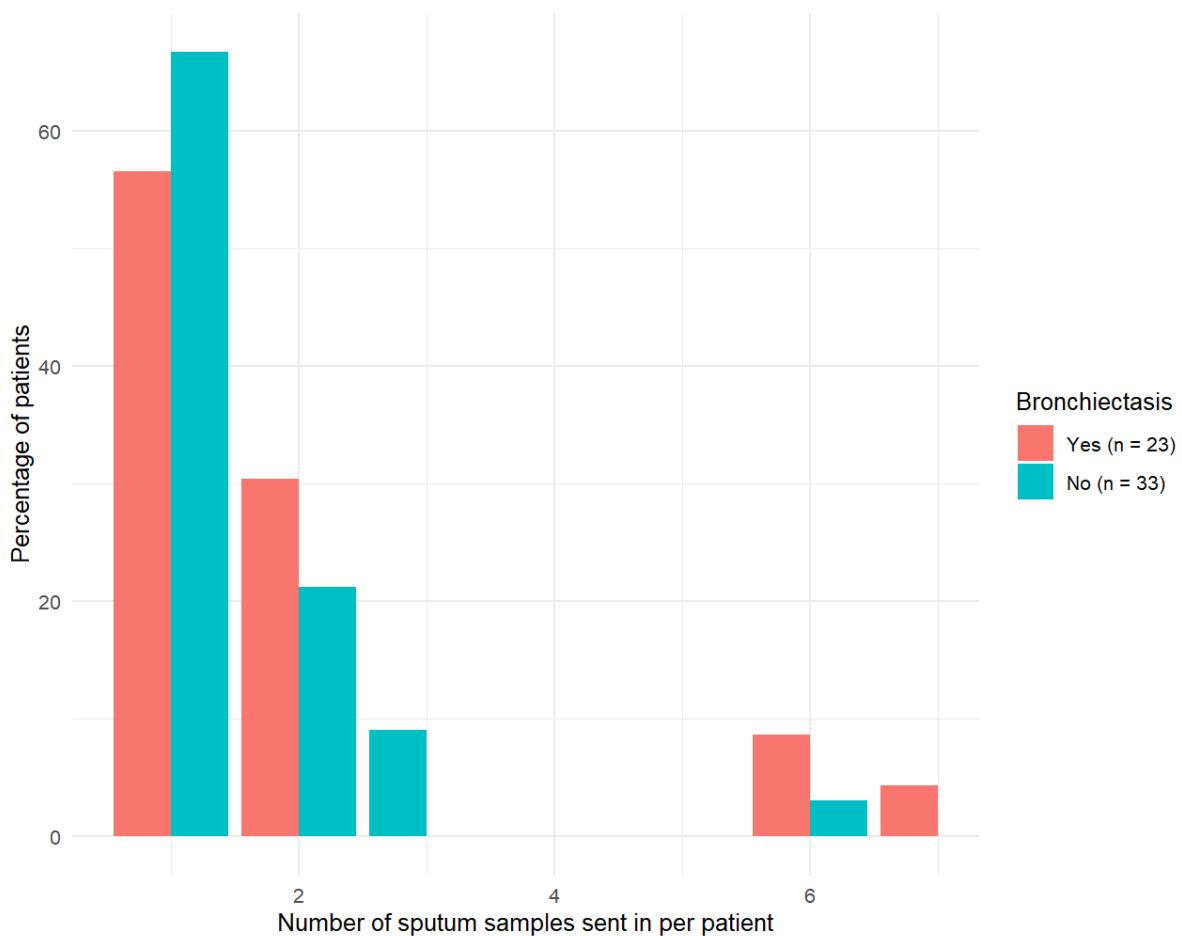
4.2 Microbiology results

N.B: methodology for the section is presented in Chapter 3 section 3.

4.2.1 New samples

69 patients with bronchiectasis were approached for sputum samples. Of these, 23 patients sent in samples. 33 control patients with AATD and without bronchiectasis were identified from other ongoing work in the department. A total of 148 stable state samples were obtained across both groups. Figure 4.4 shows the number of sputum samples sent in per patient.

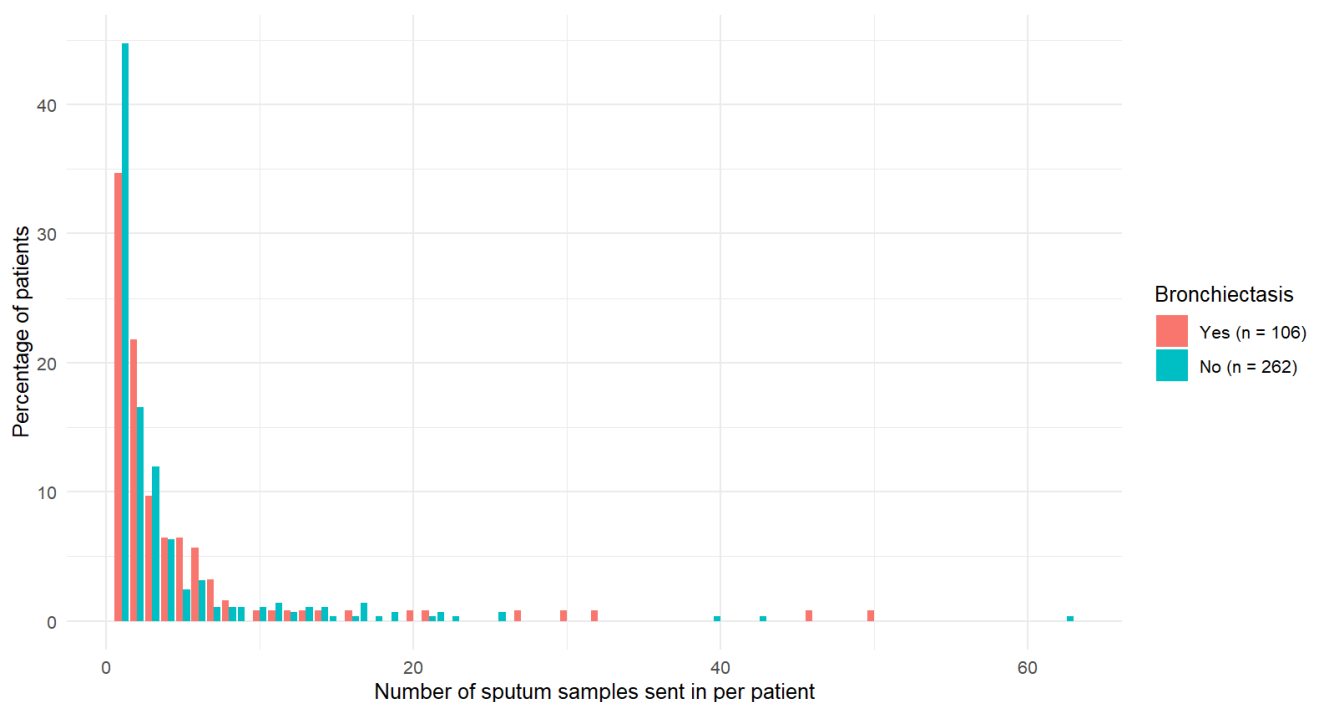
Figure 4.2: Number of sputum samples sent in per AATD patient contacted by telephone, stratified by bronchiectasis diagnosis.



4.2.2 Availability of historic data

Historic data was available from 368 patients from the registry: 106 (28.8%) of these had bronchiectasis and 262 (71.2%) did not. This data was merged with the new sputum samples to yield results from 396 patients (124 [31.3%] with bronchiectasis, 284 [68.7%] without). Number of samples sent in for these patients is shown in Figure 4.5.

Figure 4.3: Number of sputum sample results available per patient



4.2.3 Demographics

Demographics of the patients with available sputum samples are shown in Table 4.7.

Table 4.7: Demographics of patients with available sputum data

	Bronchiectasis	
	Yes	No
N (%)	130 (32.8)	266 (67.8)

	Bronchiectasis	
	Yes	No
Age (years)	55.55 (9.11)	51.56 (10.44)
Sex (Male) (%)	73 (56.2)	184 (69.2)
Genotype (%)		
Severe	116 (89.2)	228 (85.7)
Non-severe	14 (10.8)	38 (14.3)
Ever smoker (%)	80 (61.5)	217 (81.6)
COPD (%)	111 (86.7)	245 (92.5)

4.2.4 Sputum colour

312 patients had sputum colour data available. The most common sputum colour was 3 in those with bronchiectasis and those without (Figure 4.6). In both groups, colour grades 3 or higher were more common than other grades (Figure 4.6). Across all severe AATD patients, sputum colour was not associated with baseline FEV_{1pp} (n = 307, estimate -1.45, 95% CI -3.92 to -1.03, p = 0.251), FEV_{1pp} annual decline (n = 229, estimate 0.13, 95% CI -0.07 to 0.33, p = 0.190), KCO_{pp} annual decline (n = 199, estimate -0.11 95% CI -0.37 to 0.14, p = 0.386), exacerbations (n = 312, zero-inflated IRR 0.92, 95% CI 0.60 to 1.41, p = 0.709; count IRR 0.93, 95% CI 0.80 to 1.10, p = 0.408) or mean SGRQ score (n = 51, estimate -0.91, 95% CI -6.43 to 4.61, p = 0.743). No relationship between colour and mMRC score was seen (Appendix section 2.1).

Only 39 patients with sputum colour had mortality data available, so this was not assessed.

When limited the analyses to those with bronchiectasis only, findings were similar. There was no correlation between sputum colour and baseline FEV_{1pp} (n = 79, est. -1.57, 95% CI -7.01 to 3.87, p = 0.568), FEV_{1pp} annual decline (n = 55, est. 0.10, 95% CI -0.30 to 0.49, p = 0.621), KCOpp annual decline (n = 49, -0.42, 95% CI -0.96 to 0.12, p = 0.125), exacerbation rate (n = 84, zero-inflated IRR 1.80, 95% CI 0.51 to 6.38, p = 0.364; count IRR 1.10, 95% CI 0.77 to 1.59, p = 0.598), mean SGRQ score (n = 18, est. -4.82, 95% CI -14.45 to 4.80, p = 0.297) or mMRC score (Appendix section 2.1).

Figure 4.4: Histogram of sputum colour

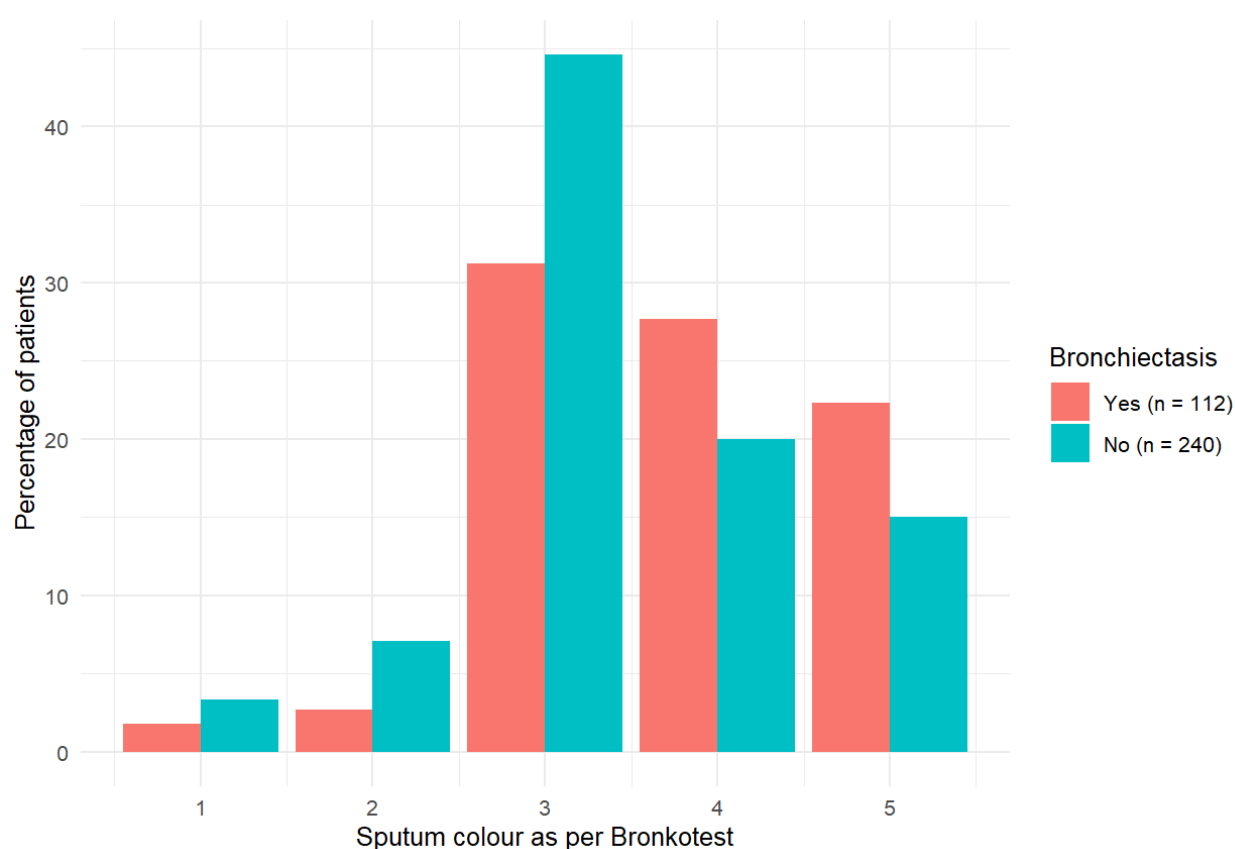


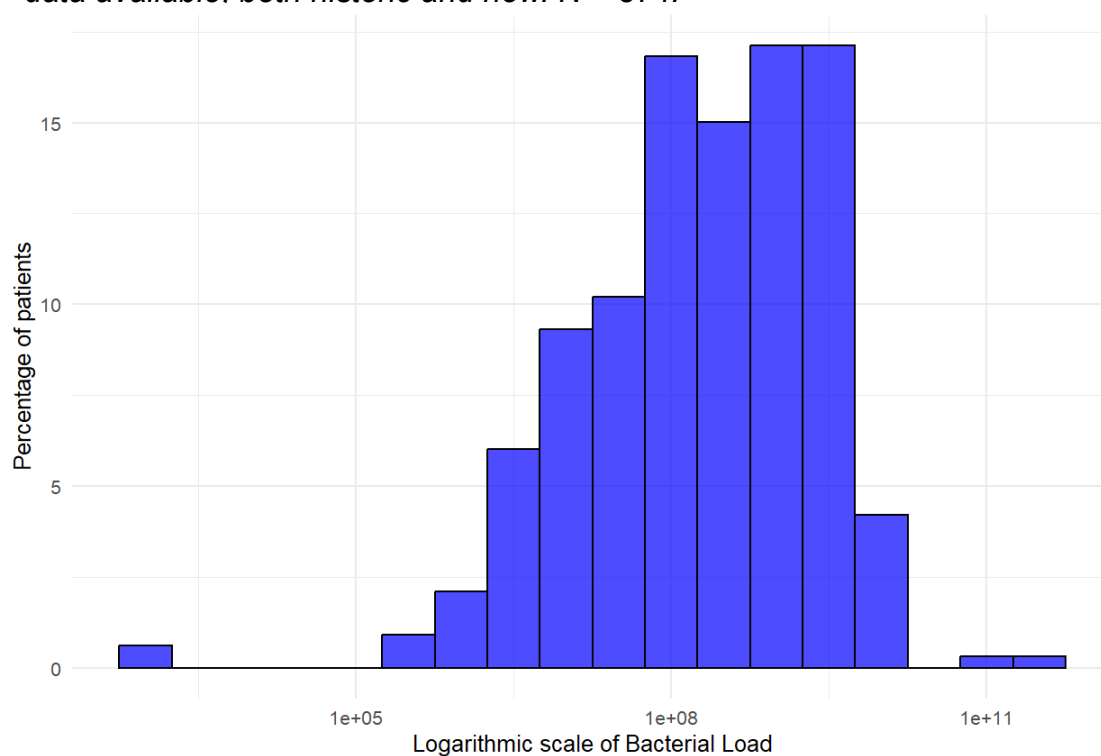
Figure 4.6.1: Bronkotest® sputum colour chart



4.2.5 Bacterial load

Bacterial load data was available from 374 patients. A histogram showing logarithmic values of mean bacterial load is shown in Figure 4.7. Due to this distribution, all statistical analyses were conducted on the logarithmic expression of the original bacterial load data.

Figure 4.5: Distribution of mean bacterial load in AATD patients with bacterial load data available. both historic and new. N = 374.



Across all severe AATD patients, bacterial load was not associated with baseline FEV₁pp (n = 279, log estimate -0.69, 95% CI -1.61 to 0.24, p = 0.144), FEV₁pp decline (n = 223, log estimate 0.04, 95% CI -0.02 to 0.11, p=0.244), KCOpp decline (n = 194, log est. -0.05, 95% CI -0.15 to 0.05, p = 0.319), or with mMRC score (Appendix section 2.2).

However, across all severe AATD patients, bacterial load was associated with exacerbation rate in those with 1 or more exacerbations per year (n = 287, log zero-inflated IRR 1.32, 95% CI 0.97 to 1.79, p = 0.074; log count log IRR 1.07, 95% CI 1.02 to 1.13 p = 0.006), with transformation of log estimates revealing a doubling of bacterial load associated with a 102% increase in exacerbation rate. This finding persisted when limiting the analysis to those with bronchiectasis, with a doubling of bacterial load associated with a 121% increase in exacerbation rate (n = 90, log count IRR 3.18, 95% CI 2.82 to 3.63, p = 0.009).

Correlation with mean SGRQ also crossed statistical significance when analysing all severe AATD patients, with a doubling of bacterial load being associated with 1.30 reduction in SGRQ score (n = 53, log est. -1.88, 95% CI -3.72 to -0.04) p = 0.046). This finding did not persist when limited to those with bronchiectasis (n = 20, log est. -2.13, 95% CI -5.69 to 1.43, p = 0.220).

4.2.6 Species

Species data is presented both as “growth”, referring to 1 or more positive sputum cultures for a particular species, and “colonisation”, referring to 2 or more positive sputum cultures for a particular species, separated by 3 months or more. Species data was available for 352 patients (Table 4.8). 141 (40.1%) of these had never grown a pathogenic species, and were recorded as mixed normal flora only. Species frequency

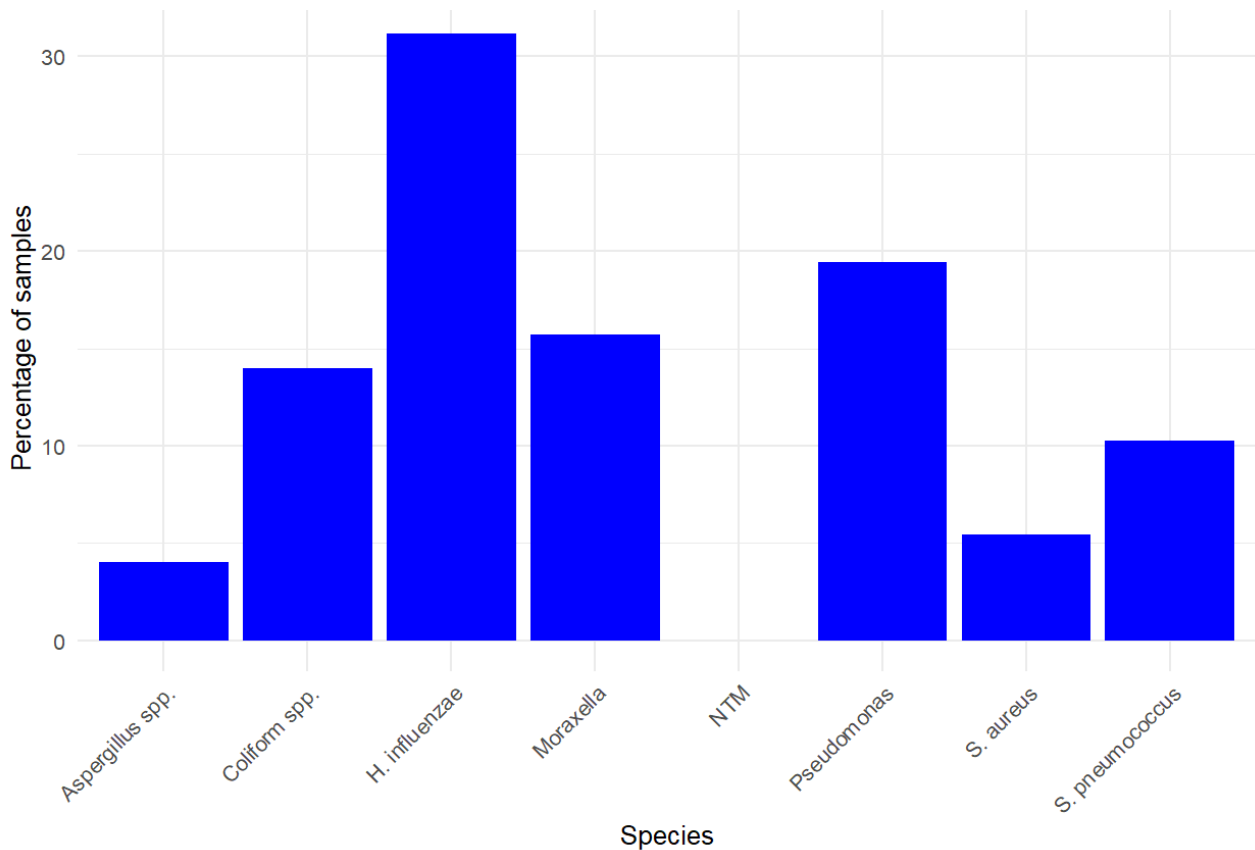
for the remaining 211 (59.9%) is shown in Figure 4.8. *Haemophilus influenzae* was the most common species isolated, followed by *Pseudomonas spp.* No patients had grown a non-tuberculous mycobacteria.

All pathogens other than *Pseudomonas* were grouped together as “other pathogens”, in accordance with Chalmers et al¹⁸⁸. 192 patients (54.5%) grew one or more of these pathogens at some point.

Table 4.8: Species frequency for AATD patients who produce sputum, stratified by bronchiectasis diagnosis. P = χ^2 test

	Bronchiectasis		p
	Yes	No	
n	112	240	
Mixed Normal Flora only	39 (34.8)	102 (42.5)	0.21
<i>Pseudomonas spp.</i> (%)	29 (25.9)	35 (14.6)	0.016
Other pathogens (%)	61 (54.5)	131 (54.6)	1
<i>Moraxella catarrhalis</i> (%)	22 (19.6)	31 (12.9)	0.138
<i>H. influenzae</i> (%)	29 (25.9)	76 (31.7)	0.328
<i>S. aureus</i> (%)	12 (10.7)	6 (2.5)	0.002
<i>S. pneumoniae</i> (%)	8 (7.1)	26 (10.8)	0.369
<i>Aspergillus</i> (%)	3 (2.7)	9 (3.8)	0.841

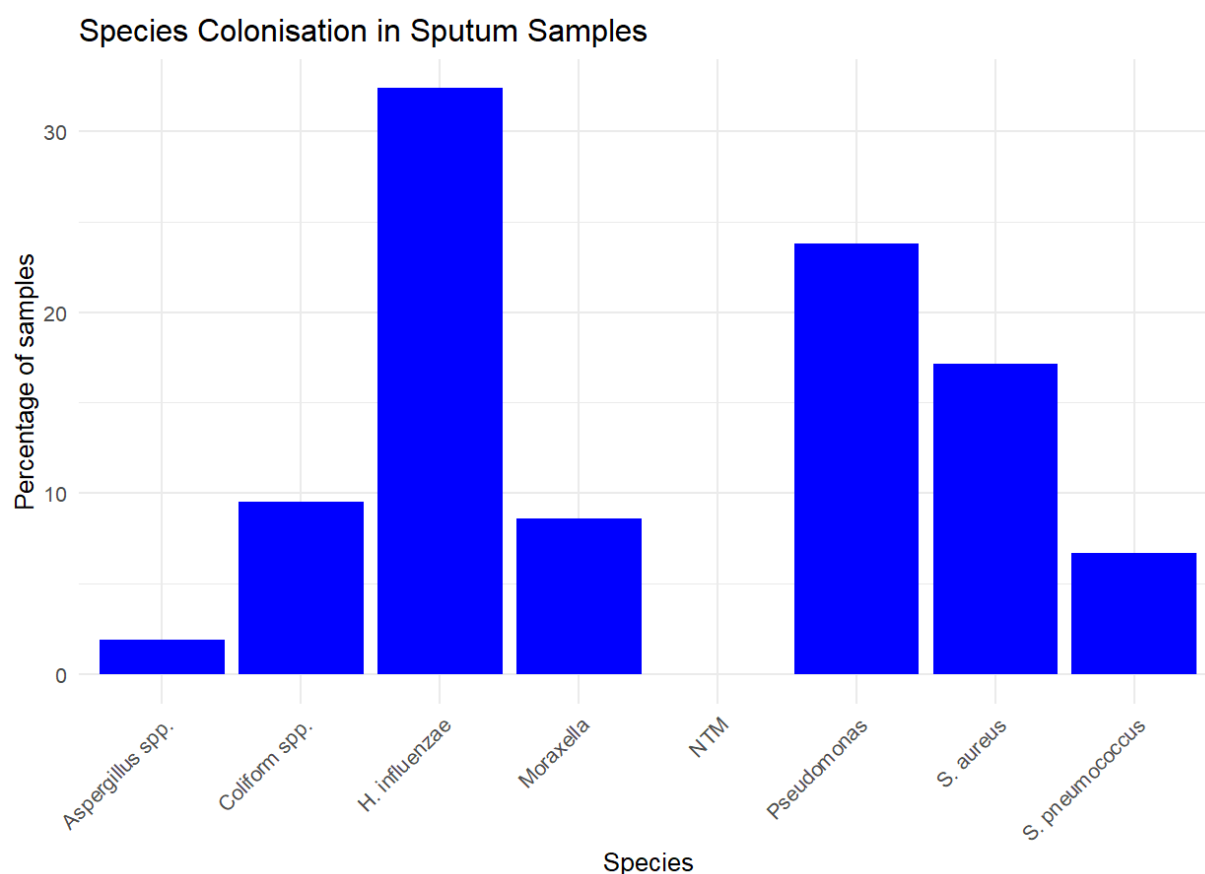
Figure 4.6: Sputum pathogen prevalence in patients with AATD who have grown a pathogen in sputum. N = 192.



4.2.6.1 Colonisation

Of the 282 patients who had data from more than one sample available, 69 (24.4%) had at some point been colonised (growth of the same organism in 2 or more samples) with one or more organisms. Frequency of colonisation with each pathogen is shown in Figure 4.9.

Figure 4.7: Sputum pathogen colonisation in patients with AATD who have demonstrated sputum bacterial colonisation. N = 69.



4.2.6.2 Correlation of *Pseudomonas* spp. with clinical outcomes

Among the 304 patients with severe AATD, 53 (17.4%) had at some point grown a *Pseudomonas*, and 22 (7.8%) had been colonised. On χ^2 testing, *Pseudomonas* was more common in those with bronchiectasis than those without ($p = 0.016$, Table 4.8). On multivariate analysis all severe AATD patients, growth of pseudomonas correlated with a 43% higher exacerbation rate in those who exacerbate ($n = 303$, count IRR 1.43, 95% CI 1.03 to 1.97, $p = 0.031$, zero-inflated IRR 1.91, 95% CI 0.70 to 5.25, $p = 0.207$, Table 4.9).

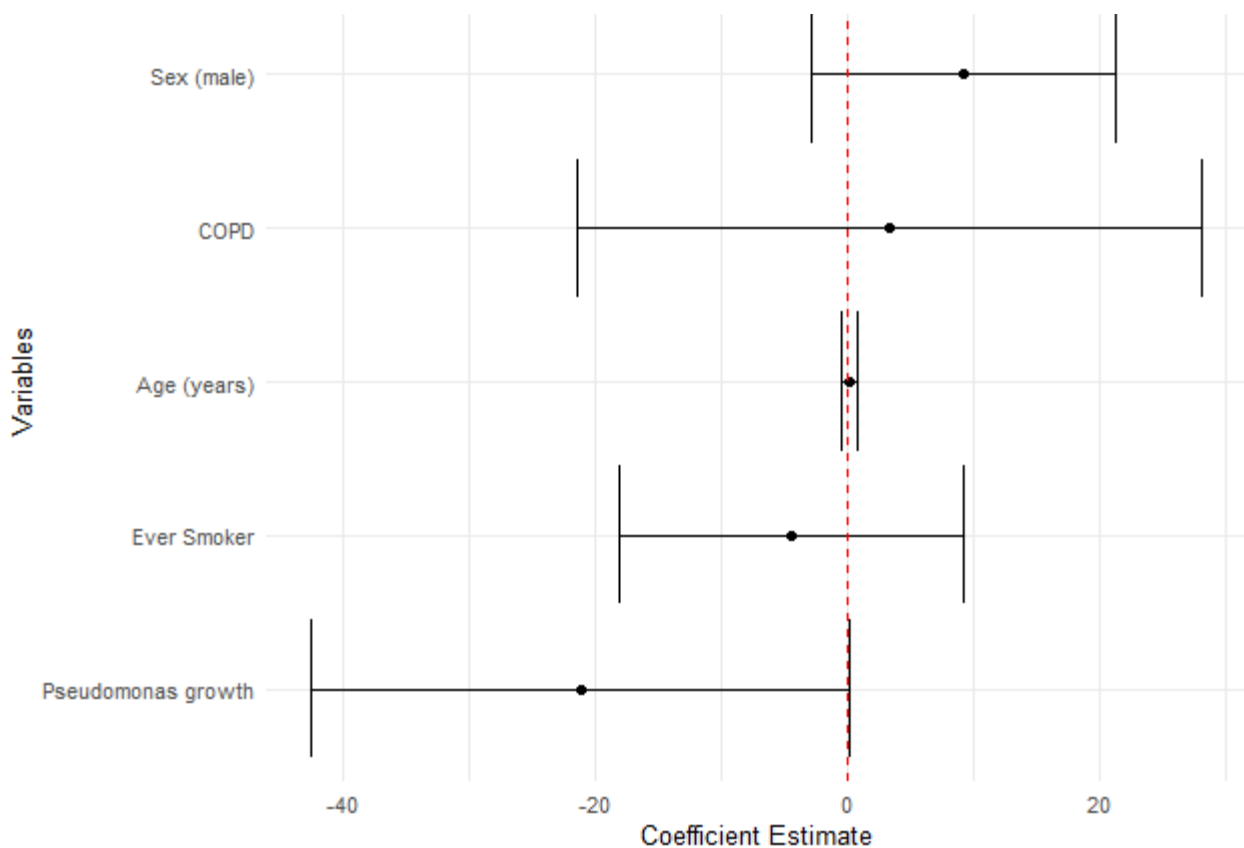
Table 4.9: Zero-inflated model estimates for the association of *Pseudomonas* growth with exacerbation rate.

Associations	Annualised exacerbation rate		
	Incidence Rate Ratios	CI	p
Count Model			
<i>Pseudomonas</i> growth	1.43	1.03 – 1.97	0.031
Age (years)	1.01	0.99 – 1.03	0.201
Sex (male)	0.84	0.61 – 1.17	0.300
COPD	0.71	0.38 – 1.33	0.285
Ever smoker	1.78	1.13 – 2.80	0.013
Zero-Inflated Model			
<i>Pseudomonas</i> growth	1.91	0.70 – 5.25	0.207
Age (years)	1.04	0.96 – 1.13	0.320
Sex (male)	2.09	0.41 – 10.50	0.372
COPD	0.33	0.05 – 2.18	0.250
Ever smoker	2.22	0.25 – 19.89	0.477
Observations	303		
R ² / R ² adjusted	0.060 / 0.041		

When analysing all patients with severe AATD, *Pseudomonas* growth was not associated with baseline FEV_{1pp} (n = 295, est. -3.30, 95% CI -9.48 to 2.87, p = 0.293), FEV_{1pp} annual decline (n = 232, est. 0.04, 95% CI -0.50 to 0.58, p = 0.881), KCOpp

annual decline ($n = 200$, est. -0.29 , 95% CI -0.98 to 0.39 , $p = 0.400$), or mortality ($n = 44$, HR 1.77 , 95% CI 0.88 to 3.54 , $p = 0.108$). Correlation with mean SGRQ score narrowly missed the statistical significance threshold, with *Pseudomonas* tending towards association with an estimated 21.22 reduction in mean SGRQ score ($n = 63$, 95% CI -42.53 to 0.09 , $p = 0.051$, Figure 4.10). Likewise, *Pseudomonas* appeared to correlate non-significantly with higher rates of mMRC score equal to 4 ($n = 295$, OR 4.08 , 95% CI 0.95 to 17.47 , $p = 0.058$).

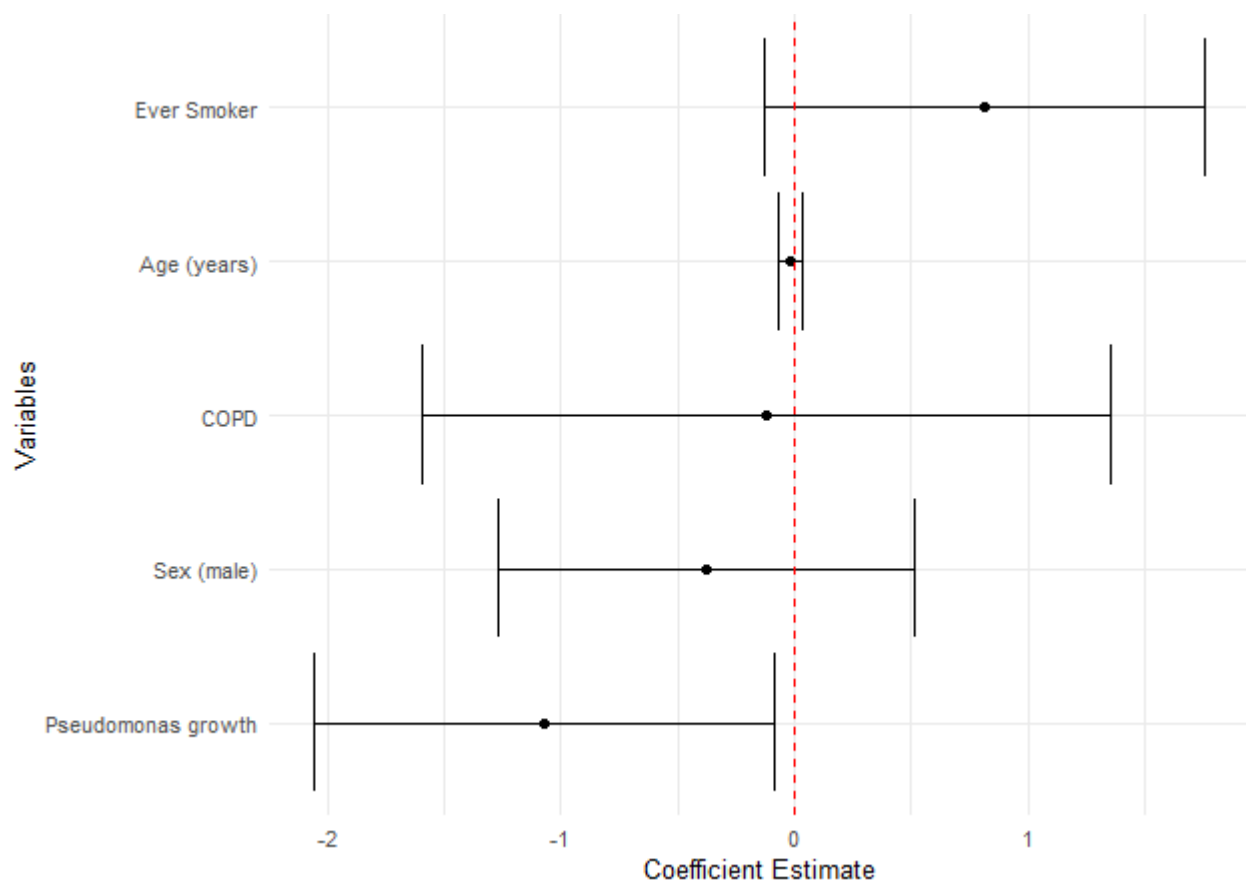
Figure 4.8: Forest plot of a multivariate linear regression for the association of SGRQ score with Pseudomonas growth correlation in patients with severe AATD.



When limited to patients with bronchiectasis only, an association with a further 1.07% KCOpp annual decline was found ($n = 60$, 95% CI -2.05 to -0.09 , $p = 0.033$, Figure 4.11), though there remained no association with baseline FEV_{1pp}, FEV_{1pp} annual decline, or mortality. The correlation with exacerbation rate in those with 1 or more

exacerbations per year persisted, with a 61% higher exacerbation rate in those with *Pseudomonas* growth (n = 60, IRR 1.61, 95% CI 1.03 to 2.50, p = 0.033). The correlation with mMRC score 4 disappeared (OR 11.3, 95% CI 0.32 to 16.89, p = 0.394). The correlation with mean SGRQ score still did not cross statistical significance thresholds (n = 27, est. -23.66, 95% CI -47.48 to 0.16, p = 0.051).

Figure 4.9: Forest plot of a multivariate linear regression for the association of Pseudomonas growth with annual gas transfer decline in patients with severe AATD. Those with Pseudomonas growth had a small (1.07%) increase in KCO %predicted annual decline compared to those without. N = 60.



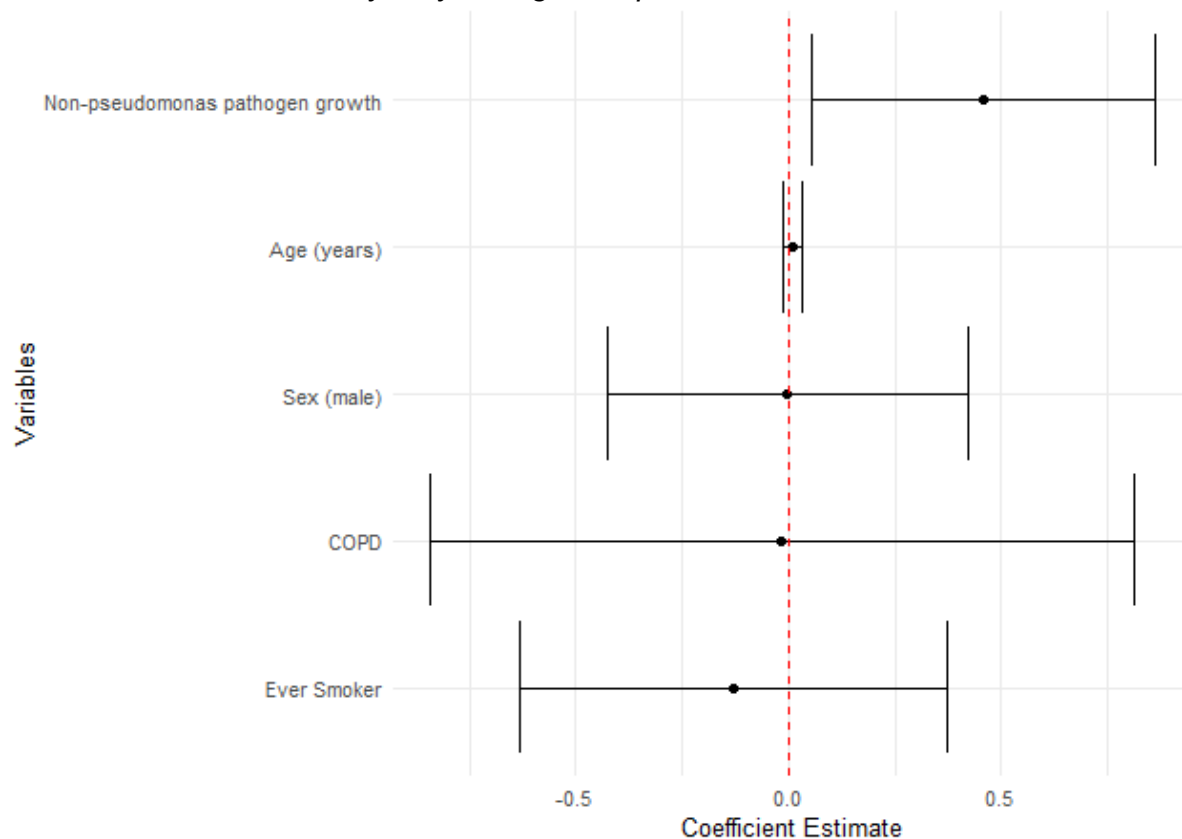
The above analyses were repeated for *Pseudomonas* colonisation, rather than growth, in all severe AATD patients, but found no associations with any of the clinical outcomes, even when limited to patients with bronchiectasis only (baseline FEV_{1pp},

FEV₁pp or KCOpp annual decline, exacerbation rate, mean SGRQ, mode mMRC, or mortality).

4.2.6.3 Correlation of other pathogens with clinical outcomes

Among the 304 patients with severe AATD genotypes, 164 (53.9%) had ever had samples with growth of one of the other pathogens, and 74 (24.3%) had been colonised. Growth of any other pathogen did not differ between those with bronchiectasis and those without ($p = 1$, Table 4.8), but growth of *Staph aureus* was more common in those with bronchiectasis than those without ($p = 0.002$). Growth of other pathogens was not correlated with baseline FEV₁pp ($n = 295$, estimate -2.05, 95% CI -6.81 to 2.72, $p = 0.399$), KCOpp annual decline ($n = 200$, estimate -0.02, 95% CI -0.53 to 0.49, $p = 0.943$), mean total SGRQ ($n = 63$, estimate -3.40, 95% CI -14.72 to 7.91, $p = 0.549$), mMRC score, exacerbation rate ($n = 304$, zero-inflated IRR 0.75, 95% CI 0.32 to 1.76, $p = 0.506$; count IRR 0.98, 95% CI 0.74 to 1.30, $p = 0.910$), or mortality ($n = 44$, HR 1.03, 95% CI 0.99 to 1.08, $p = 0.132$) but FEV₁pp annual decline was 0.46% better than those without growth of these pathogens ($n = 232$, 95% CI 0.06 to 0.86, $p = 0.026$, R^2 adj. 0.005, Figure 4.12). These analyses were repeated in patients with bronchiectasis only, and the correlation with FEV₁pp decline disappeared ($n = 68$, estimate +0.53, 95% CI -0.08 to 1.14, $p = 0.087$); furthermore, no other statistically significant correlations were found with baseline FEV₁, KCOpp decline, exacerbation rate, symptoms, or mortality.

Figure 4.10: Forest plot of a linear regression for the association of non-Pseudomonal pathogen growth correlation with FEV₁ yearly change in patients with severe AATD. Those with non-Pseudomonal pathogen growth had a small (0.46%) positive difference in annual FEV₁ yearly change compared to those without. N = 232.



Colonisation with non-pseudomonal pathogens did not correlate with baseline FEV₁pp, FEV₁pp or KCOpp decline, exacerbation rate, or mMRC score, but did appear to correlate with mortality (n = 44, HR 1.04, 95% CI 0.99 to 1.09, p = 0.031; 10-year survival 35.7% vs 60.0%, Figure 4.13). This effect persisted when limiting the analysis to those with bronchiectasis only (n = 41, HR 2.03, 95% 1.01 – 4.08, p = 0.043; 10-year survival 35.7 vs 59.2 %, Figure 4.14). Such colonisation also associated with a 19.1-point lower SGRQ score (n = 63, 95% CI -34.2 to -4.07, p = 0.014) in all 304 patients, though the effect did not persist when limited to the 100 bronchiectasis patients (n = 27, estimate -14.41, 95% CI -37.16 to 8.34, p = 0.202).

Figure 4.11: Kaplan-Meier curve stratified by non-*Pseudomonas* pathogen colonisation in patients with severe AATDnon-*Pseudomonas* pathogen colonisation had higher mortality risk over 10 years

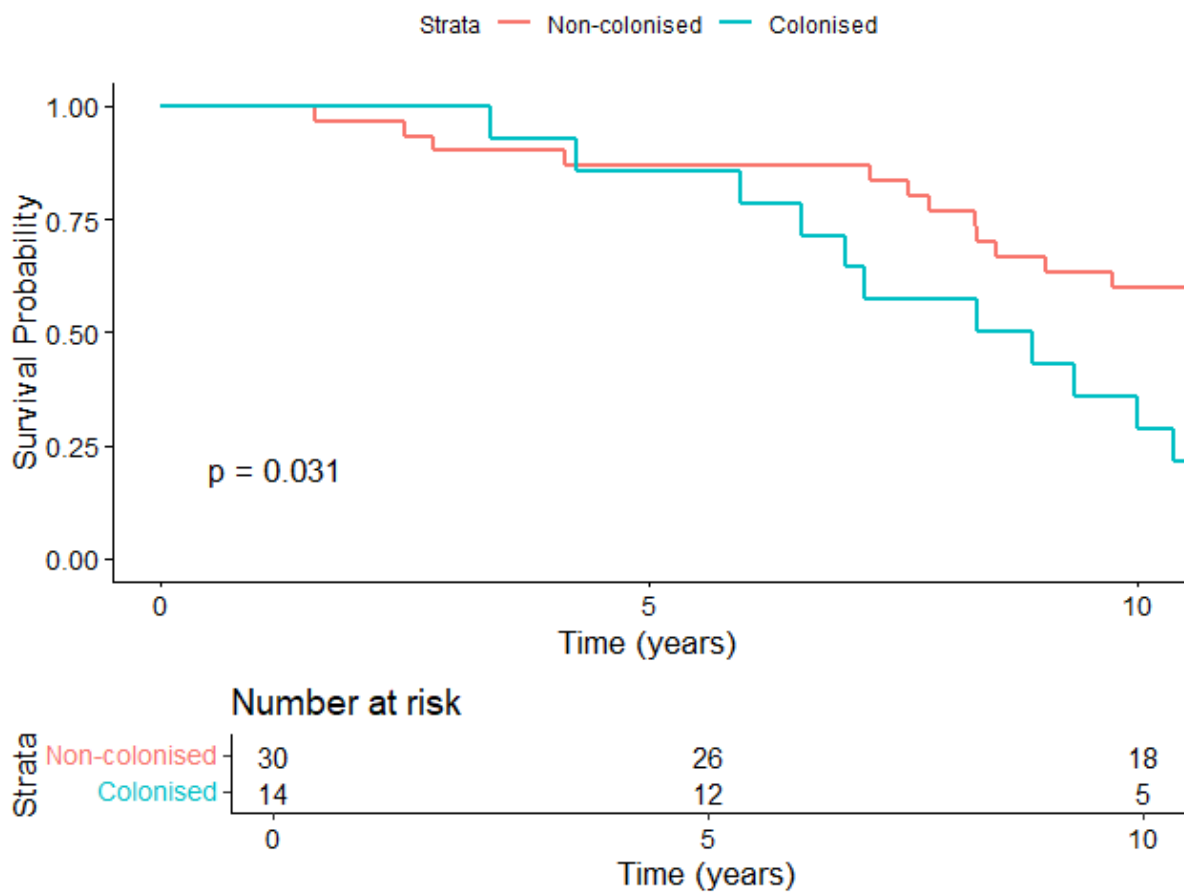
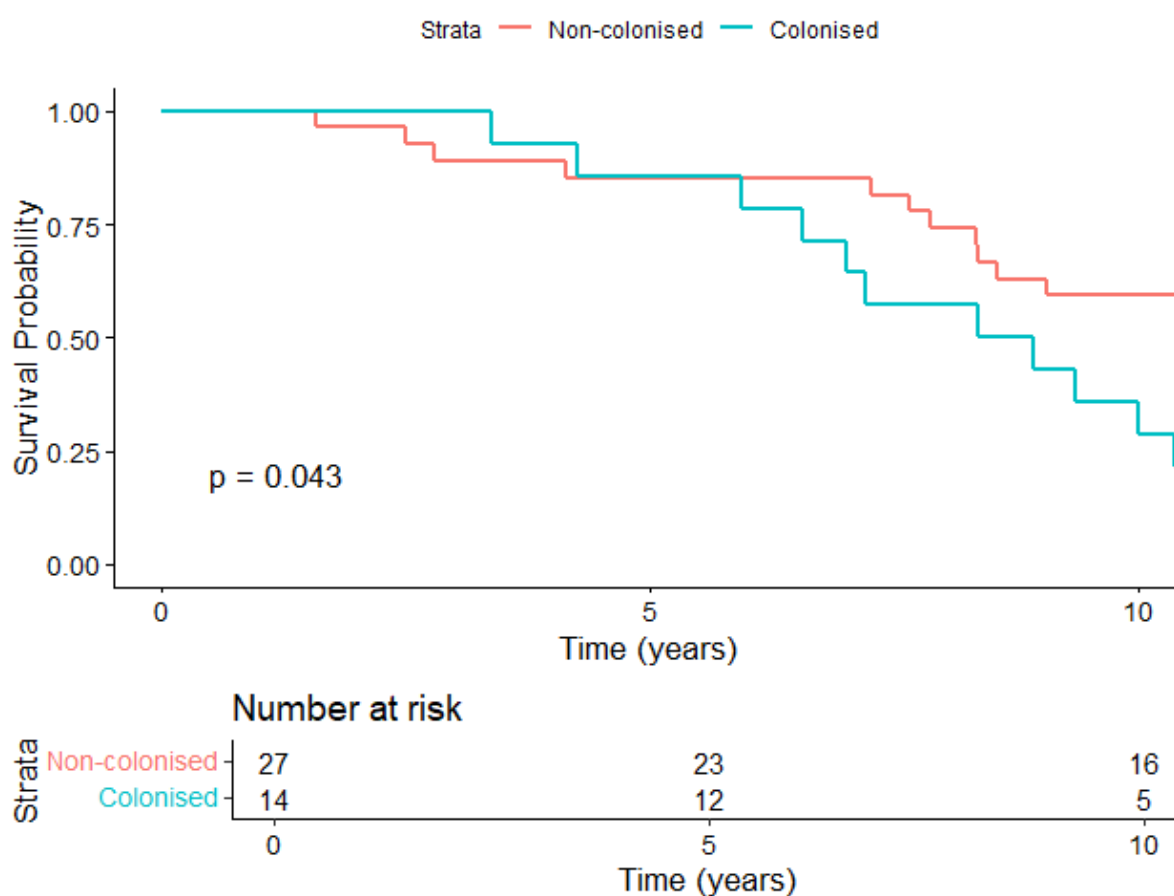


Figure 4.12: Kapan-Meier curve stratified by non-*Pseudomonas* pathogen colonisation in patients with severe AATD and bronchiectasis. Those with non-*Pseudomonas* pathogen colonisation had higher mortality risk over 10 years.



4.2.6.3.1 Correlation of *H. influenzae* with clinical outcomes

As the most frequently isolated non-*Pseudomonas* pathogen, *H. influenzae* growth and colonisation was also assessed for association with clinical outcomes. 90 (29.6%) of the 304 patients with severe-AATD had ever grown *H. influenzae* in their sputum (23 of the 100 patients with bronchiectasis), and 27 (8.9%) had been colonised with it (9 of the 100 with bronchiectasis).

H. influenzae growth did not relate to baseline FEV₁pp, FEV₁pp yearly decline, KCOpp yearly decline, mMRC score, or mortality. It did relate to mean SGRQ score, with the total score being on average 22.3-points lower ($n = 63$, 95% CI -34.33 to -10.20, $p =$

0.001), and this persisted in the bronchiectasis group (n = 27, est. -28.74, 95% CI -45.54 to -11.93, p = 0.002). Although *H. influenzae* did not correlate with exacerbation rate in the whole group of 304 patients, when limited to the 100 bronchiectasis patients, *H. influenzae* did have a negative correlation with the count estimate, even after controlling for *Pseudomonas* growth (n = 100, Count IRR 0.51, 95% CI 0.30 to 0.87, p = 0.013). No other statistically significant correlations were found between *H. influenzae* and the clinical outcomes in the bronchiectasis group.

Colonisation with *H. influenzae* did not correlate with baseline FEV_{1pp}, FEV_{1pp} or KCO_{pp} yearly decline, exacerbation rate, or mortality. In the whole group, *H. influenzae* associated with 25.98 lower SGRQ score (n = 63, 95% CI -44.65 to -7.30, p = 0.007), but this did not persist when limited to patients with bronchiectasis (n = 27, est. -17.71, 95% CI -43.33 to 7.90, p = 0.165). Insufficient variation was seen in the mMRC score among those with *H. influenzae* colonisation to create an adequate model for analysis.

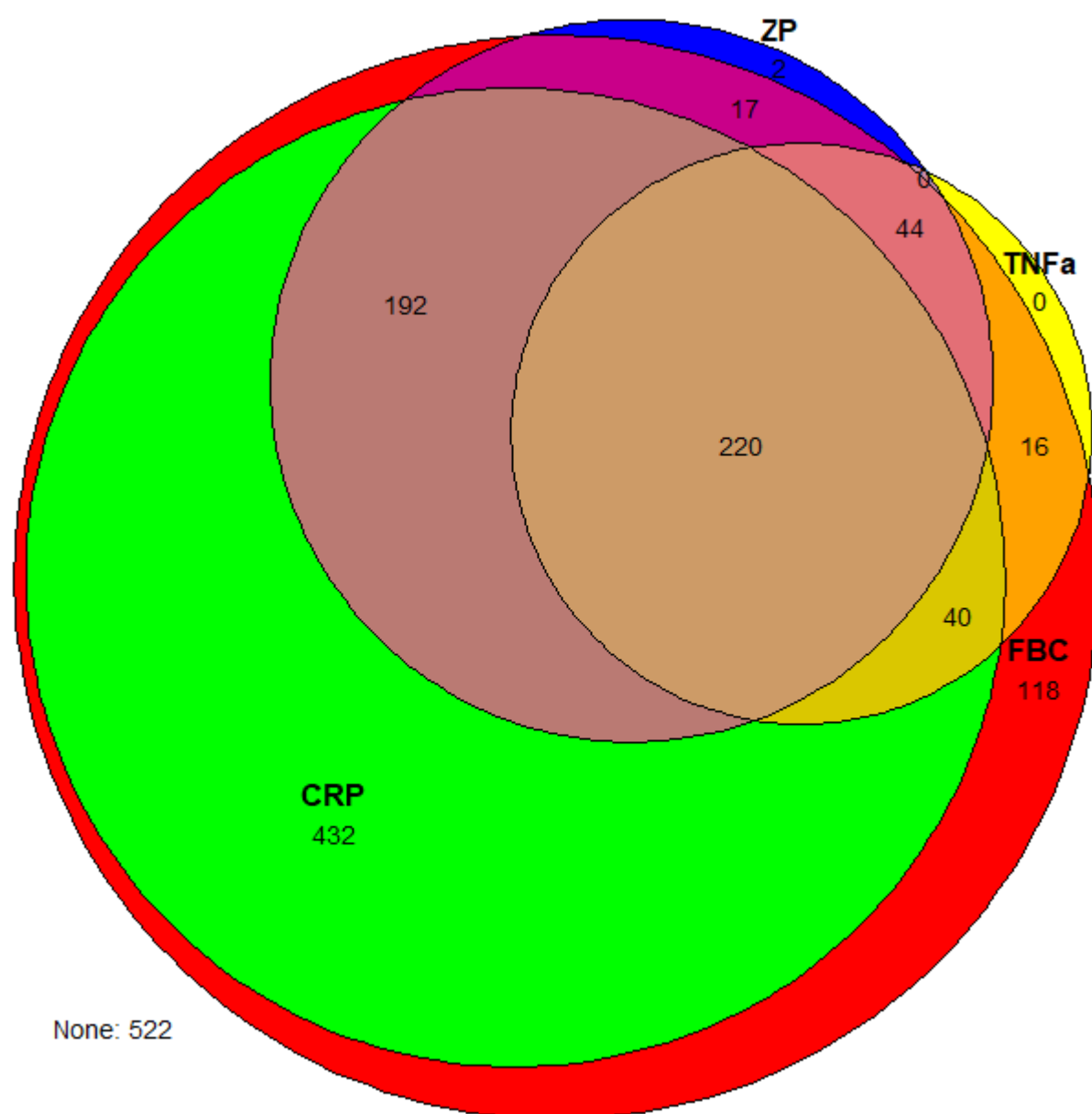
4.3 Biomarker results

N.B. Methodology for this section is shown in Chapter 3 Section 4.

4.3.1 Overlapping availability of biomarkers

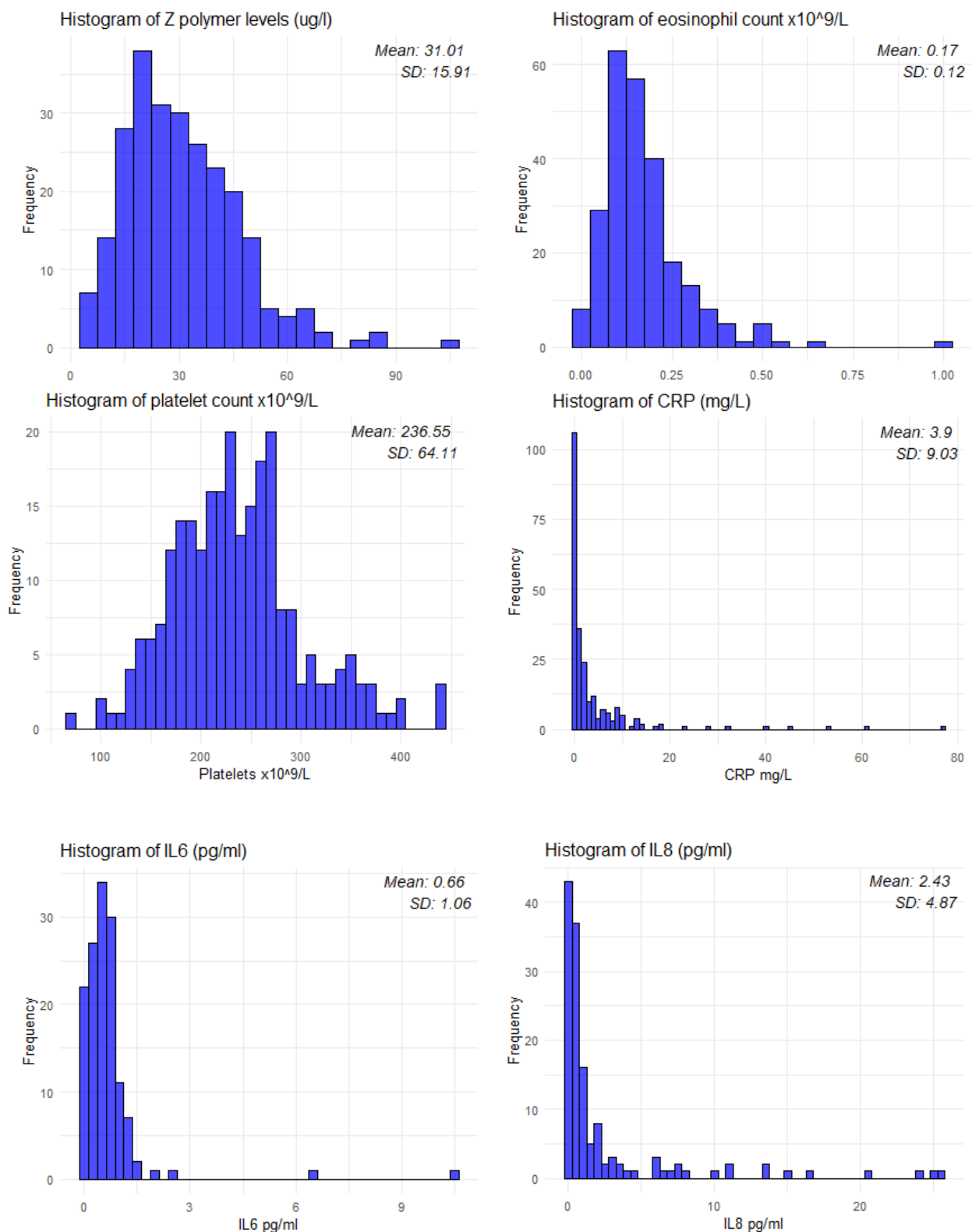
Many patients had some, but not all, of the above biomarkers tested. This complex sampling issue is shown schematically in a Euler diagram below (Figure 4.15). The largest available subset was FBC data, closely followed by CRP data. Almost all Z polymer patients had had FBC and CRP measurements. There was considerable overlap of the TNF α subset with the 3 other subsets.

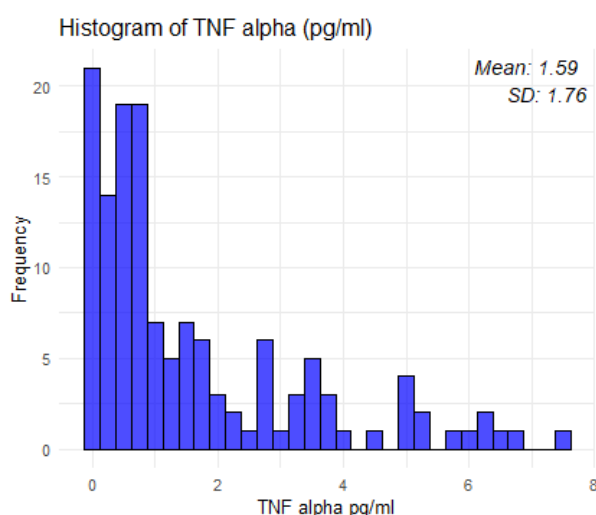
Figure 4.13: Euler diagram showing overlapping availability of biomarker data in the Birmingham AATD cohort.



4.3.2 Distribution of biochemical markers

Figure 4.14a-4.16g: Histograms of distribution of the various biochemical markers available in the Birmingham AATD cohort.





Distribution of the various biomarkers used, along with means and standard deviations, are shown in Figures 4.16a-g. Outliers were studied; their baseline characteristics are shown in Appendix section 4. Eosinophil outliers were mostly female (71.3%); platelets outliers were overwhelmingly COPD patients (91.7%). All CRP outliers had COPD. No other patterns were found.

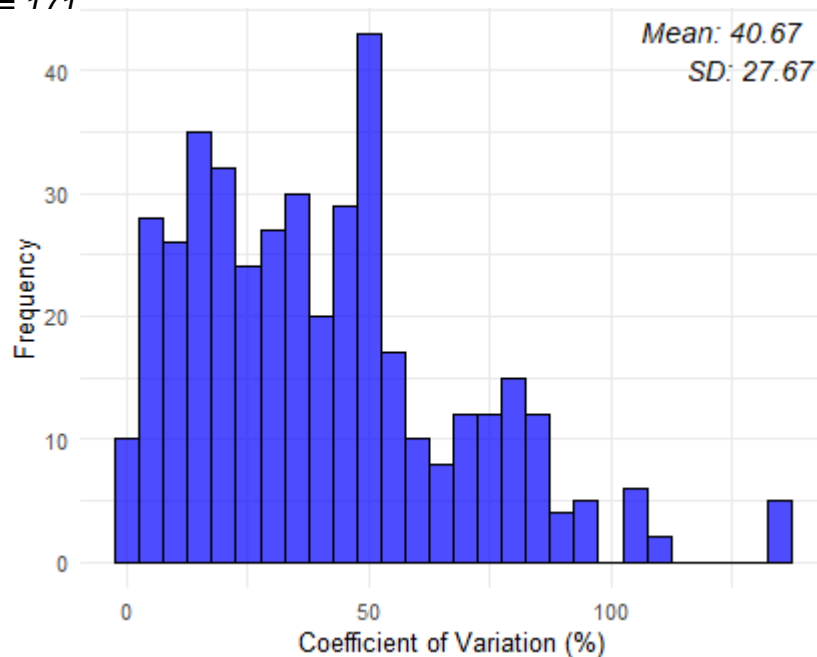
4.3.3 Stability of biochemical markers

Z polymers, eosinophil and platelet counts, and CRP levels could be assessed for stability. For TNF-alpha, IL-6, and IL-8, only 1 measurement was available per patient, and therefore stability could not be assessed.

4.3.3.1 Z polymer stability

251 patients had Z polymer measurements available. 80 patients had only one Z-polymer measurement (Figure 4.17). Coefficient of variance (CV) was calculated for the remaining 171 patients, and is displayed in Figure 3. CV was high, with mean 40.7% (SD 27.7%). Due to this high variance, a Kruskal-Wallis test was conducted to assess whether variance was higher in those with frequent exacerbations: this showed no association (χ^2 2.73, $p = 0.604$). Furthermore, when limited to patients who do not exacerbate, CV remained high (mean 40.67, SD 27.67).

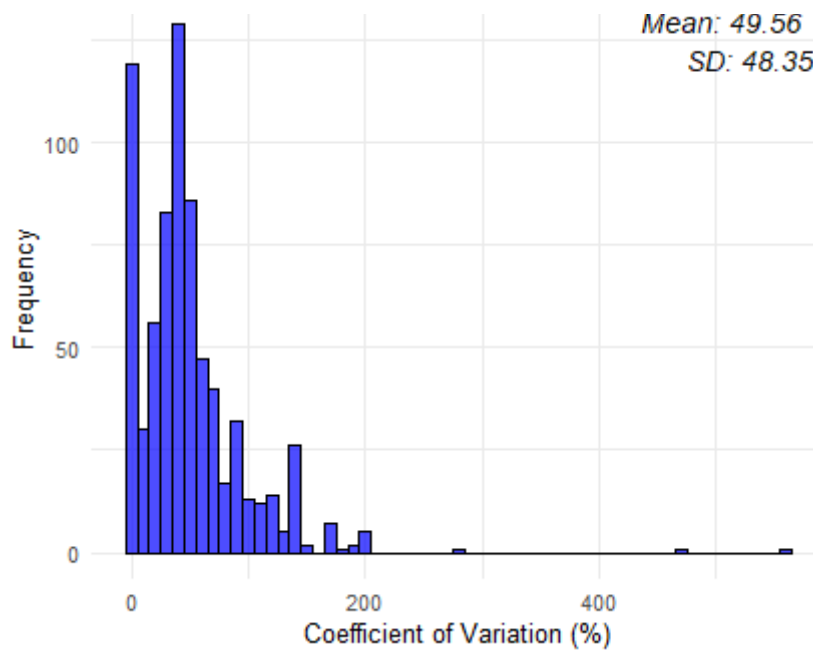
Figure 4.15: Coefficients of variance for Z polymer measurements in patients with AATD, per patient. Z polymers do not appear to be stable within the same patient over time. N = 171



4.3.3.2 Eos stability

1079 patients had eosinophil data available. 914 of these had had more than one measurement. Eosinophils showed high variability over time (Figure 4.18), with mean coefficient of variance 49.6% (SD 48.35%). This variability was not associated with annualised exacerbation rate (Kruskal-Wallis χ^2 7.01, $p = 0.526$).

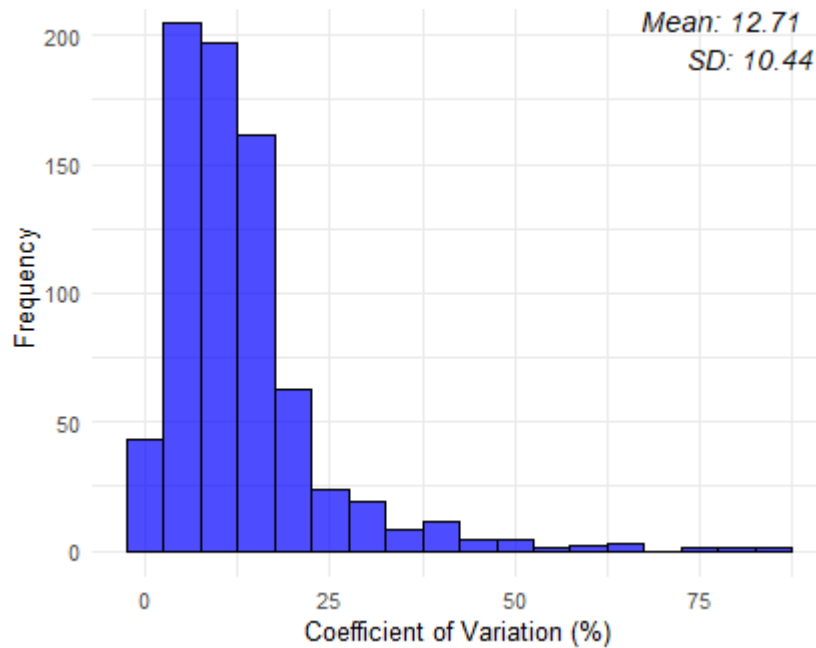
Figure 4.16: Coefficients of eosinophil variation over time in AATD patients. Eosinophils are not stable within the same patient over time.



4.3.3.3 Platelet stability

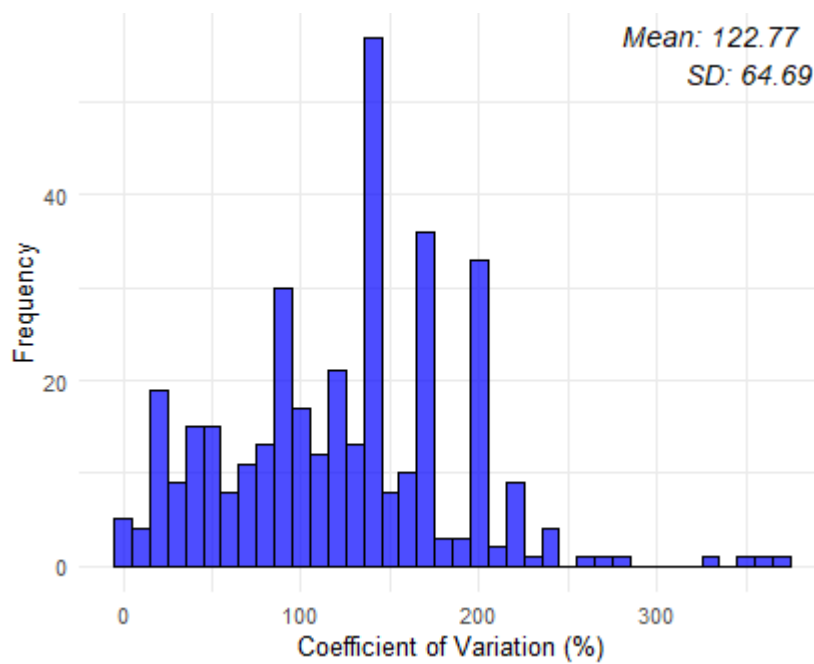
1079 patients had platelet count data available. 914 of these had had more than 1 measurement. Platelet stability was good (CV mean 12.71%, Figure 4.19).

Figure 4.17: Coefficients of variance for platelets in AATD patients, per patient. Platelets appear to be stable over time.



4.3.3.4 CRP stability

Figure 4.18: Coefficients of variance for CRP in patients with AATD, per patient. CRP varies widely within individual patients.



884 patients had CRP data available. 693 of these had had more than 1 measurement. CRP variation was high (mean CV 122.8%, SD 64.7%, Figure 4.20), and unrelated to annualised exacerbation rate (Kruskal-Wallis $\chi^2 = 5.26$, $p = 0.628$).

4.3.4 Statistical modelling

4.3.4.1 Correlation of Z-polymers with baseline characteristics

When excluding the minorities with PiMZ or PiZ-null genotypes, Z-polymers were higher in those with PiZZ vs PiSZ genotypes (Table 4.10, $p < 0.001$). Z-polymer levels did not differ between PiZZ patients with bronchiectasis and those without ($p = 0.668$, Table 4.10).

Table 4.10: Correlation of genotype with Z polymer levels

Associations	mean Z-polymer $\mu\text{g/l}$		
	Estimates	CI	p
Bronchiectasis	1.10	-3.94 – 6.13	0.668
PiZZ genotype (vs SZ)	12.82	6.16 – 19.49	<0.001
Age (years)	0.06	-0.12 – 0.25	0.515
Sex [Male]	0.78	-3.40 – 4.96	0.713
COPD	0.02	-5.26 – 5.30	0.995
Ever smoker	-0.98	-5.46 – 3.50	0.666
Observations	207		
R ² / R ² adjusted	0.081 / 0.054		

4.3.4.2 Z-polymers and clinical outcomes in AATD

On multivariate analysis of those with severe genotypes, Z-polymer levels did not associate with baseline FEV₁ pp values ($n = 180$, est. -0.04, 95% CI -0.23 to 0.16, $p = 0.712$, Table 4.11), FEV₁pp yearly decline ($n = 164$, est. 0.01, 95% CI -0.01 to 0.02, $p = 0.469$), KCOpp yearly decline ($n = 121$, est. 0.01, 95% CI -0.01 to 0.03, $p = 0.504$),

SGRQ score (n = 100, est. 0.13, 95% CI -0.13 to 0.39, p = 0.313), annualised exacerbation rate (n = 206, zero-inflated IRR 1.05, 95% CI 0.91 to 1.21, p = 0.532; count IRR 0.99, 95% 0.98 to 1.01, p = 0.382), or mMRC score (Appendix Section 3). Mortality was also unaffected (n = 9, HR 2.85, 95% CI 0.76 to 10.67, p = 0.119).

Table 4.11: Baseline FEV₁pp is not associated with Z polymer level.

<i>Associations</i>	Baseline FEV₁pp		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>
Mean serum Z polymer µg/L	-0.04	-0.23 to 0.16	0.712
COPD	-32.01	-39.81 to -24.21	<0.001
Ever smoker	-25.63	-32.08 to -19.19	<0.001
Annualised exacerbation rate	-2.84	-5.39 to -0.29	0.029
Observations	180		
R ² /R ² adjusted	0.584/0.570		

4.3.4.3 Z-polymers and clinical outcomes in AATD-bronchiectasis

When limiting the analysis to those patients with severe genotypes and bronchiectasis, Z-polymer levels did not associate with baseline FEV₁% predicted (n = 50, est. -0.09, 95CI -0.7 to 0.61, p = 0.798), or yearly lung function decline as measured by FEV₁% predicted (n = 44, est. 0.00, 95% CI -0.03 to 0.02, p = 0.660), KCOpp (n = 34, est. 0.02, 95% CI -0.01 to 0.05, p = 0.192) or TLCOpp (n = 40, est. 0.01, 95% CI -0.05 to 0.03, p = 0.622). Z-polymer levels also did not associate with exacerbation rate (n = 50, zero-inflated model IRR 0.97, 95% CI 0.88 to 1.07, p = 0.524; count model IRR 0.99, 95% CCI 0.97 to 1.02, p = 0.647), SGRQ score (n = 32, estimate -0.14, 95% CI -0.79 to 0.52, p = 0.675), or mortality (n = 4, HR 1.01 per µg/L, 95% CI 0.95 to 1.07, p = 0.851).

4.3.4.4 Eosinophil, platelet, and CRP correlation with bronchiectasis

On multivariate linear regression analysis of all those with severe AATD, eosinophils were estimated to be $0.03 \times 10^9/L$ higher in those with bronchiectasis than in those without ($n = 784$, 95% CI 0.01 to 0.05, $p = 0.001$, Table 4.12). Of note, neither COPD nor asthma were associated with a difference in eosinophil count.

Table 4.12: Eosinophils are $0.034 \times 10^9/L$ higher in those with bronchiectasis.

Associations	Eosinophil count $\times 10^9/L$		
	Estimates	CI	P
Bronchiectasis	0.034	0.014 – 0.053	0.001
COPD	0.004	-0.018 – 0.026	0.695
Ever smoker	0.004	-0.015 – 0.022	0.709
Age [years]	0.000	-0.000 – 0.001	0.294
Sex [Male]	-0.002	-0.019 – 0.014	0.762
Asthma	-0.012	-0.036 – 0.011	0.300
Observations	784		
R ² / R ² adjusted	0.021 / 0.013		

Platelets did not associate with bronchiectasis ($n = 779$, est. 6.37, 95% CI -4.54 to 17.28, $p = 0.252$); nor did CRP ($n = 696$, est. 1.93, 95% CI -1.13 to 5.00, $p = 0.216$).

CRP data was very heavily skewed (Figure 4.16d), and therefore models were adjusted to use a Yeo-Johnson transformation and a Tobit model, which adjusts for censored results such as the result of “<5 mg/dl” in clinical laboratories reporting of CRP. In this model, CRP did not associate with bronchiectasis ($n = 696$, estimate 0.09, 95% CI -0.21 to 0.40 [back transformed estimate 0.094], $p = 0.551$).

4.3.4.5 Eosinophils, platelets, and clinical outcomes in AATD

Since eosinophils and platelets were obtained from the same full blood count data, they were analysed in the same models.

Baseline FEV_{1pp} was estimated to be 0.07% lower for each platelet x10⁹/L (i.e. 7% lower per 100 platelets x10⁹/L), though was not associated with eosinophil count (Table 4.13).

Table 4.13: Platelets are associated with lower baseline FEV₁.

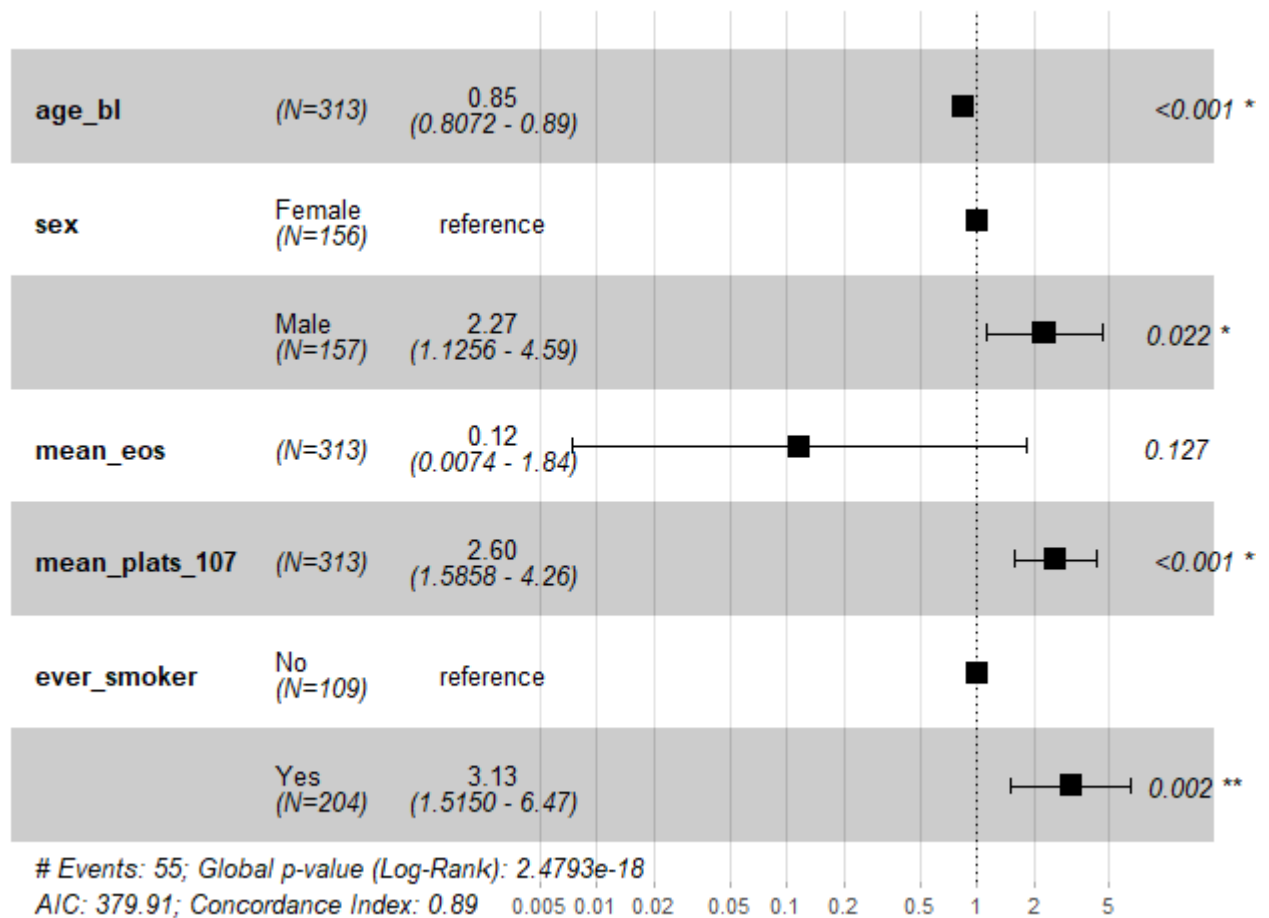
Associations	Baseline FEV ₁ % predicted		
	Estimates	CI	p
Mean Eosinophil count x10 ⁹ /L	2.25	-11.00 – 15.51	0.739
Mean Platelet count x10 ⁹ /L	-0.07	-0.09 – -0.05	<0.001
Age (years)	-0.05	-0.19 – 0.09	0.491
Sex (Male)	-8.40	-11.59 – -5.20	<0.001
Ever smoker	-21.05	-24.54 – -17.56	<0.001
COPD	-34.59	-38.88 – -30.29	<0.001
Asthma	3.85	-0.73 – 8.43	0.099
Observations	745		
R ² /R ² adjusted	0.521 / 0.516		

Eosinophil count was associated with higher exacerbation rates in those who exacerbate, (n = 171, count IRR 2.09, 95% CI 1.04 to 4.21, p = 0.038; zero-inflated IRR 0.5, 95% CI 0.04 to 6.01, p = 0.588), though platelets did not (n = 171, IRR 1, p = 0.118).

FEV₁ decline, KCO decline, mMRC and mean SGRQ score did not significantly associate with either eosinophils or platelets. Mortality was also unaffected by eosinophils on Cox proportional hazard modelling (HR 0.80 per eosinophilx10¹⁰/L,

95% CI 0.59 to 1.09, $p = 0.158$, but was affected by platelets (HR 2.60 per platelet $\times 10^7/L$, 95% CI 1.59 to 4.26, $p = <0.001$, Figure 4.21).

Figure 4.19: Cox proportional hazards model for full blood count data and mortality in patients with severe AATD. Platelet values are stored as 3 digits $\times 10^9$, and have therefore been transformed to $\times 10^7$ to allow for better comparison with the other variables.



4.3.4.6 Eosinophils, platelets, and clinical outcomes in AATD-bronchiectasis

When limiting the cohort to those with bronchiectasis, platelets did not correlate with lower baseline FEV_{1pp} values (Table 4.14).

Table 4.14: No correlation of eosinophils or platelets with baseline FEV₁ in AATD-bronchiectasis

<i>Associations</i>	Baseline FEV₁pp <i>Estimates</i>	<i>CI</i>	<i>p</i>
Eosinophils (mean, x10 ⁹)	12.07	-11.17 – 35.31	0.307
Platelets (mean, x10 ⁹)	-0.05	-0.10 – 0.01	0.089
Age (years)	0.06	-0.25 – 0.36	0.708
Sex (Male)	-10.74	-17.50 – -3.98	0.002
Ever smoker	-25.18	-32.27 – -18.08	<0.001
COPD	-30.26	-39.74 – -20.77	<0.001
Asthma	-4.70	-15.96 – 6.55	0.410
Observations	143		
R ² / R ² adjusted	0.518 / 0.497		

Eosinophils again appeared to associate with higher exacerbation rates in those who exacerbate, with rate increasing by a factor of 1.18 (after transformation of IRR, i.e. 5.54^{0.1}) for each 0.1 rise in eosinophils, this time with a higher degree of statistical significance, and a 10 units change in platelet count x10⁹ being associated both with a 32% lower chance of being an exacerbator, and a 4% lower exacerbation rate in those who exacerbate (Table 4.15).

The negative correlation with platelets was unexpected, so 2 further models were constructed, whereby exacerbation rates of those with low (<150 x10⁹/L) and high (>300 x10⁹/L) platelets were compared against those with platelets in the normal range. In the first model, low platelets were associated with a 143% increase in annual exacerbation rate in those with a rate ≥1 (Table 4.16). High platelets were not associated with any difference in exacerbation rate (n = 171, zero-inflated IRR 0.20, 95% CI 0.01 to 2.95, p = 0.243; count IRR 0.73, 95% CI 0.42 to 1.26, p = 0.257; COPD not included as a covariate due to high collinearity between COPD and high platelets).

Table 4.15: Associations of full blood count data with exacerbation rate in AATD-bronchiectasis

<i>Associations</i>	Annualised exacerbation rate		
	<i>Incidence Rate Ratios</i>	<i>CI</i>	<i>p</i>
Count Model			
Eosinophils (mean, x10 ⁹)	5.54	1.20 – 25.51	0.028
Platelets (mean, x10 ⁸)	0.96	0.93 – 0.99	0.011
Age (years)	0.98	0.96 – 1.00	0.103
Sex (Male)	0.37	0.25 – 0.56	<0.001
Ever smoker	0.69	0.45 – 1.08	0.108
COPD	2.72	1.41 – 5.26	0.003
Asthma	1.22	0.66 – 2.25	0.524
Zero-Inflated Model			
Eosinophils (mean, x10 ⁹)	0.30	0.00 – 43.83	0.637
Platelets (mean, x10 ⁸)	0.68	0.51 – 0.91	0.010
Age (years)	0.88	0.79 – 0.99	0.034
Sex (Male)	0.00	0.00 – 0.27	0.010
Ever smoker	0.01	0.00 – 0.25	0.007
COPD	1538.30	6.56 – 360546.77	0.008
Asthma	0.40	0.01 – 29.21	0.675
Observations	171		
R ² / R ² adjusted	0.099 / 0.055		

Table 4.16: Association of low platelets with exacerbation rate in AATD-bronchiectasis

Associations	Annualised Exacerbation Rate		
	Incidence Rate Ratios	CI	p
Count Model			
Low platelets (<150 x10 ⁹ /L)	2.43	1.22 – 4.82	0.011
Age (years)	0.98	0.96 – 1.00	0.092
Sex (Male)	0.42	0.28 – 0.61	<0.001
Ever smoker	0.67	0.45 – 1.02	0.060
COPD	2.98	1.70 – 5.24	<0.001
Zero-Inflated Model			
Low platelets (<150 x10 ⁹ /L)	23.13	0.48 – 1121.65	0.113
Age (years)	0.92	0.85 – 1.00	0.058
Sex (Male)	0.04	0.00 – 1.09	0.057
Ever smoker	0.05	0.01 – 0.41	0.005
COPD	155.14	2.74 – 8793.27	0.014
Observations	171		
R ² / R ² adjusted	0.077 / 0.046		

Platelets did not associate with mMRC score, and there was insufficient variation in mMRC score to construct an adequate multinomial model for the association of eosinophils with mMRC score. Again, no association was seen for either eosinophils or platelets in the case of FEV₁ decline, KCO decline, or mean SGRQ. Platelets were not associated with mortality in this subset on Cox proportional hazards models (n = 50, HR 1.14 per cell x10⁷, 95% CI 0.61 to 2.12, p = 0.687).

4.3.4.7 CRP and clinical outcomes in AATD

In the cohort as a whole, CRP was associated with baseline FEV_{1pp} (-0.14, 95% CI -0.23 to -0.05, p = 0.003, Table 4.17), FEV_{1pp} decline (estimate -0.02, 95% CI -0.03 to

-0.01, $p = 0.001$, Table 4.18) and mean SGRQ score (estimate -0.14, 95% CI -0.27 to -0.01, $p = 0.035$, Table 4.19).

Table 4.17: Correlation of mean CRP with baseline FEV₁% predicted values in AATD.

<i>Associations</i>	Baseline FEV₁pp		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>
CRP (mean, mg/L)	-0.14	-0.23 – -0.05	0.003
Age (years)	-0.03	-0.19 – 0.12	0.677
Sex (male)	-5.13	-8.41 – -1.86	0.002
Ever smoker	-21.00	-24.73 – -17.27	<0.001
COPD	-36.39	-41.06 – -31.71	<0.001
Observations	663		
R ² / R ² adjusted	0.493 / 0.489		

Table 4.18: Correlation of CRP with annual FEV₁ decline in AATD.

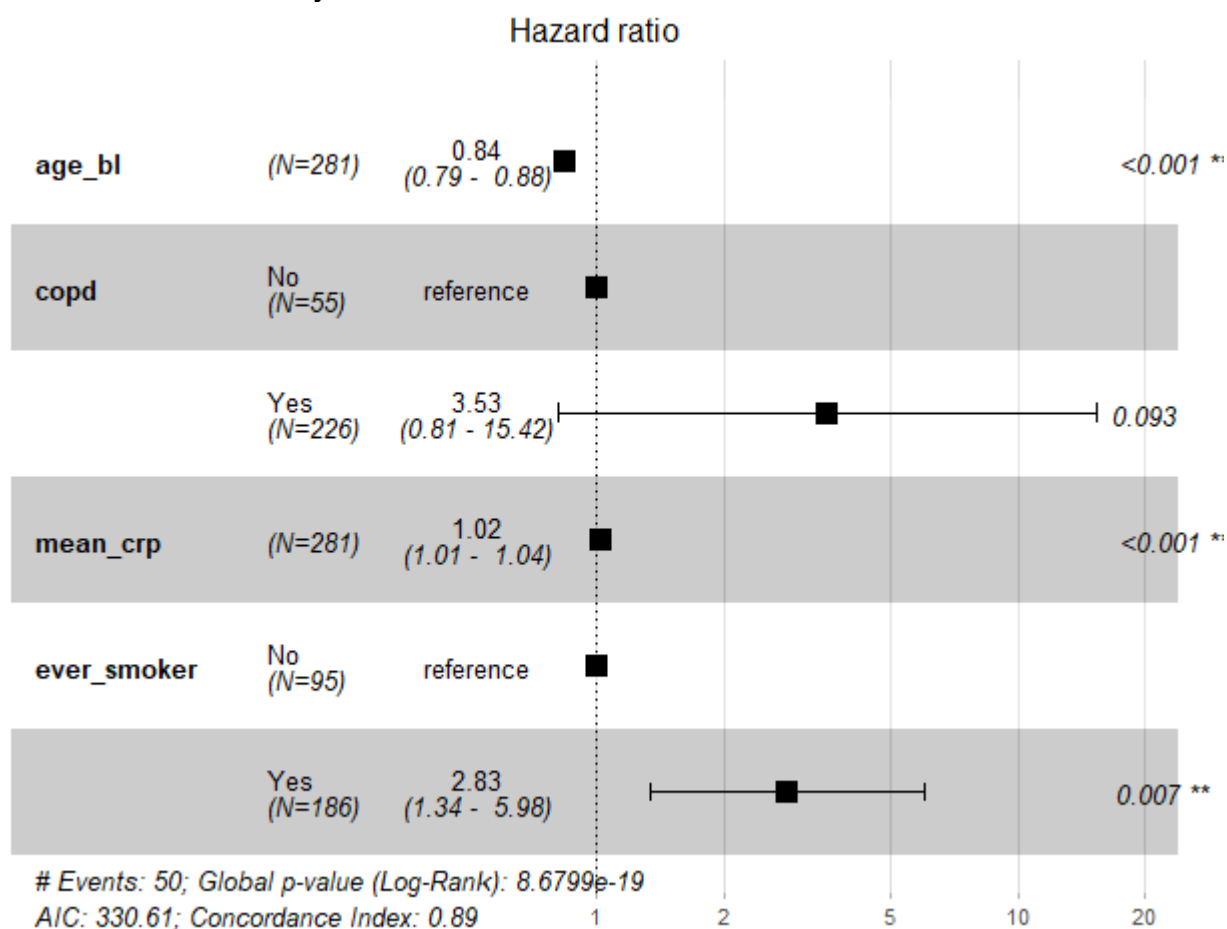
<i>Associations</i>	FEV₁pp annual decline		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>
CRP (mean, mg/L)	-0.02	-0.03 – -0.01	0.001
Age (years)	0.01	-0.01 – 0.02	0.238
Sex (male)	-0.16	-0.43 – 0.10	0.228
Ever smoker	0.28	-0.01 – 0.58	0.062
COPD	-0.30	-0.68 – 0.08	0.118
Observations	598		
R ² / R ² adjusted	0.030 / 0.021		

Table 4.19: Correlation of CRP with mean SGRQ score in AATD.

<i>Associations</i>	mean SGRQ score		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>
CRP (mean, mg/L)	-0.12	-0.24 – -0.01	0.039
Age (years)	0.33	0.08 – 0.58	0.009
Sex (male)	6.78	1.78 – 11.78	0.008
Ever smoker	9.01	3.11 – 14.91	0.003
COPD	-10.58	-17.78 – -3.37	0.004
Observations	289		
R ² / R ² adjusted	0.081 / 0.065		

Mean CRP level was associated with an increased risk of mortality (HR 1.02, 95% CI 1.01 to 1.04, $p < 0.001$, Figure 4.22).

Figure 4.20: Cox proportional hazards model for the association of CRP with mortality in patients with severe AATD. CRP has a small but statistically significant positive effect on mortality.



KCOpp decline and exacerbation rate did not associate with CRP level. CRP increased the odds of mMRC 4, but no other mMRC score (OR 1.04 per CRP mg/L, 95% CI 1.01 to 1.07, $p = 0.005$).

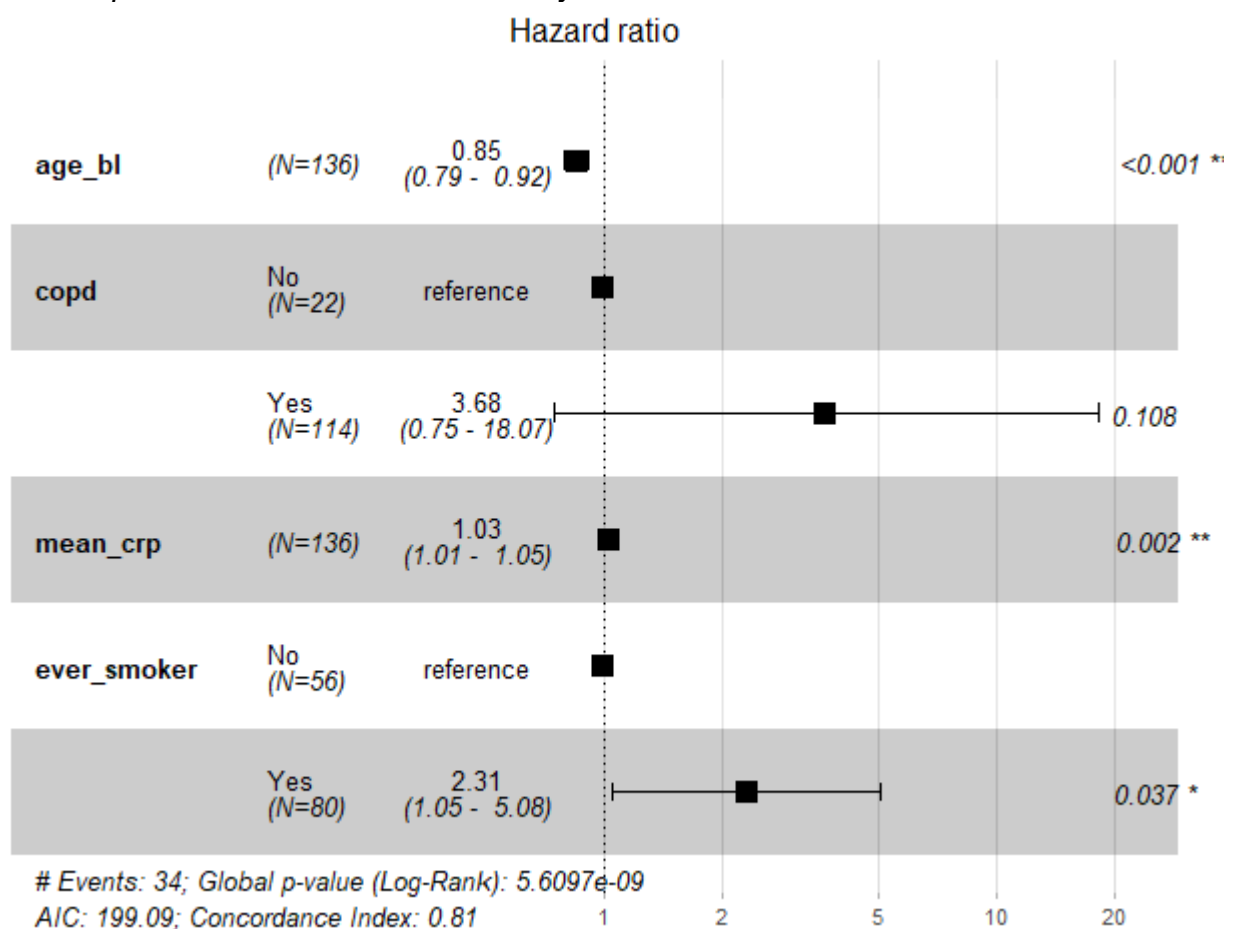
4.3.4.8 CRP and clinical outcomes in AATD-Bronchiectasis

When limited to those with bronchiectasis, many of the above findings did not persist. CRP was not associated with baseline FEV₁pp, FEV₁pp decline, KCOpp decline, or exacerbation rate, though an association with mean SGRQ score was again seen, albeit with a very small effect ($n = 327$, est. -0.16, 95% CI -0.30 to -0.03, $p = 0.021$).

The association between CRP and mMRC score 4 disappeared (n = 698, OR 1.07, 95% CI 0.98 to 1.16, p = 0.111).

The relationship of CRP to mortality persisted in those with bronchiectasis (HR 1.03, 95% CI 1.01 to 1.05, p = 0.002) on a backward-fit Cox proportional hazards model (Figure 4.23).

Figure 4.21: Cox proportional hazards model for the association of CRP with mortality in patients with AATD and bronchiectasis. CRP has a small but statistically significant positive correlation with mortality.



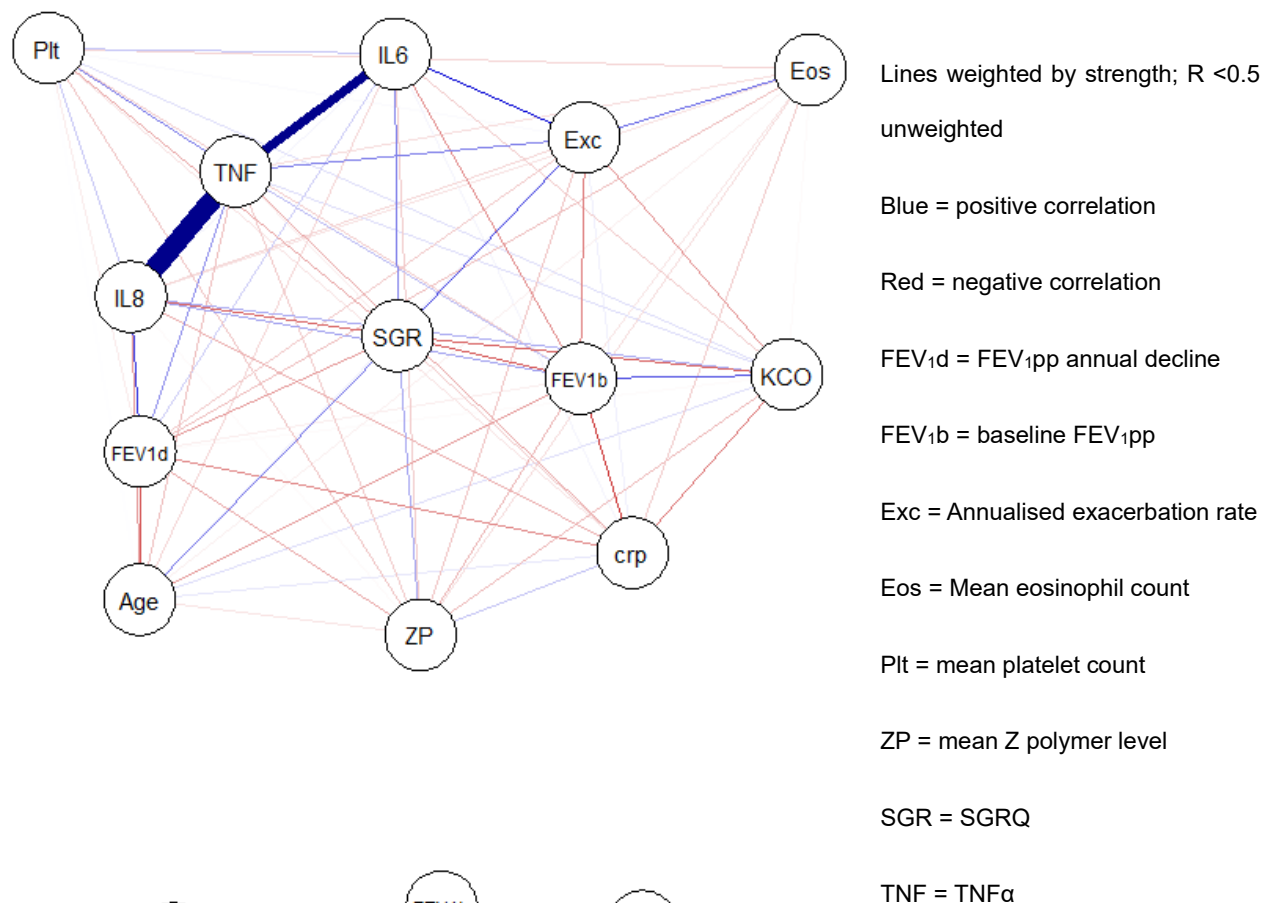
4.3.4.9 Network analysis

Matrix analysis of biomarkers with clinical features in the whole cohort revealed correlations ranging from -0.38 to 0.73. The only correlation coefficients > 0.5 or < -0.5 were for TNF alpha correlating positively with both IL-6 and IL-8. However, in matrix

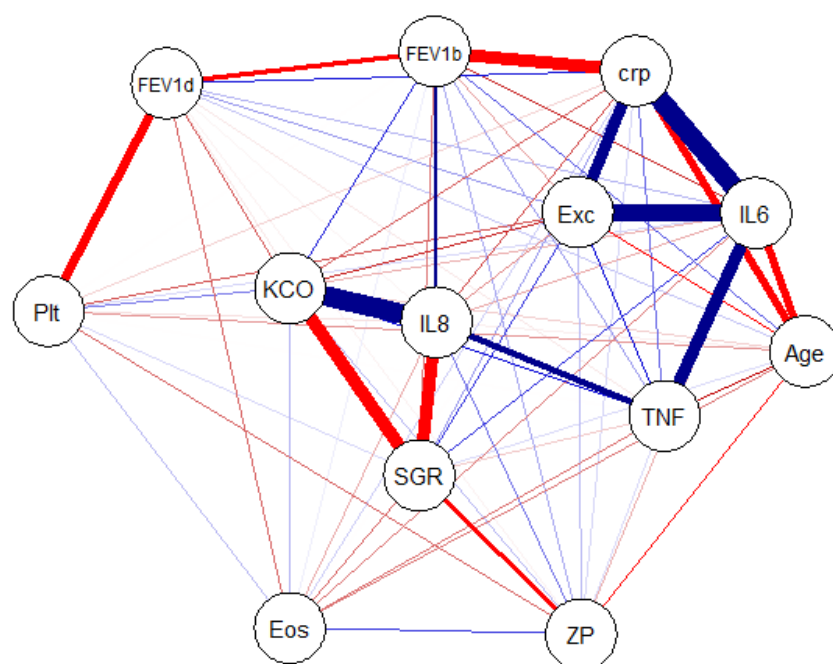
analysis limited to bronchiectasis patients, several correlations >0.5 or < -0.5 were seen (Figures 24a and b, and Appendix section 5).

Figure 4.22a and 24b: String network diagrams demonstrating interactions between selected continuous variables in patients with AATD (4.24a) and patients with AATD and bronchiectasis (4.24b).

4.24a



4.24b



4.3.4.10 $TNF\alpha$, Interleukin-6 and -8, and clinical outcomes

$TNF\alpha$, IL-6 and IL-8 values were available for 320 patients. $TNF\alpha$, IL-6 and IL-8 values did not differ between those with bronchiectasis and those without (Table 4.20).

Table 4.20: No difference in $TNF\alpha$, IL-6 and IL-8 with bronchiectasis diagnosis. $p = T$ -test.

	Bronchiectasis	No Bronchiectasis	p
$TNF\alpha$	1.97 +/- 2.19	1.35 +/- 1.60	0.16
IL-6	1.13 +/- 2.36	0.51 +/- 0.41	0.15
IL-8	3.17 +/-5.88	2.00 +/- 4.33	0.32

In the cohort as a whole, relationships between $TNF\alpha$, IL-6, IL-8 and clinical outcomes were not examined further with statistical models as no strong relationships were found in matrix analyses (Figure 22a). When limited to bronchiectasis only (and severe genotypes), the following statistical models were constructed.

There was no relationship between annualised exacerbation rate and IL8 ($n = 51$, zero-inflated IRR 0.58, $p = 0.607$; count IRR 0.95, $p = 0.263$), although the correlation of IL-6 with exacerbation rate in those who exacerbate did cross the statistical significance threshold ($n = 51$, IL6 zero-inflated IRR 0.12, $p = 0.711$; count IRR 1.1, $p = 0.159$).

KCOpp yearly change was associated with IL-8, with a small magnitude positive association ($n = 89$, estimate +0.12, 95% CI 0.06 to 0.18, $p < 0.001$, figure 24b), and with $TNF\alpha$ ($n = 89$, est. +0.18, 95% CI 0.01 to 0.35, $p = 0.043$), though the IL-8 model was disproportionally affected by 3 outliers (Figures 4.25). Mean SGRQ score associated with IL-8, with a small magnitude association, and only 27 patients could

be included in the model ($n = 27$, est. -1.79 , 95% CCI -2.72 to -0.87 , $p < 0.001$, Figure 4.26).

Figure 4.24: Correlation of IL-8 with KCOpp decline in patients with severe AATD

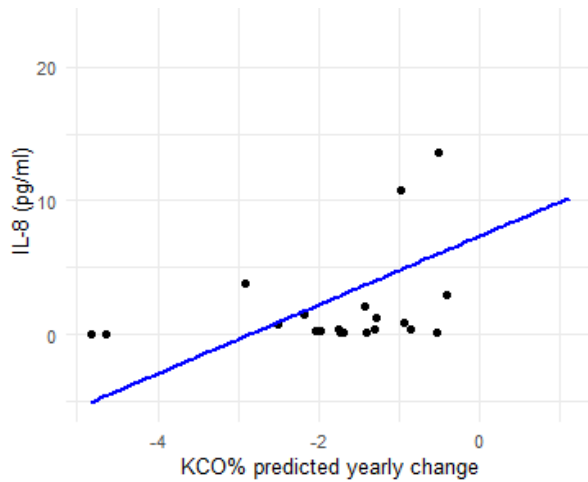
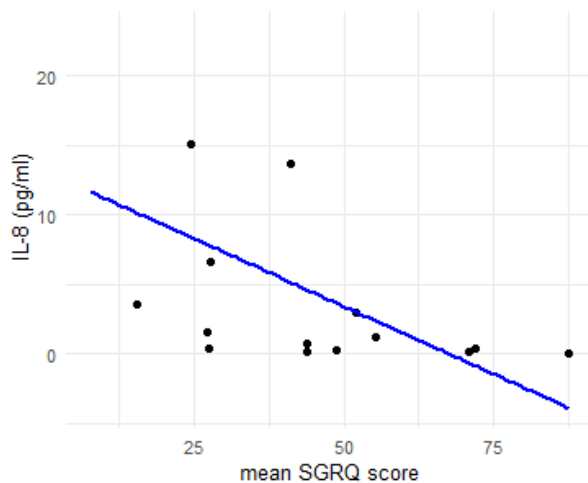


Figure 4.23: Correlation of IL-8 with mean SGRQ score in patients with severe AATD



4.4 Discussion

4.4.1 Prevalence of Bronchiectasis in AATD

Prevalence of bronchiectasis in our PiZZ cohort, at 20.9%, is lower than in a recently published summary of the EARCO database, which found a prevalence of 36.1%³⁵⁵. However, that study used a radiological definition of bronchiectasis, whereas the

present work has used a clinical code, which will likely underestimate the prevalence of radiological bronchiectasis, as demonstrated in our incidental finding of bronchiectasis in 7.5% of those not previously known to have it. Notably, radiological bronchiectasis of any cause does not necessarily bear clinical relevance unless subcategorised further¹⁸⁸, which is why we chose to look at clinical diagnosis as our primary means of assessment. The lack of associations between bronchiectasis and lung function decline or exacerbation rate in our cohort is explained by ours being a tertiary cohort of a much rarer disease, with a proportionally lower threshold for CT scanning and consequently more incidental findings of mild bronchiectasis.

4.4.2 Evidence for an independent relationship between bronchiectasis and AATD

In 2012, 211000 of an estimated 51.7 million adults in the UK had bronchiectasis, a prevalence of 0.4% ^{356,357}. This would confirm that prevalence of bronchiectasis in our cohort is much higher than the general population, but this figure alone does not necessarily support an independent association of bronchiectasis with AATD: it is consistent with some estimates of the prevalence of bronchiectasis in usual COPD ³⁵⁸, though such estimates vary widely, from 0.03% ²⁵⁴ to 57.6% ²⁵⁵. We therefore studied bronchiectasis in those without a diagnosis of COPD; the prevalence of bronchiectasis of 18.6% in this group is also much higher than in the general population, even after removing those with other clear causes, which supports an independent association of AATD with bronchiectasis. Further analysis was therefore warranted.

In our analysis of the associations of bronchiectasis diagnosis, we found that bronchiectasis occurs independently of COPD in our PiZZ patients, instead being

related to age at baseline, smoking history, and inversely related to serum AAT levels, which raises suspicion of a direct relationship between AATD and bronchiectasis. However, Low R^2 values in our models suggested these variables only explained a small part of the variance in the dataset. Differences in age may be explained by the fact that bronchiectasis has a slow onset, and therefore those presenting to clinic later can be expected to have more advanced disease. The difference in smoking history would appear counter-intuitive, but the analysis of EARCO found a similar pattern³⁵⁵, and there is a well-established poorer prognosis of smokers in those with AATD^{47,158}.

4.4.3 Relationship of unclassified bronchiectasis and clinical outcomes

Bronchiectasis in AATD patients was not definitively proven to be related to lung function decline or exacerbation rate in this analysis. To our knowledge, no other studies have examined the effect of bronchiectasis on exacerbations in AATD cohorts. Like our study, the recent analysis of bronchiectasis in the EARCO database found no link with lung function, although these were static baseline measurements rather than the temporal changes assessed in the present study³⁵⁵. A Portuguese team recently studied temporal lung function decline and also found no association with bronchiectasis diagnosis, although their study was limited to 43 patients³⁵⁹. However, they found their patients with bronchiectasis to have more severe breathlessness as measured by mMRC score, findings replicated in our study. More severe symptoms, along with the non-association of bronchiectasis with COPD in our cohort, suggest the diagnosis of bronchiectasis in AATD may represent a clinically relevant phenotype, and warrants specific assessment. Whilst the finding of exacerbation rate being no higher in bronchiectasis patients was unexpected, there are several possible reasons this may have occurred, including mild disease, recall bias when reporting events at annual reviews, and different triggers to exacerbation. Consistent with the latter

interpretation, preliminary microbiology data from the cohort shows that exacerbation events are not associated with expansion of prior colonising organisms³⁶⁰. Further research into characteristics of AATD exacerbations was therefore warranted.

4.4.4 Limitations of the analyses

One limitation of this study is its use of a historic database. Whilst a significant proportion of COPD and bronchiectasis diagnoses have been assessed for accuracy, with low levels of errors, not all patients had spirometry and CT scans available for direct analysis. Ongoing data collection for our database now ensures spirometry is recorded, and CT scans are transferred from secondary care centres in digital format.

Milder disease is unlikely to have an impact on disease progression unless measured over very long time periods or with larger sample sizes, since in other causes of bronchiectasis only more severe dilatation, morphology or more widespread disease have been observed to impact disease progression^{180,187,188}. In smaller sample sizes or shorter durations of follow-up, this will therefore reduce statistical power. Both these limitations are often present in research in AATD, due to its nature as a rare and slowly progressive disease.

Our cohort is one of the largest in Europe, which increases generalisability of our results, but despite this it only contains 239 bronchiectasis patients, not all of whom met our criteria for lung function decline assessment, leading to lower statistical power of regression analysis. However, within those who did have longitudinal data available many were seen for over 5 years, as shown by the distribution of follow up times, suggesting we should have captured slow progression. Finally, we did not have a contemporaneous, PiMM database for comparison of all characteristics in our study. This may be the subject of future research.

4.4.5 Clinical implications of unclassified bronchiectasis in AATD

Bronchiectasis is present in a significant minority of PiZZ AATD patients, independently of concurrent COPD diagnosis. These patients are more likely to have more severe shortness of breath. Appropriate management of bronchiectasis in AATD is therefore essential. Without subcategorisation, bronchiectasis does not predict lung function decline or exacerbation rate, and further study of the radiology, microbiology and blood biomarkers within AATD-bronchiectasis was conducted, as described in this and following chapters.

4.4.6 Sputum sampling in AATD

Obtaining new samples was a challenging process. Patients in AATD are scattered around the country, and aside from their annual clinic reviews, communication is reliant on telephone and post. Patients may not respond to these communication methods, or their production of sputum may not be reliable enough to send in a sample. These methods also introduce selection bias – producing and sending in a sample requires organisation and mobility, which may filter out the most unwell or socially isolated. Using the same registry in 2012, Stone et al could only recruit 12 patients to send in 9 repeat samples³⁶¹, and other studies of sputum in AATD have recruited similarly low numbers³⁶². The largest primary study in the evidence base for the treatment of chronic infection in cystic fibrosis includes 520 patients, but this is a far commoner genetic pulmonary disease, and the study was coordinated across 69 cystic fibrosis centres in the US³⁶³. Considering this, the work presented here collects a large amount of data. Broncho-alveolar lavage could theoretically address the limitation of insufficient sputum production by patients, but this comes with ethical considerations,

as bronchoscopy is an invasive procedure requiring sedation and (brief) hospital admission, which may not be appropriate for research purposes only. Nasal lavage has also been suggested as a substitute for sputum in other respiratory disorders^{364,365}, however there is no direct evidence to support its correlation with sputum microbiology in bronchiectasis or COPD, and it can be uncomfortable for patients.

4.4.7 Sputum colour

Sputum colour was not associated with any clinical outcome. This contrasts with a study of usual bronchiectasis patients in the EMBARC registry which used a very large number (>13000) of patients to find a correlation with FEV₁% predicted, exacerbations, and quality of life³⁶⁶. This discrepancy is unexpected, since sputum colour has been found to correlate with various granulocyte-secreted enzymes, among them neutrophil elastase, the activity of which is greatly increased by AATD³⁶⁷. The relationship of colour with clinical outcomes in COPD is less clear: although Anthonisen's original study of COPD exacerbations found an enhanced treatment effect in those with purulent sputum, the definition of "purulent" was not standardised by colour²⁷. Two more recent studies have disagreed on whether sputum colour is correlated with bacterial load in COPD^{368,369}. The co-existence of COPD in the majority of patients in this work may therefore account for the lack of a clear relationship between clinical outcomes and sputum colour. The clinical implication of this finding is that sputum colour may not be so reliable a marker of clinical outcomes in AATD-bronchiectasis as it appears to be in other bronchiectasis aetiologies.

4.4.8 Bacterial load

Bacterial load associated with increased exacerbation rates in those who are known to exacerbate, which is consistent with findings in all-cause bronchiectasis associating stable-state bacterial load with exacerbation rate²³⁶. It did not associate with FEV₁ decline, unlike in one study of usual-COPD³⁷⁰; however, the main driver of FEV₁ decline in AATD is likely to be emphysema, unlike in usual-COPD, where a greater proportion also have chronic bronchitis, which is likely to be more influenced by bacterial load than is emphysema.

One study has also found stable bacterial load to be associated with poorer quality of life, and predictive of better response to inhaled antibiotics³⁷¹. However, in the present work, the correlation with mean SGRQ score was very small, only just crossed statistical significance, and did not occur when limiting the analysis to those with bronchiectasis. In context of the multiple analyses conducted in this work, this is likely to be a chance finding.

4.4.9 Species

Species analysis has one major limitation, which is the definition of “colonisation”. Colonisation in bronchiectasis is usually defined as 2 or more positive sputum samples separated by 3 months in time. An attempt has been made here to replicate this criteria, but most patients approached for a new sputum sample did not supply more than one sample. Historical data was merged to help mitigate this difficulty, but the analysis would have more robust had every patient supplied more than one sample. Furthermore, it is entirely possible that many patients had further sputum samples with their primary or secondary care services, as opposed to the tertiary care database used here, and therefore many false negatives will be present in the database whereby

patients who are not recorded as having been colonised with an organism may in fact have been, but this information is with local services. Collection of all this historic data from a multitude of secondary care services throughout the country is impractical. Accordingly, single-sample growth was used instead of colonisation to increase sample sizes, but this may introduce bias from contaminants or exacerbations erroneously labelled as “stable”. Nonetheless, many biologically plausible results were found in this work.

4.4.9.1 *Pseudomonas*

Frequency of pathogen identification broadly followed findings in all-cause bronchiectasis⁹⁷. *Pseudomonas* growth was more common in those with bronchiectasis, and was also associated with a higher exacerbation rate and a small difference in KCO annual decline, although colonisation was not associated with either of these two outcomes, most likely due to the low numbers of *Pseudomonas* colonisation seen. *Pseudomonas* has long been known to be associated with increased exacerbation rate in both bronchiectasis and COPD^{188,332}, so this finding is not unexpected. There is also a known relationship with mortality^{188,330} which was not observed here, though mortality data was only available for a minority of patients. A relationship between *Pseudomonas* growth and colonisation has also been suggested, but the evidence base is so far inconclusive⁹⁷, and so the negative finding here is also not unexpected. A non-significant relationship with quality of life seen in this work is also consistent with (statistically significant) results in other causes of bronchiectasis³³².

4.4.9.2 Other pathogens

The association of other-pathogen growth with a marginally improved FEV₁ decline can be dismissed as an artefact of the many statistical models conducted in this work, considering statistical significance was not overwhelmingly high. However, in bronchiectasis patients, the growth of other pathogens was associated with mortality in the small number who had mortality data available: this is consistent with the findings of the bronchiectasis severity index group¹⁸⁸. However, *Haemophilus influenzae* growth counter-intuitively seemed to associate with a lower rate of exacerbations. This has no plausible aetiology, but the effect of *H. influenzae* on clinical outcomes in bronchiectasis or COPD is unclear, with some studies suggesting a relationship to mild exacerbations³⁷², and a relationship between *H. influenzae* and other inflammatory markers³⁷³, but otherwise no clear evidence that colonisation, rather than infection, by *H. influenzae* is associated with poorer clinical outcomes. Alternatively, this finding may reflect the fact that sputum sample testing automatically selects the most engaged patients, who may have fewer exacerbations due to better engagement with exacerbation-avoiding behaviours (vaccination, hand hygiene, etc.), and *H. influenzae* growth in fact has no effect on exacerbations. As such, the finding in this work is probably artefactual, and the lack of a negative association is not at odds with the literature.

Since samples can only be obtained from those who produce sputum, it remains unknown whether the presence of respiratory pathogens in the lungs of those without phlegm production bears clinical relevance. As mentioned above, the use of sputum surrogates could mean all patients could be tested regardless of phlegm production, but this would only be of use if these are found to correlate with sputum. Furthermore, pathogens grown in traditional culture methods, such as those used here, do not fully

represent all organisms present within the lungs. Advances in DNA sequencing have allowed study of the entire microbiome, and it is known that the distribution of other airway microorganisms, including non-pathogens, influences the behaviour of the dominant pathogens, and influences bronchiectasis severity³⁷⁴, exacerbation rate and mortality¹⁷¹. Similar clinical relevance has been shown in COPD³⁷⁵, but to date no data on the microbiome in AATD has been published. Such study could delineate subtypes of AATD who may benefit from emerging treatments.

Overall, these microbiology findings suggest, as is already known in other respiratory diseases, that *Pseudomonas* is the most clinically relevant pathogen in AATD and AATD-bronchiectasis. Furthermore, quantitative culture also provides useful information, which highlights the importance of prompt sputum sample analysis. Outstanding questions for future work includes whether microbiome differences among patients with AATD have clinical relevance, and whether surrogates for sputum availability may be available.

4.4.10 Biomarkers

The case for a specific endotype underlying AATD-bronchiectasis is not strengthened by this data. Although eosinophils were slightly higher in those with bronchiectasis, the relationship was not strong, and therefore unlikely to be clinically useful. Other biomarkers did not differ between the two groups.

4.4.10.1 Z-polymers

Z polymer measurements in this work were highly variable, and varied even among samples taken from the same patients. As expected, they were higher in the PiZZ phenotype than the PiSZ phenotype, but were not associated with any other baseline characteristic, unlike in Tan et al's work, which found higher levels in COPD and male

patients, although these relationships were not overwhelming strong ($p = 0.02$ in both cases) and their study only took a single measurement of Z polymer from each patient. The high variability of Z polymer levels probably accounts for this discrepancy. However, like the present work, they also found no association with smoking.

Z polymers were not higher in those with bronchiectasis than those without. A difference might have been expected, since bronchiectasis constitutes a distinct phenotype of inflammatory lung damage, i.e. airway damage rather than alveolar or interstitial lung disease, and Z polymers are known to be produced in bronchial epithelial cells (rather than alveolar)³⁷⁶. However, local production of Z polymers could simply affect both airway tissue and alveolar tissue equally.

Z polymer variability was also found not be associated with exacerbation rate, and variability remained high even in those who never exacerbate. Such a relationship could reasonably have been expected, as Z polymers have been shown to be pro-inflammatory in vitro¹⁰¹ and in murine lung tissue¹⁰². However, since Z polymers also form in the lungs³⁷⁶, serum measurements may not reflect levels in the alveoli. Thus, a more effective way of establishing a link between Z polymerisation and exacerbation rate would be to correlate an exacerbation diary with Z polymer measurement, take measurements during exacerbations, and to assess Z polymer levels in sputum rather than serum. These are promising areas for future work which may lead to clinical utility of Z polymer levels.

4.4.10.2 Utility of full blood count data in AATD-bronchiectasis

Eosinophil counts also showed considerable intra-patient variability. An association of mean eosinophils with higher exacerbation rates is to be expected, as has been found in all-cause bronchiectasis^{175,232}. Since only a minority of the patients here had

bronchiectasis without COPD, patients with eosinophilia in the clinic are likely to already be prescribed inhaled corticosteroids, but since COPD was one of the covariates accounted for in the analyses, this finding still suggests eosinophils are an important risk factor for exacerbation rate in AATD-bronchiectasis. Inhaled steroids are the usual treatment strategy for eosinophilic lung diseases, however, the weight of evidence suggests inhaled corticosteroids do not reduce exacerbation rate in bronchiectasis without co-existent COPD, asthma or ABPA, and therefore this finding should not change clinical practice²⁴⁴.

Platelets were associated with mortality in the whole cohort, a finding shown in all-cause bronchiectasis²³⁴; but in the present work this effect disappeared when limited to bronchiectasis only. In usual-COPD, high platelets have been associated with mortality, with a suggested aetiology of increased inflammation and higher rates of cardiovascular comorbidity³⁷⁷. Cardiovascular comorbidities are present in AATD-COPD, but are lower than in controls³⁷⁸.

Higher platelets were associated with a lower baseline FEV_{1pp} in the whole cohort, but not when limited to bronchiectasis, which matches consistent findings in the literature of higher platelets in the usual-COPD population compared to healthy controls^{379,380}.

Platelets were inversely correlated with exacerbation status and rate, which is contrary to findings in all-cause bronchiectasis²³⁴. Further analysis found an association of abnormally low platelets with higher exacerbation rates. This is consistent with findings of thrombocytopenia predicting poorer prognosis in acute exacerbation^{381,382}, but not with one study which found a two-tailed effect of platelet count on mortality, but not exacerbation rate³⁷⁷. Platelets are known to secrete chemotaxins which increase

neutrophil migration³⁸³, and it may be that a pathologically low platelet count results in an insufficient immune response, and could therefore result in more frequent symptomatic infective exacerbations. Interestingly, it has been hypothesised that the lung may be a major centre of platelet *formation*³⁸⁴. The effect of protease activity on this process in AATD has not been published, and could be an area of future research. Alternatively, any correlation found here may be a false positive. Therefore, clinical management may not be changed by the above findings, but thrombocytopenia should not be ignored where found.

4.4.10.3 CRP

The CRP data used here has limitations. The excess of 0 values, related to the inability of clinical laboratories to report values <5 mg/dl, results in an excessive positive distribution. Nonetheless, the associations of CRP with poorer baseline FEV₁, FEV₁ decline, mean SGRQ, and a small increase in mortality, the latter 2 of which were also present in the bronchiectasis-only group, fit with the knowledge that increased inflammation in bronchiectasis and COPD is associated with poorer outcomes^{385,386}. So too does the association of IL-8 with increased symptoms. However, CRP has also previously been linked with increased severe exacerbations in bronchiectasis, which was not seen here²³⁷. The positive associations of KCO change with TNF α and IL-8 are likely to be chance findings due to outliers. The relationship between CRP and mortality found here suggests that stable-state CRP could be of value in routine blood testing, particularly since its wide intra-patient variation means an average of several measurements would be more valuable than a single measurement.

A major weakness of the network analysis is its pooling of data from different sources including routine clinical blood data, and biomarker panels for research purposes. Each of these had different inclusion criteria. Future biomarker research will be

improved by analysing the same biomarkers in a larger pool of patients, allowing more complex network analyses to be undertaken, and to confirm or refute the findings of the inflammatory biomarkers above. Of particular interest will be whether the CRP findings persist when values less than 5mg/dl are accurately reported.

4.5 Conclusions

Overall, this chapter has shown that bronchiectasis exists in AATD independently of COPD diagnosis, and that *Pseudomonas* and CRP may be the most clinically useful ways of identifying those who are likely to have more severe disease. However, since unclassified bronchiectasis was not associated with any changes in most clinical outcomes, it remains to be seen whether radiological subclassification may identify further groups of patients with clinical differences. This work is presented in the next chapter.

5 The radiological classification of bronchiectasis in alpha-1 antitrypsin deficiency

The data in section 5.1 was published in the following papers/abstracts:

1. J De Soyza, P Ellis, M Newnham, L Rickard, AM Turner. *Bronchiectasis Occurs Independently of Chronic Obstructive Pulmonary Disease in Alpha-1 Antitrypsin Deficiency. Chronic Obstr Pulm Dis. 2024 Sep 27;11(5):507-514. doi: 10.15326/jcopdf.2024.0526. PMID: 39213377.*
2. J De Soyza, P Ellis, M Newnham, L Rickard, AM Turner. *Bronchiectasis vs clinical outcomes in Alpha-1 Antitrypsin Deficiency. (Oral presentation at BTS winter 2024)*

5.1 Visual assessment of CT bronchiectasis

5.1.1 Summary statistics

193 CT scans were available from the 256 patients with bronchiectasis in the database. 142 of these were available from digital NHS systems (PACS), and 51 were available from CD-ROM storage. A further 97 CT scans were obtained from those without a prior diagnosis of bronchiectasis. Baseline characteristics of the entire group are shown in Table 5.1.

Table 5.1: Demographic characteristics of AATD patients with CT scans assessed for bronchiectasis.

Characteristic	Overall
<i>n</i>	290
Baseline age (years)	53.33 (+/- 10.57)
Sex (Male, %)	151 (52.1)
Genotype: (%)	
ZZ	259 (89.3)
SZ	21 (7.2)
Other severe	9 (3.1)
Other non-severe	2 (0.7)
Serum AAT (µm)	4.88 (3.01)
Ever smoker (%)	195 (67.5)
Pack-year smoking history	13.73 (17.50)
COPD (%)	234 (80.7)
Bronchiectasis (Clinical diagnosis, %)	193 (66.6%)

Summary statistics of the clinical outcomes assessed, as well as data availability, are shown in Table 5.2. Missing data was particularly high in KCO and TLCO yearly decline: whereas most patients in the clinic have yearly nurse-led spirometry, gas transfer assessment is performed less often, as it is not used for monitoring in most cases, and requires a physiologist. Therefore, much fewer patients had the minimum 3 readings necessary to calculate a decline measurement.

Table 5.2: Clinical outcomes in AATD patients with CT scans assessed for bronchiectasis

Clinical outcome	Overall	Missing data (%)
<i>n</i>	290	
Baseline FEV ₁ pp	58.59 (+/- 28.08)	6.6
FEV ₁ pp annual decline	-0.91 (+/- 1.96)	30.0
FEV ₁ ml yearly decline	-46.65 (+/- 61.73)	30.0
KCOpp yearly decline	-1.68 (+/- 2.07)	50.3
TLCOpp yearly decline	-2.10 (+/- 2.12)	50.7
Annualised exacerbation rate	1.01 (+/- 1.33)	0.0
Mode mMRC score (%)		1.0
0	47 (16.4)	
1	69 (24.0)	
2	67 (23.3)	
3	74 (25.8)	
4	30 (10.5)	
SGRQ score (mean (SD))	43.39 (22.63)	49.3

Lobar involvement, bronchial dilatation scores, bronchial wall thickening scores and morphology are shown in Figures 5.1, 5.2, and Table 5.3. Among the 97 patients without a prior clinical diagnosis of bronchiectasis, 23 had radiological evidence, including 7 with 3 or more lobes affected. Bronchiectasis was overall mild, and of a predominantly lower lobe distribution. An example image is shown in Figure 5.3.

Figure 5.1: Lobar involvement of bronchiectasis in AATD patients with and without a previous clinical bronchiectasis diagnosis. The majority of both groups have less extensive disease.

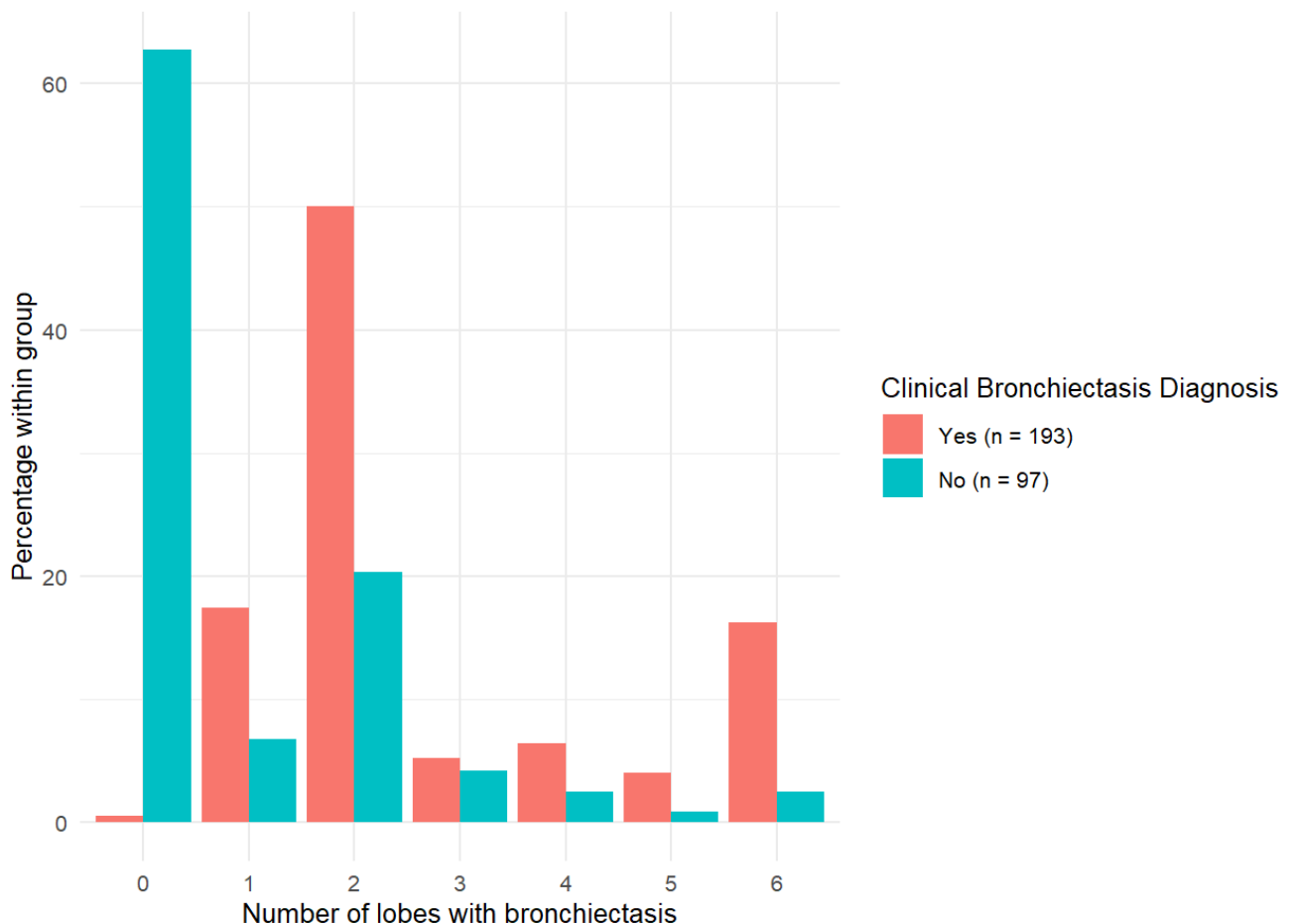


Figure 5.2: Lobar distribution of radiological bronchiectasis is predominantly lower lobe in AATD patients with and without a prior clinical diagnosis of bronchiectasis.

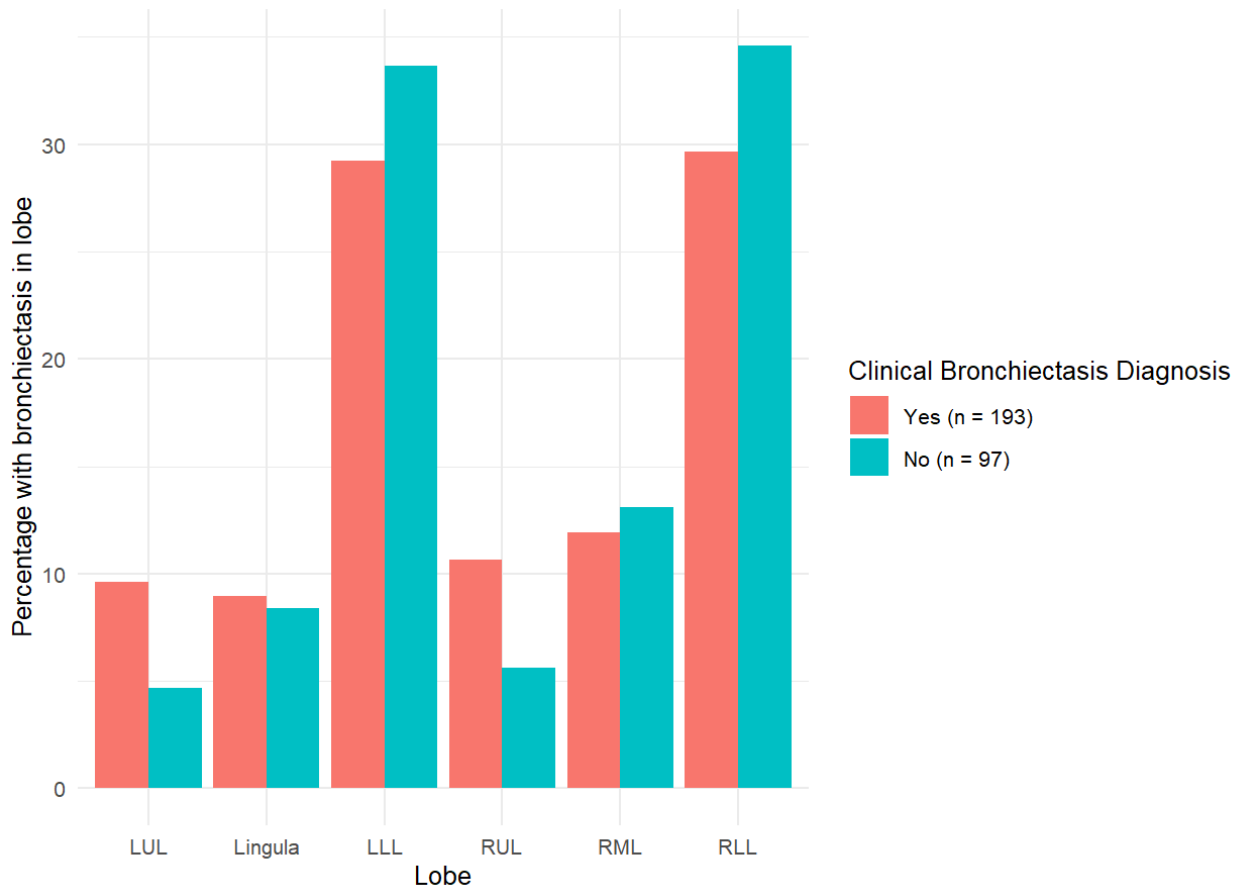
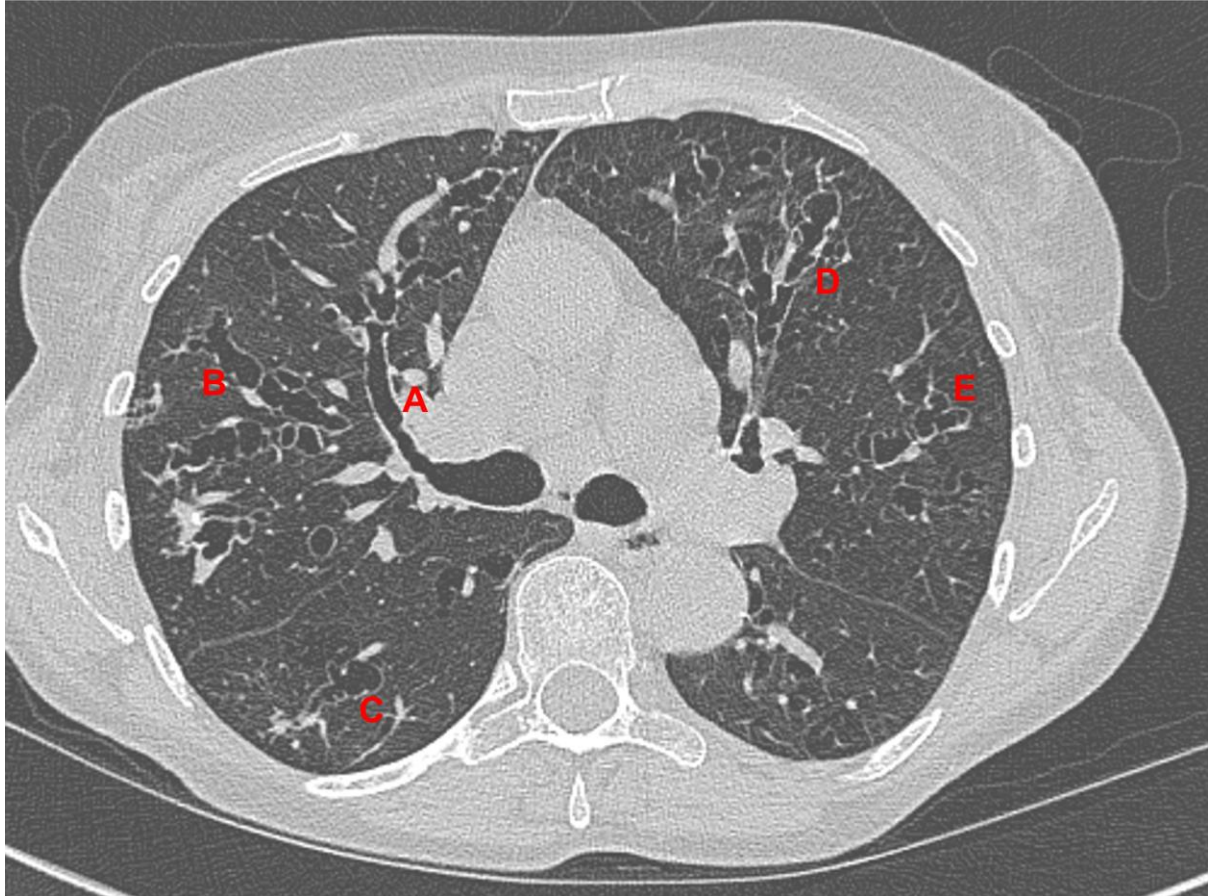


Table 5.3: CT features of AATD patients with and without a clinical diagnosis of bronchiectasis

	Clinical Bronchiectasis diagnosis	No clinical bronchiectasis diagnosis
n	193	97
Left upper lobe (%)	48 (24.9)	3 (3.1)
Lingula (%)	47 (24.4)	5 (5.2)
Left lower lobe (%)	161 (83.4)	15 (15.5)
Right upper lobe (%)	54 (28.0)	3 (3.1)
Right middle lobe (%)	63 (32.6)	8 (8.2)
Right lower lobe (%)	162 (83.9)	17 (17.5)
Lobar involvement (n) (%):		
0	0 (0.0)	74 (76.3)
1	31 (16.1)	8 (8.2)
2	102 (52.8)	8 (8.2)
3	10 (5.2)	4 (4.1)
4	13 (6.7)	1 (1.0)
5	7 (3.6)	1 (1.0)
6	30 (15.5)	1 (1.0)
Bronchial dilatation score (%):		
0	0 (0.0)	74 (76.3)
1	135 (69.9)	16 (16.5)
2	35 (18.1)	7 (7.2)
3	23 (11.9)	0 (0.0)

	Clinical Bronchiectasis diagnosis	No clinical bronchiectasis diagnosis
Bronchial wall thickness score (%):		
0	5 (2.6)	71 (73.2)
1	133 (68.9)	20 (20.6)
2	39 (20.2)	1 (1.0)
3	16 (8.3)	5 (5.2)
Morphology (%):		
No bronchiectasis	0 (0.0)	74 (76.3)
Cylindrical only	146 (75.6)	19 (19.6)
Varicose or cylindrical	32 (16.6)	4 (4.1)
Cystic	15 (7.8)	0 (0.0)

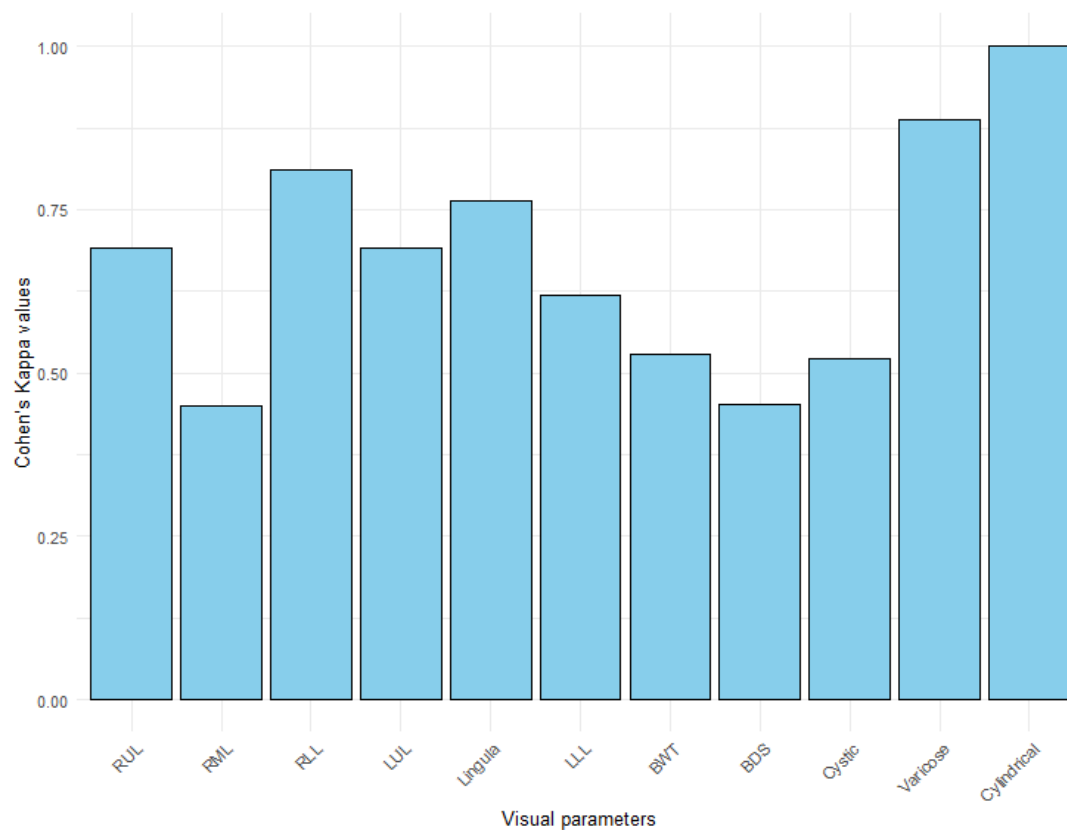
Figure 5.3: Example CT image exhibiting:
A: cylindrical bronchiectasis morphology
B: varicose bronchiectasis morphology
C: cystic bronchiectasis morphology
D: bronchial dilatation score = 2
E: wall thickness score = 1



5.1.2 Inter-rater reliability

Inter-rater reliability was at least moderate, and strong in the cases of presence of varicose and cylindrical (Figure 5.4). Mean Cohen's κ for all parameters was 0.64.

Figure 5.4: Inter-rater reliability for CT bronchiectasis features.



5.1.3 Exclusion of patients with alternative bronchiectasis causes

Only 22 (11.3%) patients with bronchiectasis had an acceptable documentation of exclusion of other bronchiectasis causes, as defined by the BTS guidelines for bronchiectasis investigation⁹⁷. Four had documented alternative explanations for their bronchiectasis: one had allergic broncho-pulmonary aspergillosis, 1 had traction bronchiectasis secondary to interstitial lung disease, and 2 had previous pertussis infection. The remaining patients were included in the statistical models. These patients' CT characteristics are shown in Table 5.4.

Table 5.4: Patients with alternative causes of bronchiectasis.

Cause	Genotype	n lobes	BWT	BDS	Morphology
ABPA	ZZ	2	1	1	1
Traction	ZZ	2	1	1	1
Pertussis 1	ZZ	6	2	2	1
Pertussis 2	ZZ	5	1	2	2

5.1.4 Lobar involvement and clinical outcomes

More extensive lobar involvement was associated with higher baseline FEV₁% predicted values (n = 259, 2.01, 95% CI 0.48 to 3.54, p = 0.01). Ever-smoking status was associated with a significantly lower baseline FEV₁ % predicted value (estimate - 31.55, 95% CI -37.62 to -25.49, p < 0.001). On binary categorisation, ≥3 lobe involvement was associated with higher FEV₁ predicted values (estimate 7.48, 95% CI 0.93 to 14.04, p = 0.025), as were ≥4 lobes (est. 10.99, p = 0.002), ≥5 lobes (est. 18.42, p <0.001), and 6 lobes (est. 19.98, p < 0.001).

However, lobar involvement was not associated with FEV₁ yearly decline as measured by % predicted or ml values, nor KCO or TLCO percent predicted yearly decline. Among covariates, COPD was associated with TLCO decline, with a further 1.61% decline per year (n = 143, 95% CI 0.40 to 2.81, p=0.009), but no other covariates associated with lung function decline in these models.

As shown in Table 5.5, lobar involvement did not associate with exacerbator status (n = 261, incidence rate ratio (IRR) 0.95, 95% CI 0.72 – 1.25, p = 0.719) or exacerbation rate (n = 261, IRR 1.01, 95% CI 0.93 – 1.09, p = 0.880) in zero-inflated models, though ever-smokers were much more likely to exacerbate (IRR 15.74, 95% CI 1.25 to 197.8, p = 0.033). Among those who had any exacerbations, those who had ever smoked had moderately higher exacerbation rates (IRR 1.74, 95% CI 1.21 to 2.50, p = 0.003). Unexpectedly, those with COPD appeared less likely to exacerbate (IRR 0.08, 95% CI

0.01 to 0.51, $p = 0.007$), though among those who did exacerbate, COPD had no effect on number of exacerbations (IRR 0.77, 95% 0.47 – 1.26, $p = 0.295$). However, this must be interpreted in context: as shown in Table 5.1, the majority (80.7%) of patients had concurrent COPD; furthermore, the covariates in the model only encompassed a minority of the variability seen (adjusted R^2 0.035).

Table 5.5: Number of lobes with bronchiectasis is unrelated to exacerbation rate

Annualised exacerbation rate			
<i>Associations</i>	<i>Incidence Rate Ratios</i>	<i>CI</i>	<i>p</i>
Zero-Inflated Model			
n lobes	0.95	0.72 – 1.25	0.719
Baseline age	0.95	0.88 – 1.02	0.172
Sex [Male]	2.06	0.67 – 6.36	0.209
Ever smoker	15.74	1.25 – 197.80	0.033
COPD	0.08	0.01 – 0.51	0.007
Count Model			
n lobes	1.01	0.93 – 1.09	0.880
Baseline age	0.98	0.97 – 1.00	0.024
Sex [Male]	0.85	0.63 – 1.16	0.312
Ever smoker	1.74	1.21 – 2.50	0.003
COPD	0.77	0.47 – 1.26	0.295
Observations	261		
R^2 / R^2 adjusted	0.057 / 0.035		

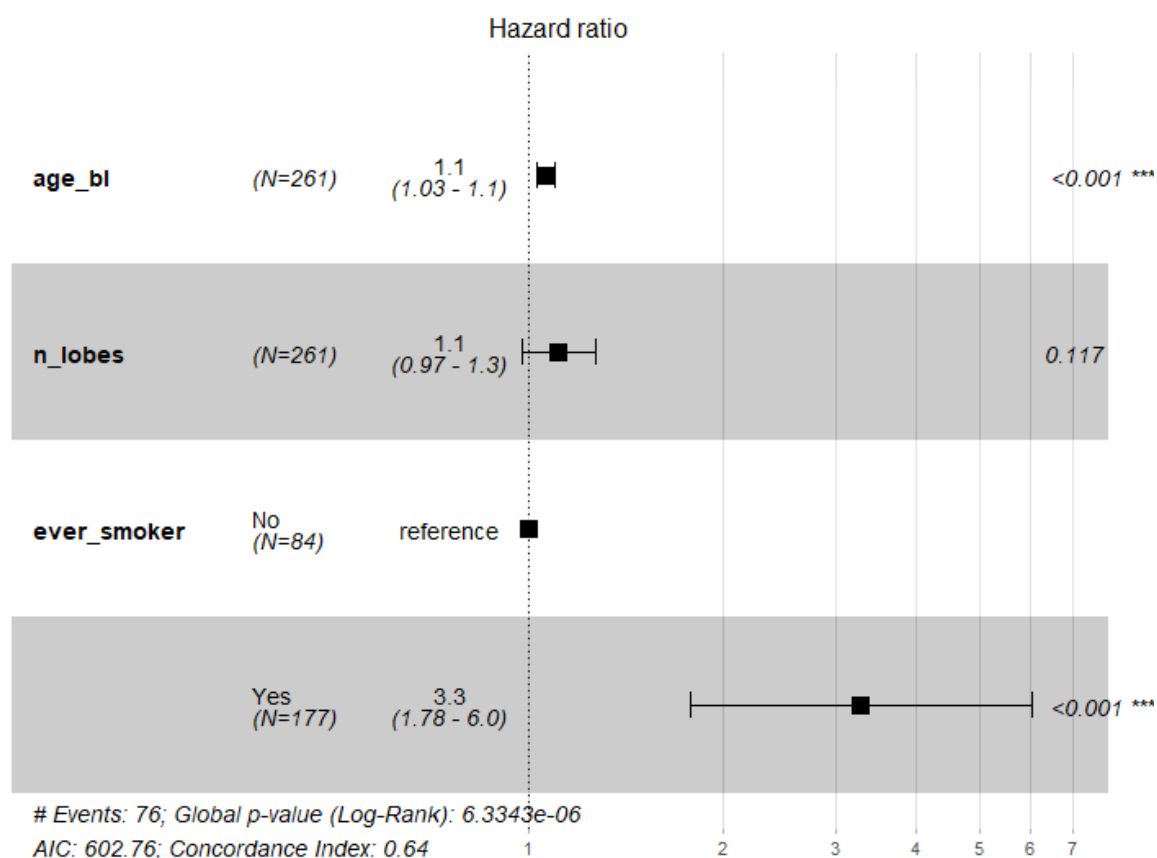
Lobar involvement also did not associate with mMRC score, although age increased the odds of mMRC 1 or 2, and ever-smoking status or COPD diagnosis increased the odds of mMRC 2, 3 or 4. R^2 was higher at 0.113 (adjusted). Full details of this model

are given in Appendix section 6. High collinearity was demonstrated for the covariates of age and COPD diagnosis, possibly since COPD correlated with ever-smoker status (Pearson's coefficient 0.36), but a Lasso regularised model (used in order to reduce the influence of collinearity) returned similarly non-significant results at each level of mMRC score, with the influence of lobar involvement being 0. This means collinearity, despite being high, was unlikely to have influenced the model output.

No association was found between SGRQ score and lobar involvement ($n = 129$, estimate -0.65 per lobe, 95% CI -3.06 to 1.75, $p=0.592$), or any of the covariates.

Lobar involvement was not associated with survival from baseline ($n = 261$, hazard ratio 1.11, 95% CI 0.97 – 1.27, $p = 0.117$), though ever-smoker status was associated with a 3-fold increase in risk of death (HR 3.28, 95% CI 1.78 to 6.04, $p < 0.001$, Figure 5.5).

Figure 5.5: Cox proportional hazards model for the association of survival with number of lobes affected with bronchiectasis in severe AATD patients. Lobar extent does not associate with survival.



5.1.5 Bronchial dilatation score and clinical outcomes

Higher (i.e., more dilated) bronchial dilatation scores were associated with higher baseline FEV_{1pp} values (n = 259, estimate 3.89, 95% CI 0.81 to 6.98, p = 0.014), but did not associate with FEV_{1pp}/year decline (n = 187, estimate 0.07, 95% CI -0.28 to 0.43, p = 0.674), FEV_{1ml}/year decline (n = 187, estimate 0.42, 95% CI -10.78 to 11.62, p = 0.941), KCO_{pp}/year decline (n = 133, estimate 0.09, 95% CI -0.36 to 0.54, p = 0.696), TLC_{Opp}/year decline (n = 133, estimate -0.12, 95% CI -0.57 to 0.34, p = 0.619), or SGRQ score (n = 129, estimate 1.11, 95% CI -3.77 – 6.00, p = 0.652) on multivariate linear regression modelling.

No association was seen between bronchial dilatation score and exacerbation rate on multivariate zero-inflated regression modelling (n = 261, zero-inflated model IRR 0.83, 95% CI 0.46 to 1.50, p = 0.539; count model IRR 1.03, 95% CI 0.88 to 1.21, p = 0.691). No association was seen between bronchial dilatation and mMRC score on multinomial regression, or a Lasso regularised model.

Survival analysis showed a difference in mortality risk on Kaplan-Meier curve visualisation, but this was not significant (p = 0.24), even when all bronchial dilatation scores ≥ 1 were combined (n = 262, 10-year survival 71.7% vs 100%, p = 0.055, Figure 5.6 and 5.7).

Figure 5.6: Kaplan-Meier curve showing an apparent association of Bronchial Dilatation Score (BDS) with survival which does not reach statistical significance.

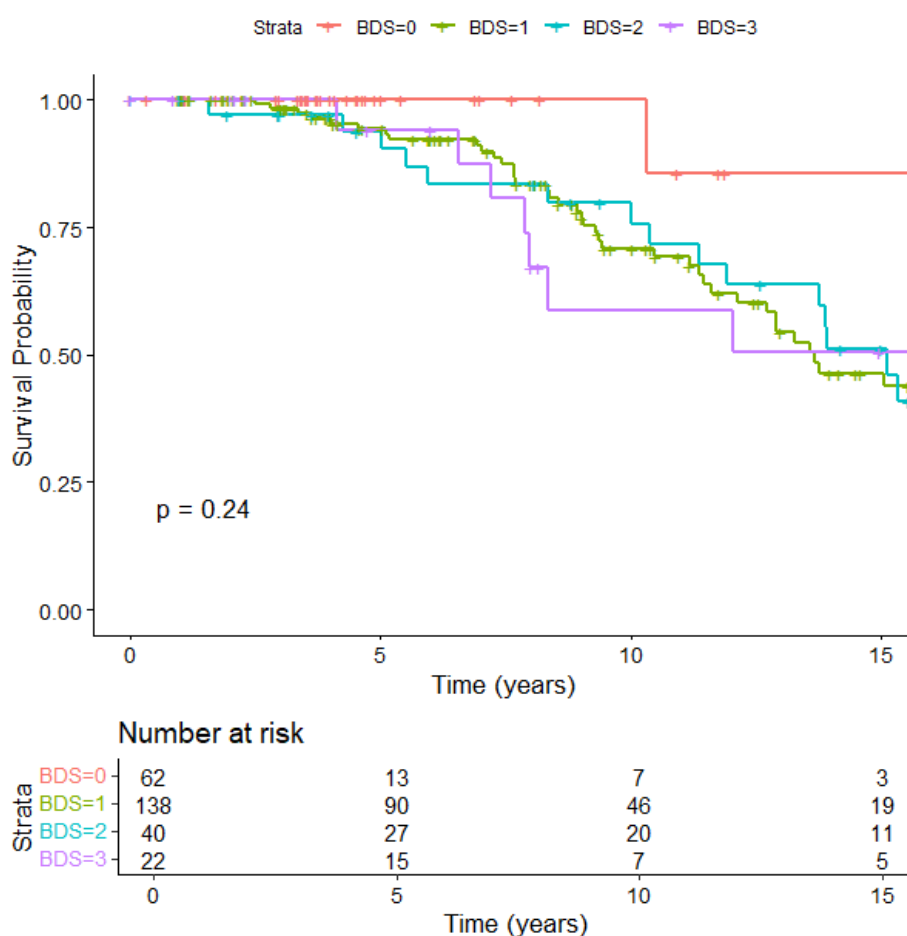
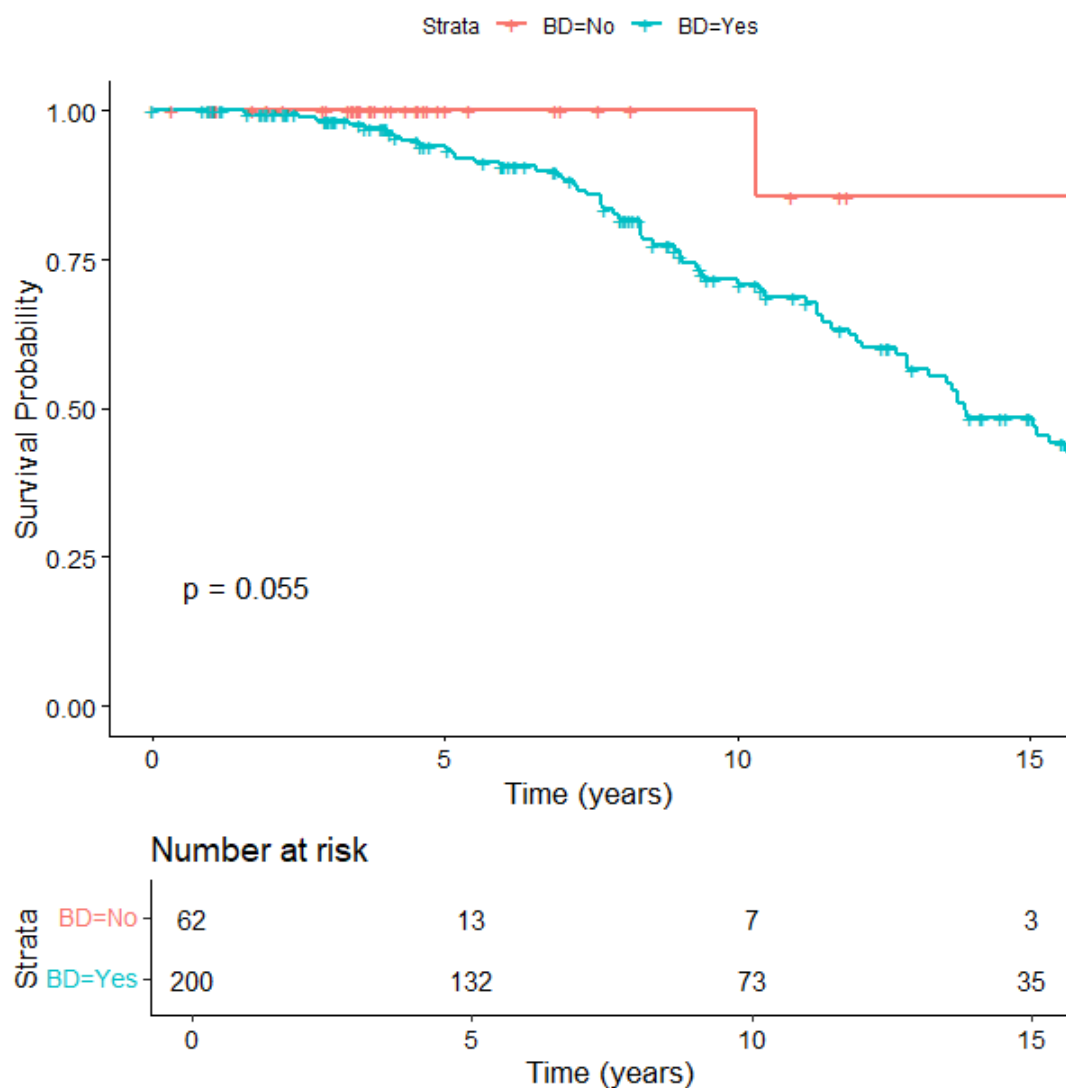
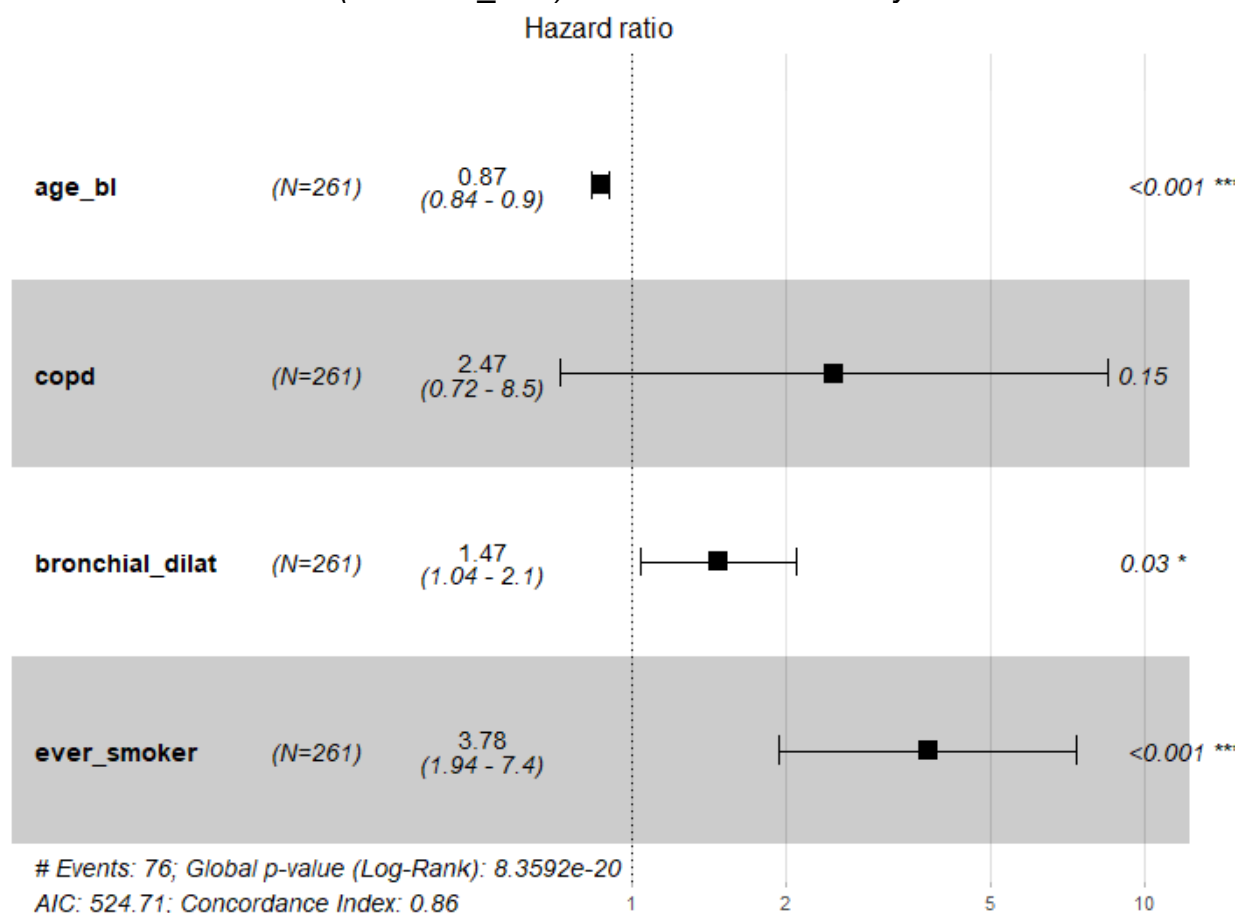


Figure 5.7: Kaplan-Meier curve for Bronchial Dilatation Score (BDS), scores 1-3 combined as BD (Bronchial Dilatation), showing an apparent association of BD with survival which does not reach statistical significance.



In a Cox proportional hazards model adjusting for age, sex, COPD diagnosis and smoking status, bronchial dilatation was associated with an increased risk of death ($n = 261$, HR 1.47, 95% CI 1.04 – 2.09, $p = 0.03$, Figure 5.8).

Figure 5.8: Multivariate Cox proportional hazards model showing the association of Bronchial Dilatation Score (bronchial_dilat) with increased mortality.



5.1.6 Bronchial wall thickening and clinical outcomes

Bronchial wall thickening was not associated with baseline FEV_{1pp} (n = 259, estimate 1.43, 95% CI -1.73 to 4.58, p = 0.374), FEV_{1pp}/year decline (n = 187, estimate 0.12, 95%CI -0.26 to 0.49, p = 0.536), FEV_{1ml}/year decline (n = 187, estimate 2.82, 95% CI -9.16 to 14.8, p = 0.643), KCO_{pp}/year decline (n = 133, estimate -0.04, 95% CI -0.54 to 0.47, p = 0.879), TLC_{Opp}/year decline (n = 133, estimate -0.08, 95% CI -0.60 to 0.44, p = 0.768), or SGRQ score (n = 129, estimate -1.96, 95% CI -6.8 to 2.88, p = 0.425).

No association was seen between exacerbation rate and bronchial wall thickening on zero-inflation modelling (n = 261, zero-inflated IRR 0.97, 95% CI 0.57 – 1.66, p =

0.920; count model IRR 1.07, 95% CI 0.91 to 1.25, $p = 0.410$). No significant association between mMRC and bronchial wall thickening was seen, including after regularising via the Lasso method.

On Kaplan-Meier curve analysis, a small difference in mortality between BWT = 0 and all other BWT scores was apparent visually, but this was not significant ($p = 0.24$), even when BWT scores ≥ 1 were combined (10-year survival 71.7% vs 95%, $p = 0.12$, Figures 5.9 and 5.10).

Figure 5.9: Kaplan-Meier curve demonstrating lack of an association of Bronchial Wall Thickness score (BWT) with mortality in AATD patients.

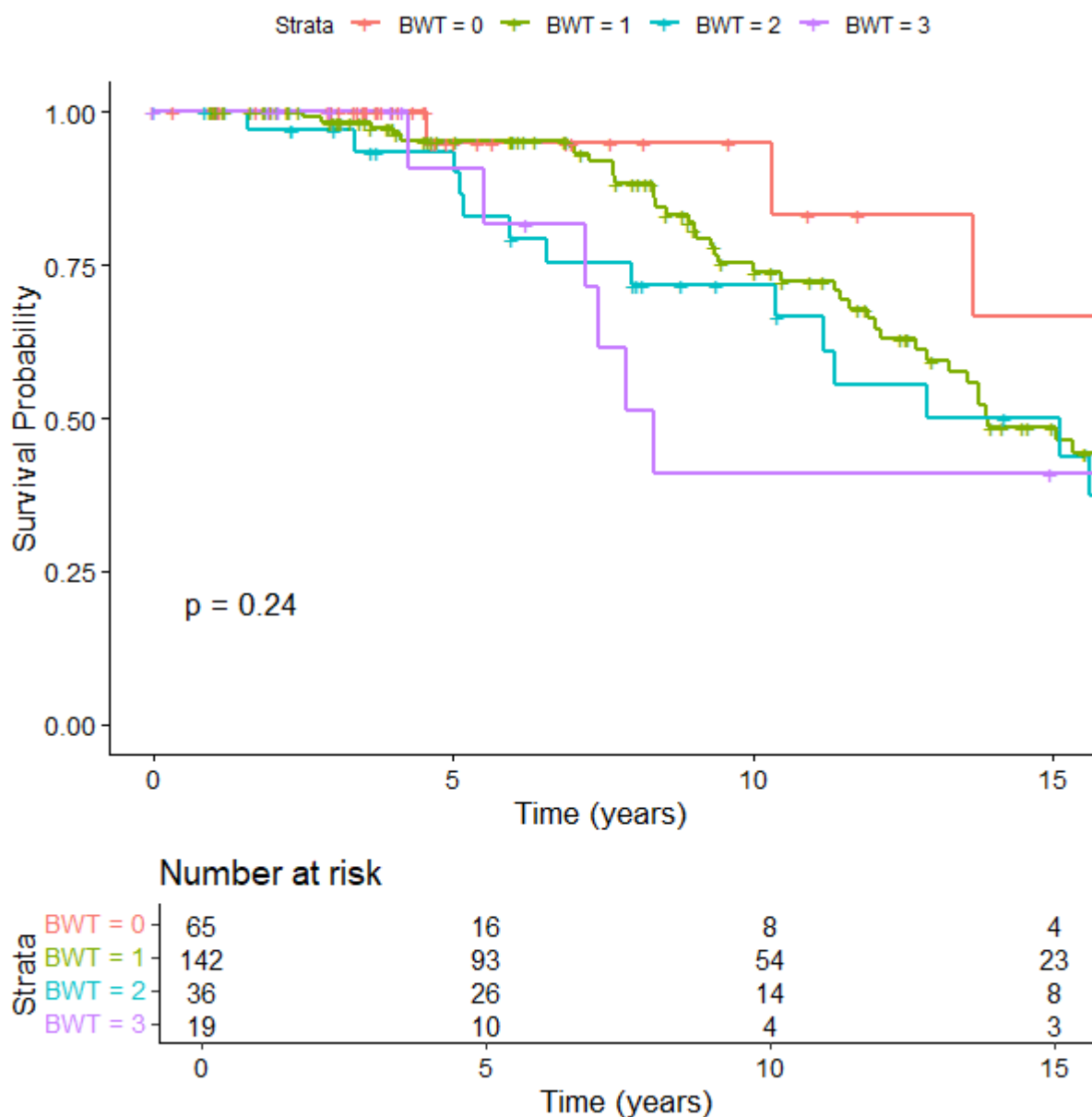
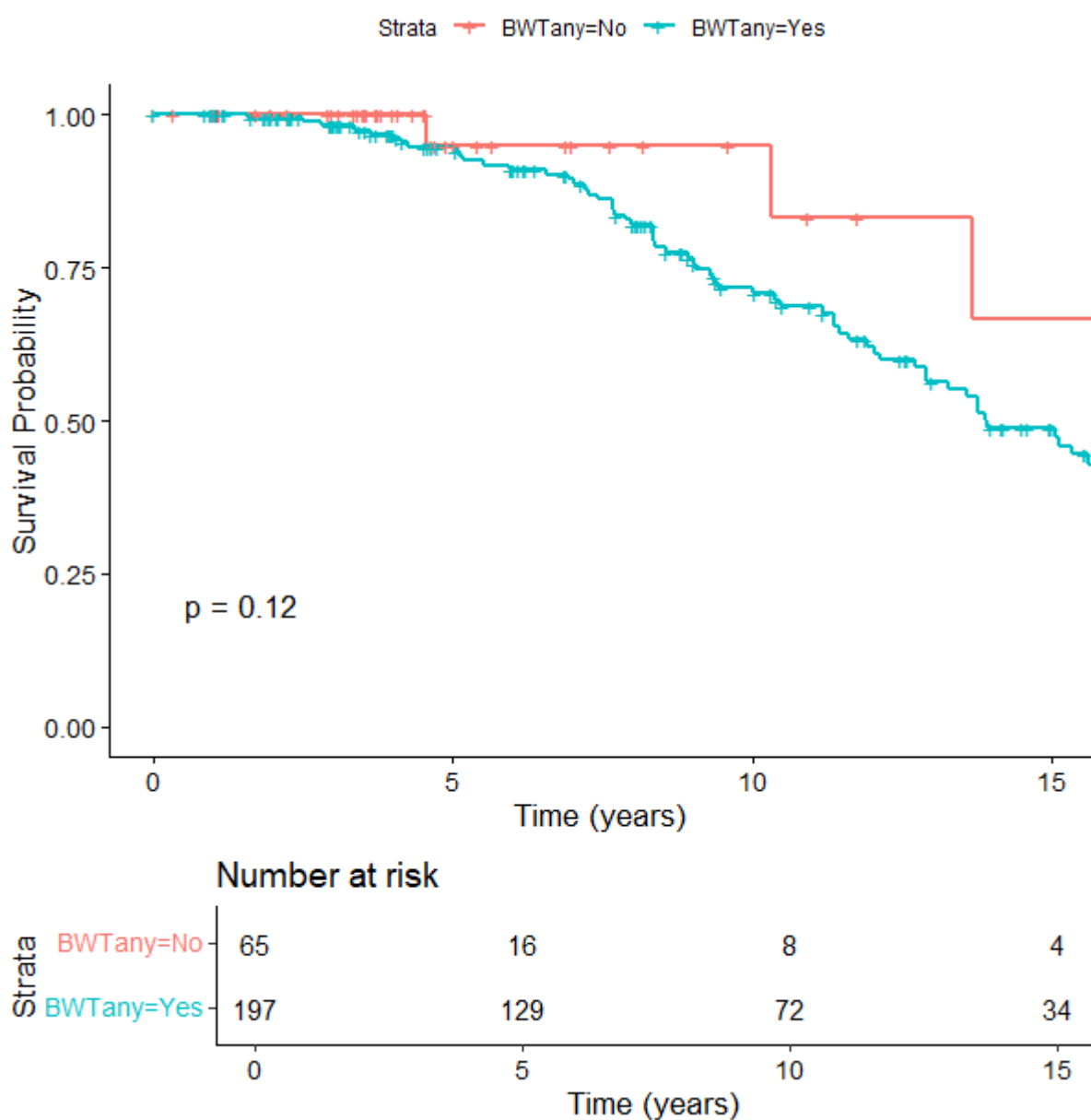


Figure 5.10: Kaplan-Meier curve for Bronchial Wall Thickness scores 1-3 vs 0, showing no association with mortality.

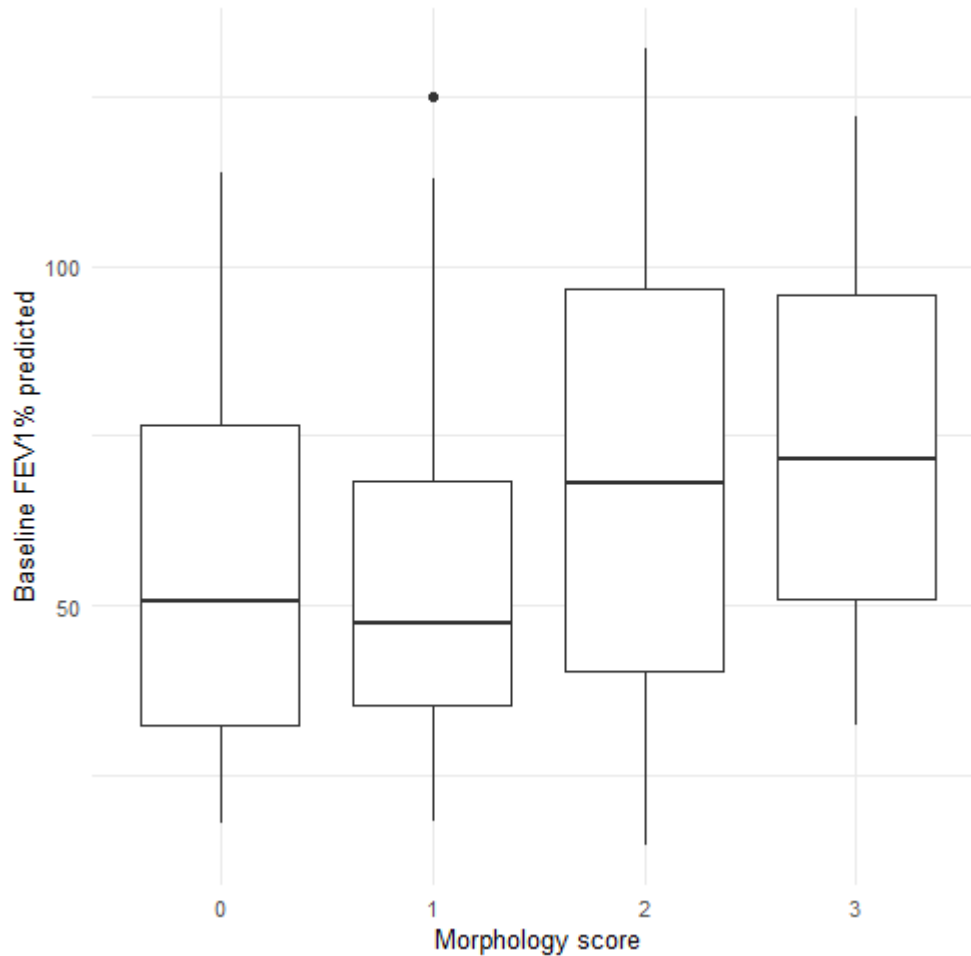


No effect of bronchial wall thickness on mortality was found on Cox proportional hazard modelling (HR 1.3, 95% CI 0.92 to 1.84, $p = 0.133$).

5.1.7 Bronchiectasis morphology and clinical outcomes

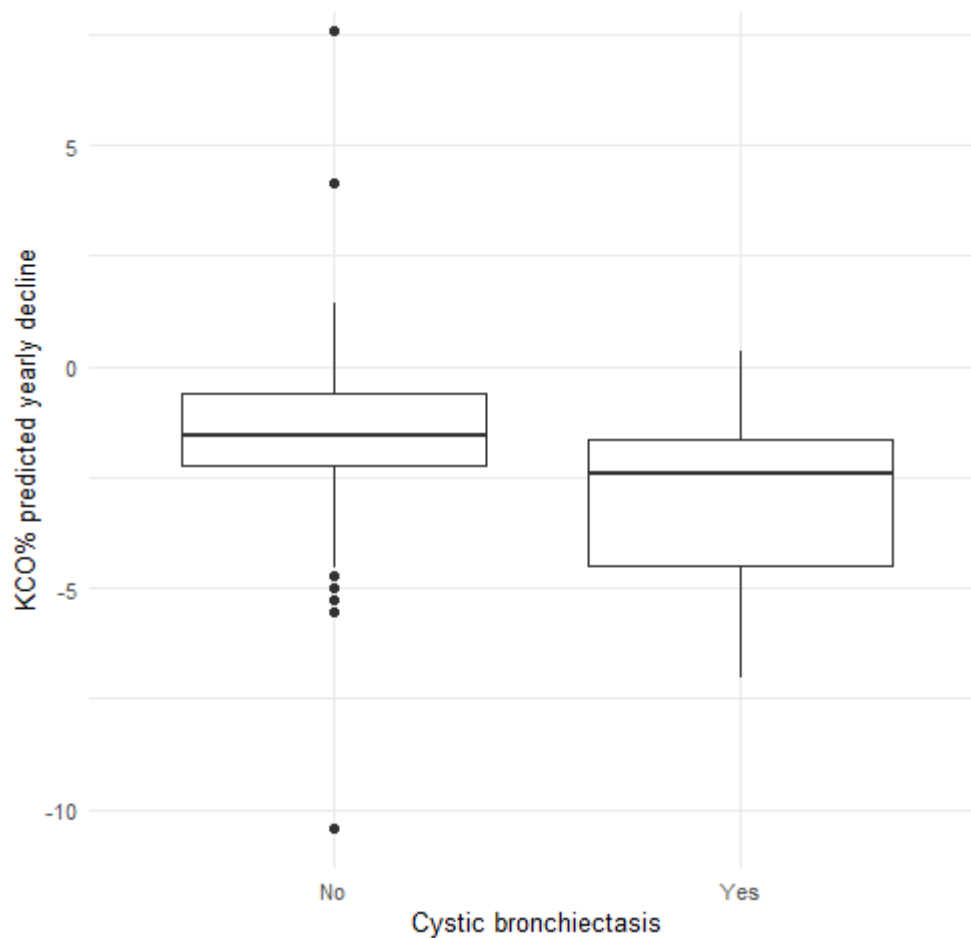
A non-significant positive influence of morphology score on baseline FEV_{1pp} was observed ($n = 259$, estimate 3.41, 95% CI -0.01 to 6.82, $p = 0.051$, Figure 5.11).

Figure 5.11: Boxplot of baseline FEV₁ values in AATD patients by morphology score: 0 = no bronchiectasis, 1 = cylindrical bronchiectasis only, 2 = cylindrical or varicose bronchiectasis, 3 = cystic bronchiectasis



However, no association was seen between morphology and FEV₁pp/year decline (n = 187, estimate 0.16, -0.23 to 0.54, p = 0.428), FEV₁ml/year decline (n = 187, estimate 3.23, 95% CI -9.18 to 15.63, p = 0.608), KCOpp/year decline (n = 133, estimate -0.04, 95% CI -0.54 to 0.46, p = 0.885), TLCOpp/year decline (n = 133, estimate -0.14, 95% CI -0.67 to 0.38, p = 0.591), or SGRQ (n = 129, estimate -1.24, 95% CI -6.64 to 4.17, p = 0.652). However, when patients with cystic morphology were compared with all others, a small but significant effect on KCOpp/year decline was seen (n = 133, estimate -1.52, 95% -2.81 to -0.23, p = 0.021, Figure 5.12), though the effect on baseline FEV₁pp became much less significant (n = 259, estimate 7.06, 95% -3.78 to 17.91, p = 0.201).

Figure 5.12: Boxplot of KCO% predicted yearly decline by presence of cystic bronchiectasis in AATD patients.



Similarly to the other parameters, no association was seen between morphology score and exacerbation rate ($n = 261$, zero-inflated estimate 0.87, 95% CI 0.47 to 1.61, $p = 0.661$; count estimate 1.09, 95% CI 0.92 to 1.28, $p = 0.323$). However, when comparing patients with cystic bronchiectasis with all other patients, although the model exhibited mild (21%) overdispersion of residuals, there was a positive association of cystic bronchiectasis with exacerbation rate among those who exacerbate ($n = 261$, count estimate IRR 1.95, 95% CI 1.31 to 2.91, $p = 0.001$).

No association was seen between mMRC and morphology score or cystic bronchiectasis presence.

No significant difference in mortality between morphologies was observed via Kaplan-Meier curve analysis (10 year-survival present vs absent cystic bronchiectasis: 63.6% vs 74.9%, $p = 0.91$, Figures 5.13 and 5.14) or Cox proportional hazard modelling (Morphology score HR 1.15, 95% CI 0.79 – 1.66, $p = 0.471$; cystic bronchiectasis HR 1.03, 95% CI 0.42 to 2.56).

Figure 5.13: Kaplan-Meier curves for demonstrating no association of bronchiectasis morphology on survival. MS = Morphology Score.

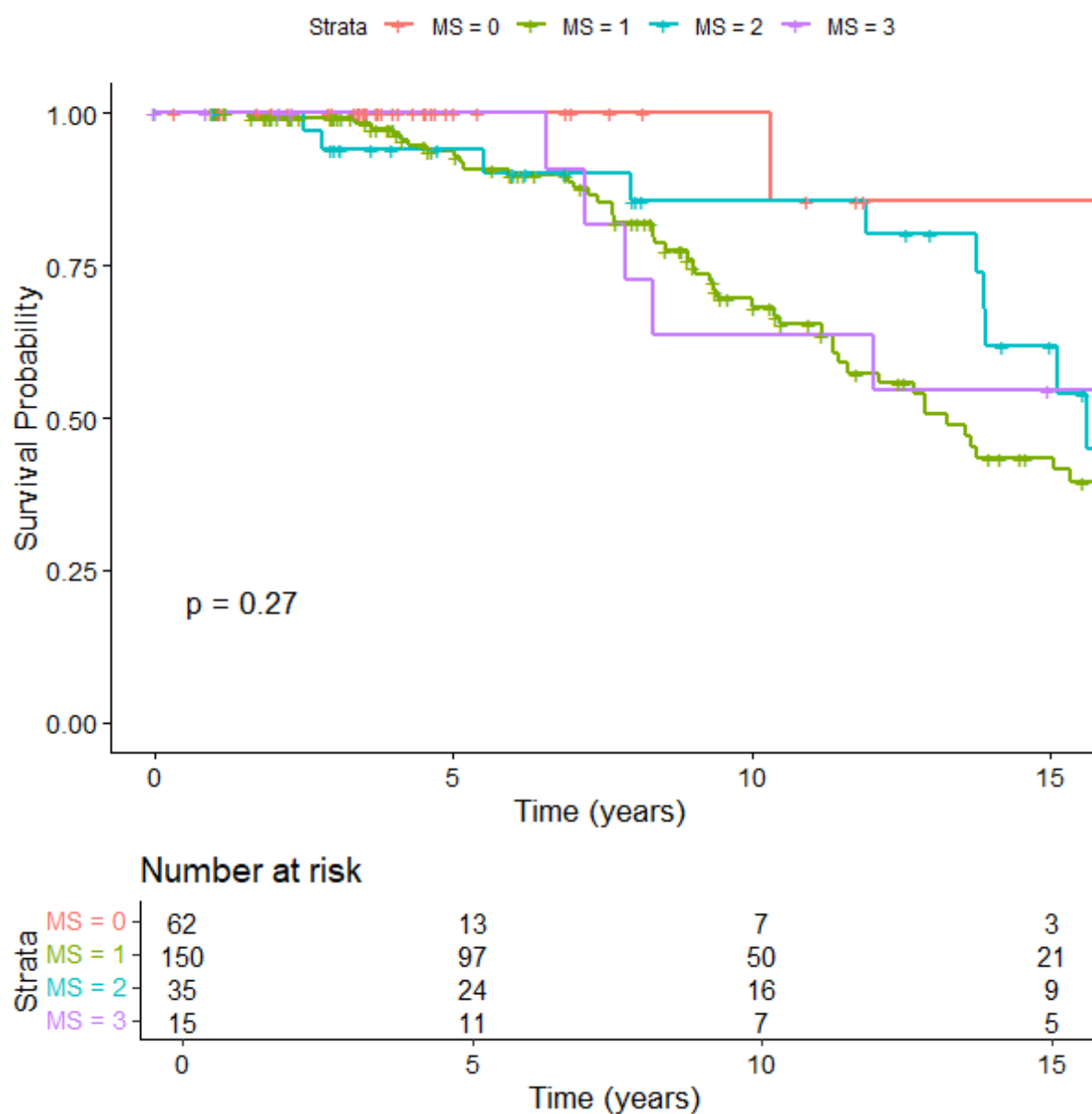
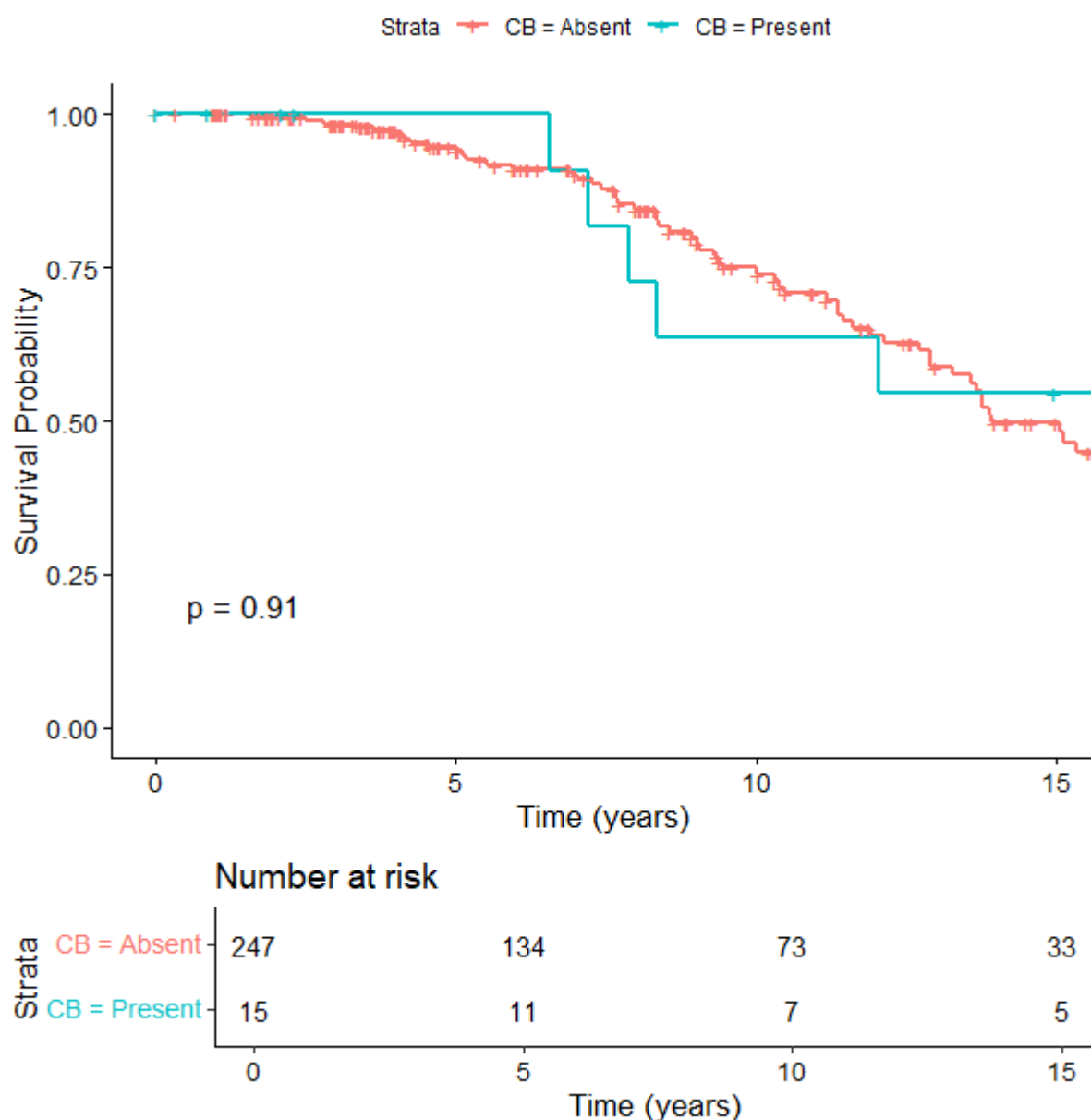


Figure 5.14: Kaplan-Meier curves demonstrating no association of cystic bronchiectasis morphology on survival. CB = cystic bronchiectasis.



5.2 Bronchiectasis severity index scores

With the information from the microbiology in Chapter 4 Section 2, and radiology from Chapter 5 Section 1, BSI scores could be calculated for the majority of cases, with some limitations as detailed in chapter 3 section 5.5. Of the 290 patients with radiology data in section 5.1, 7 lacked backline spirometry in the database, and 2 lacked mMRC

data. The remained 281 were analysed for correspondence with mortality. 259 had severe genotypes, 22 did not. Median BSI score among severe genotypes was 5, with range 0 to 14 (Figure 5.15). Among severe genotypes, survival differed significantly between the 3 groups (Figure 5.16, $p < 0.0001$). For mild, moderate and severe disease, 1 year mortality was estimated at 0.9, 6.1 and 16.9% respectively, and 4-year mortality was estimated at 4.3, 21.5, and 27.8% respectively. A Cox proportional hazard ratio confirmed this difference, with the backward selection model giving a hazard ratio of 1.13 (95% CI 1.05 – 1.22, $p = 0.001$).

Figure 5.15: Distribution of Bronchiectasis Severity Index scores in patients with severe AATD and bronchiectasis

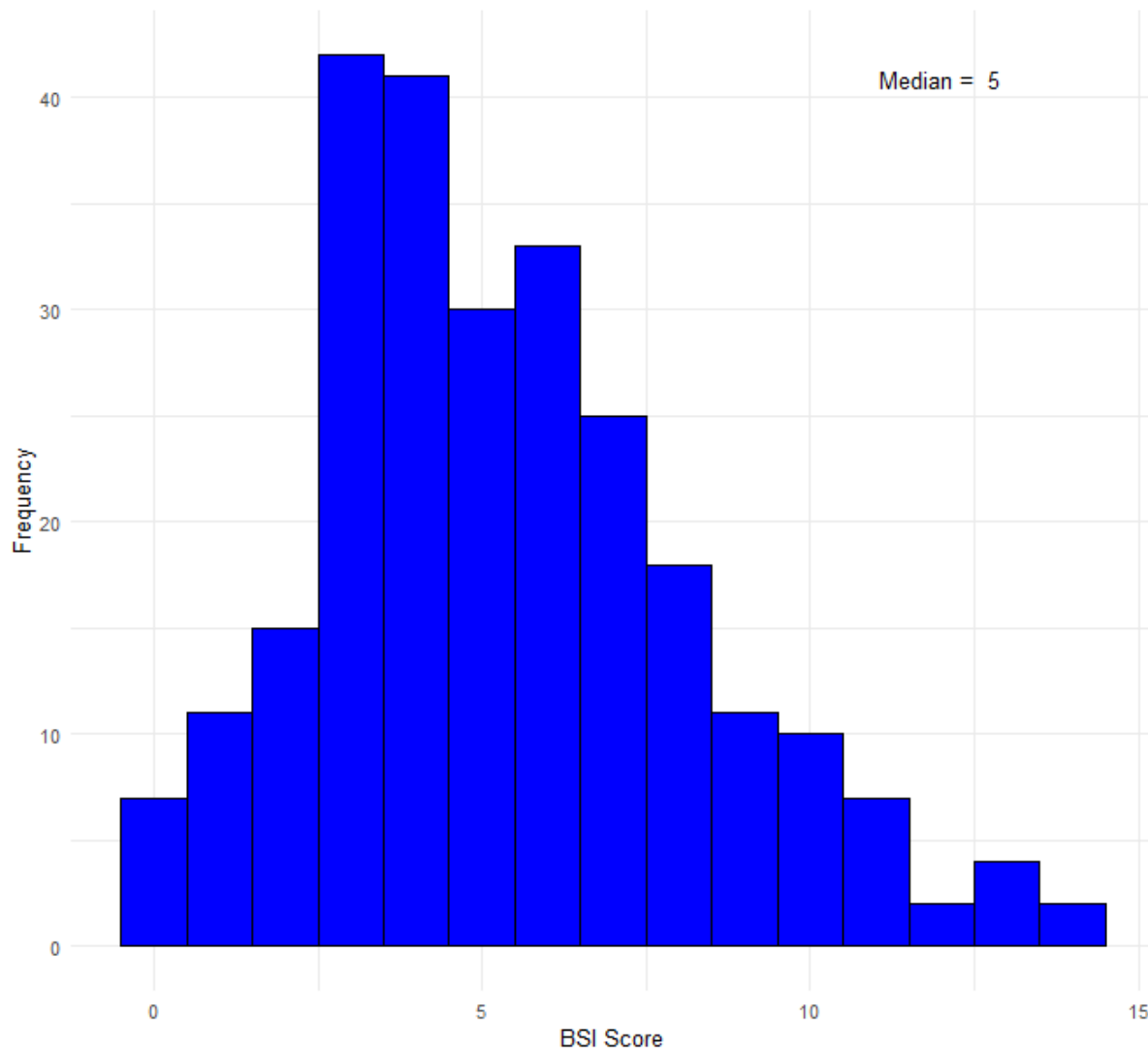
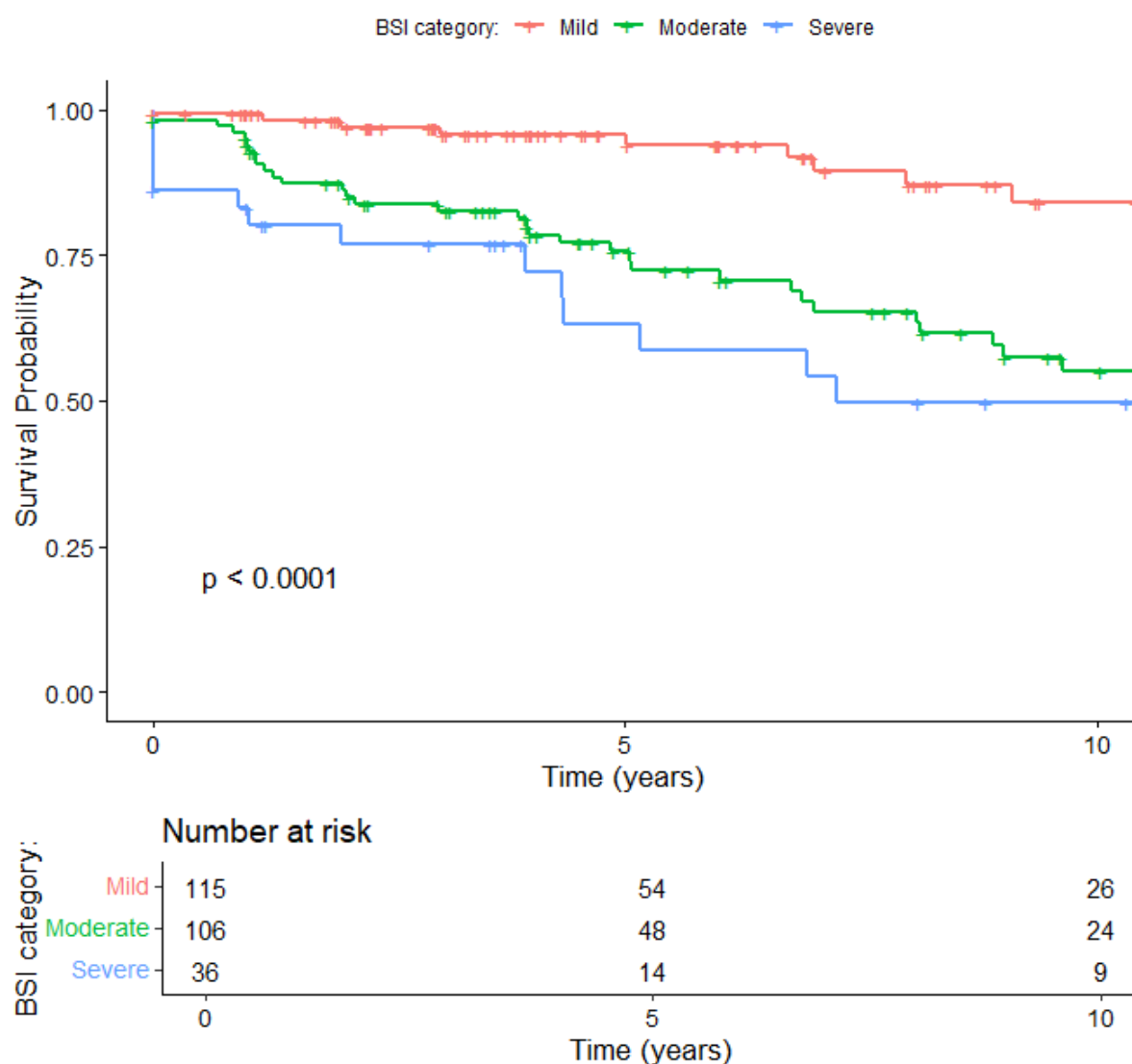


Figure 5.16: Kaplan-Meier survival curves demonstrating the association of BSI category with reduced survival from baseline in severe AATD.



5.3 Algorithmic analysis of CT bronchiectasis

5.3.1 Summary Statistics

154 scans were compatible with the YACTA software. Summary statistics are provided in Table 5.6.

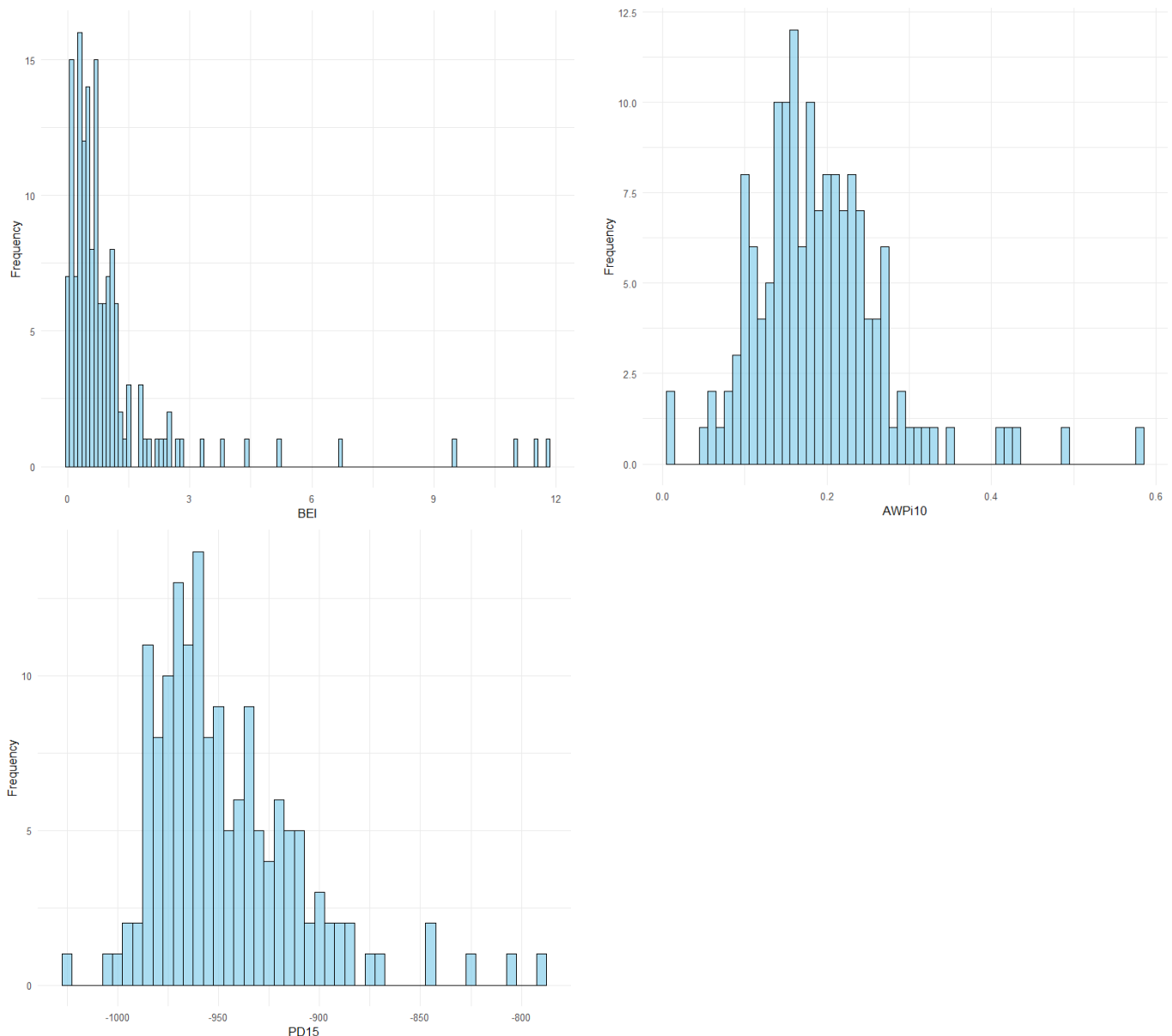
Table 5.6: Summary statistics for patients with CT scans assessed by YACTA

	Overall
n	154
Age (years)	52.46 (10.57)
Sex (Male, %)	73 (47.4)
Phenotype (%)	
ZZ	138 (89.6)
SZ	11 (7.1)
Other severe	3 (1.8)
Other non-severe	2 (1.2)
Ever smoker	107 (69.9)
Visual Bronchial Dilatation Score (%)	
0	55 (35.7)
1	75 (48.7)
2	17 (11.0)
3	8 (5.2)
Visual Bronchial Wall Thickness Score (%)	
0	55 (35.7)
1	76 (49.4)
2	18 (11.7)
3	5 (3.2)
Number of lobes affected (%)	
0	55 (35.7)
1	15 (9.7)
2	57 (37.0)
3	6 (3.9)
4	6 (3.9)

	Overall
5	1 (0.6)
6	14 (9.1)
Visual Morphology score (%)	
0: None	54 (35.1)
1: Cylindrical only	78 (50.6)
2: Varicose (+/- cylindrical)	19 (12.3)
3: Cystic (+/- varicose or cylindrical)	3 (1.9)
Bronchiectasis Index (BEI) (median (IQR))	0.63 (0.78)
AWPi10 (mean (SD))	0.19 (0.08)
PD15 (mean (SD))	-946.5 (37.03)

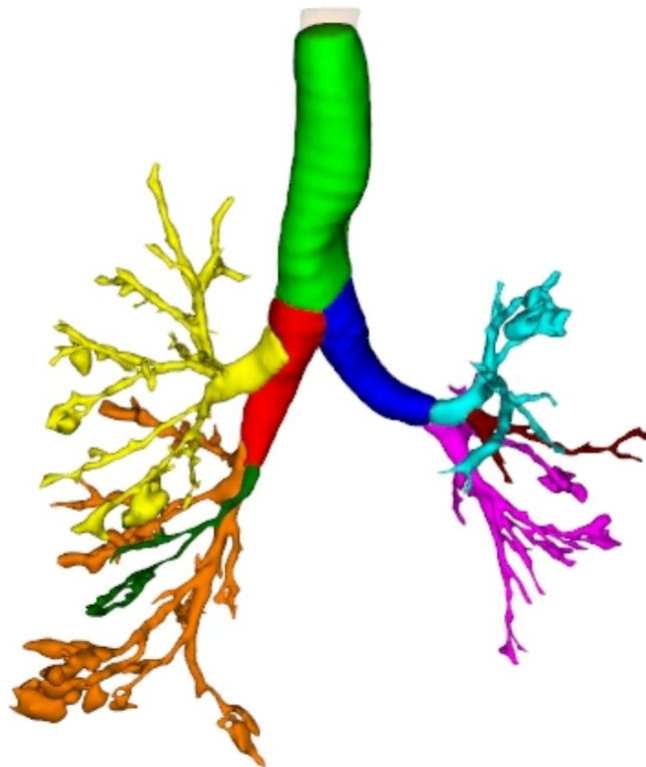
Bronchiectasis index (BEI) was not normally distributed in the population (Figure 5.17a); accordingly, non-linear models were used for this variable in the statistical analysis. However, AWPi10 and PD15 did follow normal distributions (Figure 5.17b and 17c).

Figure 5.17a-c: Histograms of Bronchiectasis Index (BEI, 2a), AWPi10 (2b) and PD15 (2c) distribution in AATD patients with CTs analysed by automated quantitative algorithm (YACTA).



For patients with outlying BEI values, 3-dimensional models generated by the algorithm were visually inspected to ensure the algorithm had appropriately identified the airway. This element of quality control was recommended by the original authors of the software. An example of such a 3-dimensional model is given in Figure 5.18.

Figure 5.18: Algorithm-generated 3-dimensional airway model exhibiting bronchiectasis in all 6 lobes.



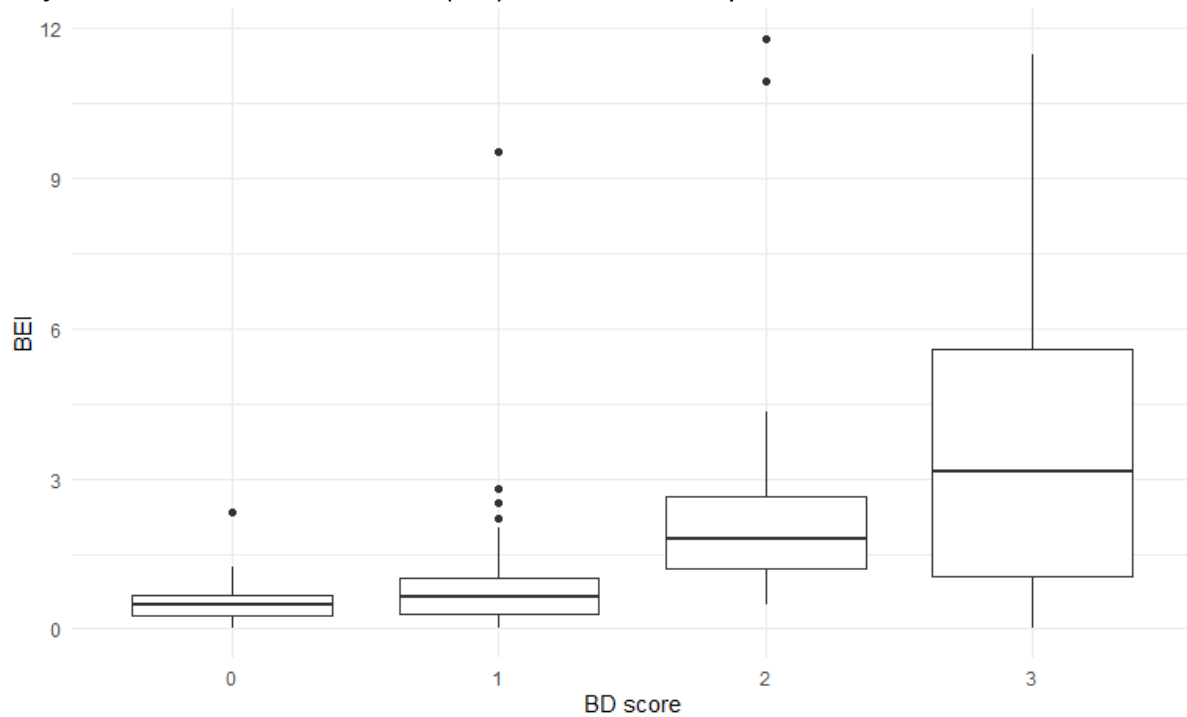
5.3.2 Comparison of algorithmic with visual data

Bronchiectasis Index (BEI) of the whole lung was significantly higher in those with any bronchiectasis identified on visual assessment vs those without (Median BEI 0.88 vs 0.50, Wilcoxon rank sum estimate -0.26, 95% CI -0.46 to -0.09, $p = 0.002$).

5.3.2.1 Comparison with visual bronchial dilatation score

BEI was suspected to correlate well with visual bronchial dilatation score (BD) after visualisation with a boxplot (Figure 5.19). This was confirmed on non-parametric testing (Kruskal-Wallis $\chi^2 = 38.47$, $p < 0.001$).

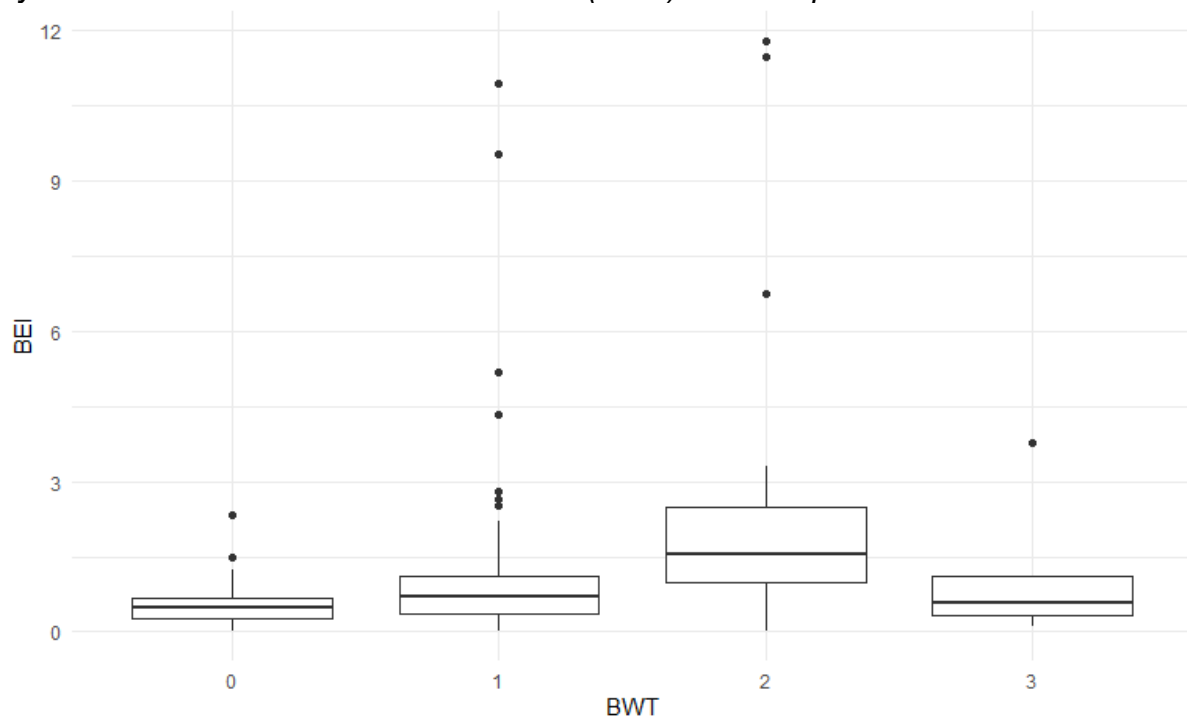
Figure 5.19: Box plot of algorithm-derived tapering index (Bronchiectasis Index, BEI) by visual Bronchial Dilatation (BD) score in AATD patients.



5.3.2.2 Comparison with visual bronchial wall thickness score

BEI also correlated with visual bronchial wall thickness score (BWT) (Figure 5.20, $\chi^2 = 20.1$, $p < 0.001$). AWPi10 did not correlate with visual bronchial wall thickness score (BWT) (Figure 17, $\chi^2 = 3.88$, $p = 0.28$).

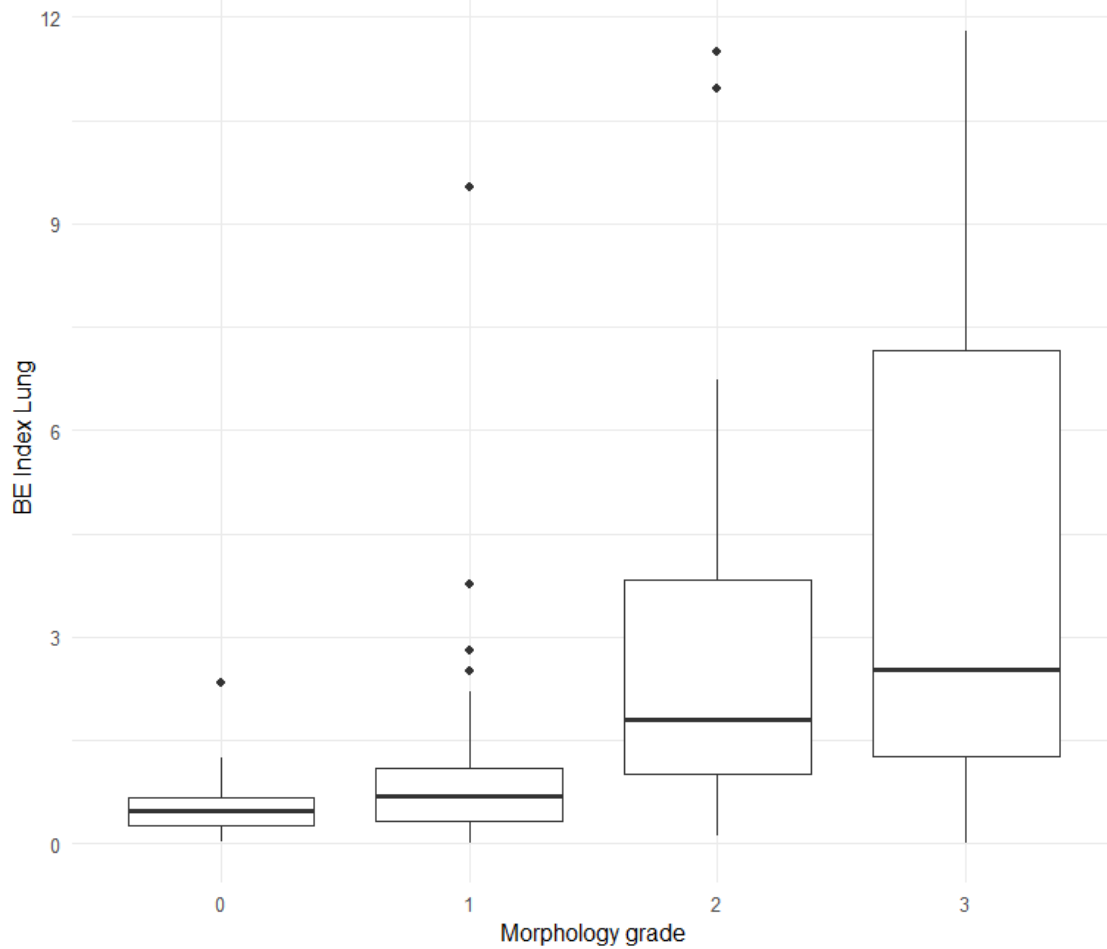
Figure 5.20: Box plot of algorithm-derived tapering index (Bronchiectasis Index, BEI) by visual Bronchial Wall Thickness score (BWT) in AATD patients.



5.3.2.3 Comparison with morphology

BEI did not differ significantly between those with cystic morphology on visual assessment vs those without (median BEI 2.53 vs 0.628 respectively, $p = 0.39$). Additionally, morphology type was converted to an ordinal variable with value 0 if no bronchiectasis present; 1 if only cylindrical morphology present; 2 if varicose but not cystic morphology present, and 3 if cystic morphology present. Using this variable, BEI correlated with morphology, with $\chi^2 = 21.883$ (Figure 5.21, $p < 0.001$).

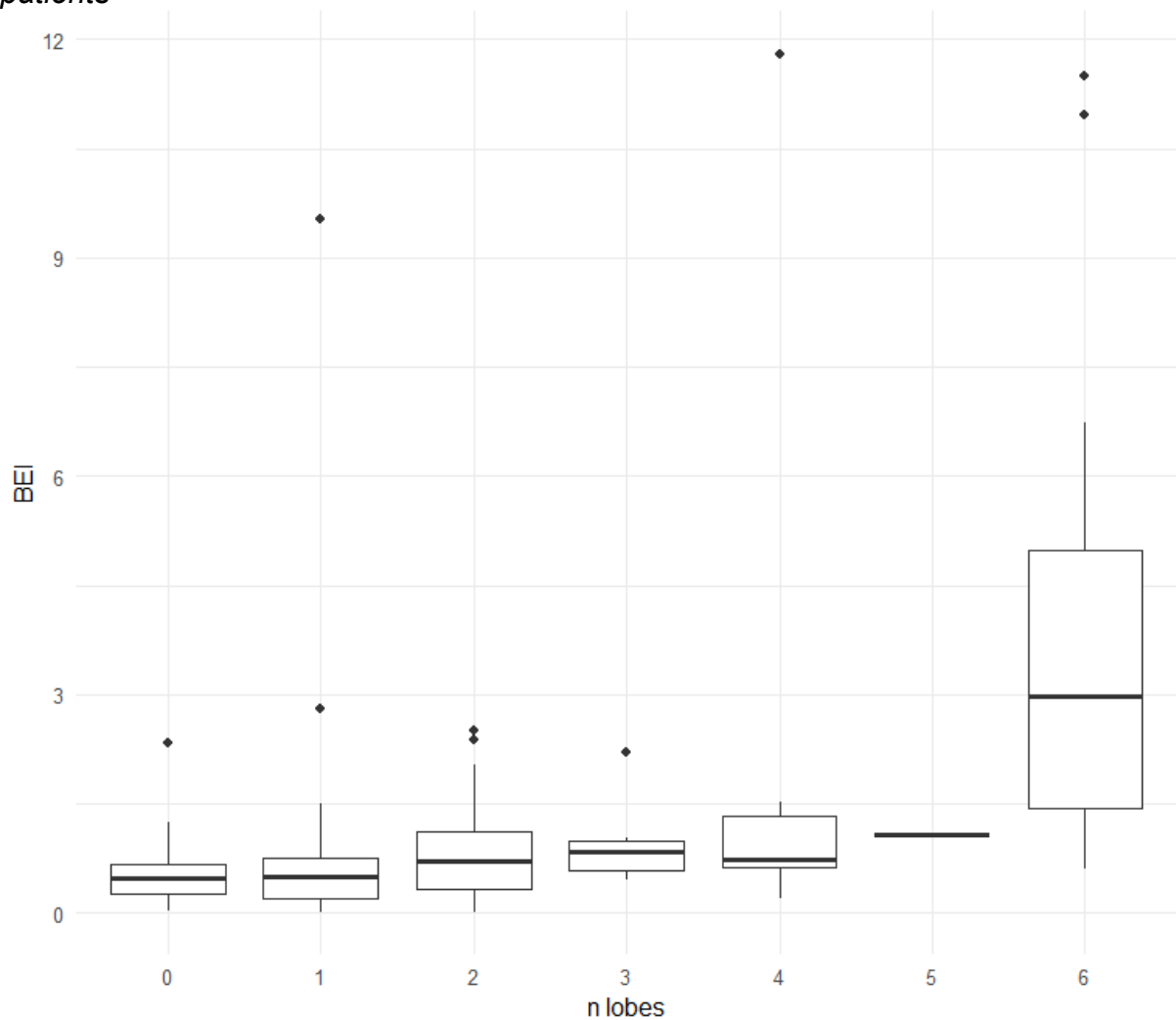
Figure 5.21: Box plot of algorithm-derived tapering index (Bronchiectasis Index, BEI) by Bronchiectasis Morphology as per Reid et al, 1950, in AATD patients.



5.3.2.4 Comparison with lobar distribution

BEI also associated with number of lobes affected ($\chi^2 = 0.37$, $p = <0.001$). Univariate linear regression analysis revealed an increase of 0.51 in BEI for each extra lobe affected (95% CI 0.36 – 0.66, $p <0.001$, R^2 / R^2 adjusted 0.236 / 0.231).

Figure 5.22: Box plot of algorithm-derived tapering index (Bronchiectasis Index, BEI) by number of lobes affected with bronchiectasis on visual assessment, in AATD patients



BEI was also analysed in relation to the cumulative number of affected lobes, simplified into binary metrics indicating the involvement of at least 'n' lobes. For instance, BEI was compared to whether at least 2, 3, 4, 5, or all 6 lobes were affected. Results of this analysis are shown in Table 5.7. The effect size increased with each additional lobe involvement, with a high degree of statistical significance at all levels.

Table 5.7: BEI association with cumulative lobar involvement on visual assessment by Wilcoxon rank sum test.

BEI			
<i>Lobar involvement</i>	<i>Estimate</i>	<i>95% CI</i>	<i>p</i>
≥ 2	0.38	0.20 - 0.58	<0.001
≥ 3	0.82	0.48 - 1.57	<0.001
≥ 4	1.25	0.64 – 2.49	<0.001
≥ 5	2.14	1.02 – 3.27	<0.001
6	2.33	1.24 – 3.54	<0.001

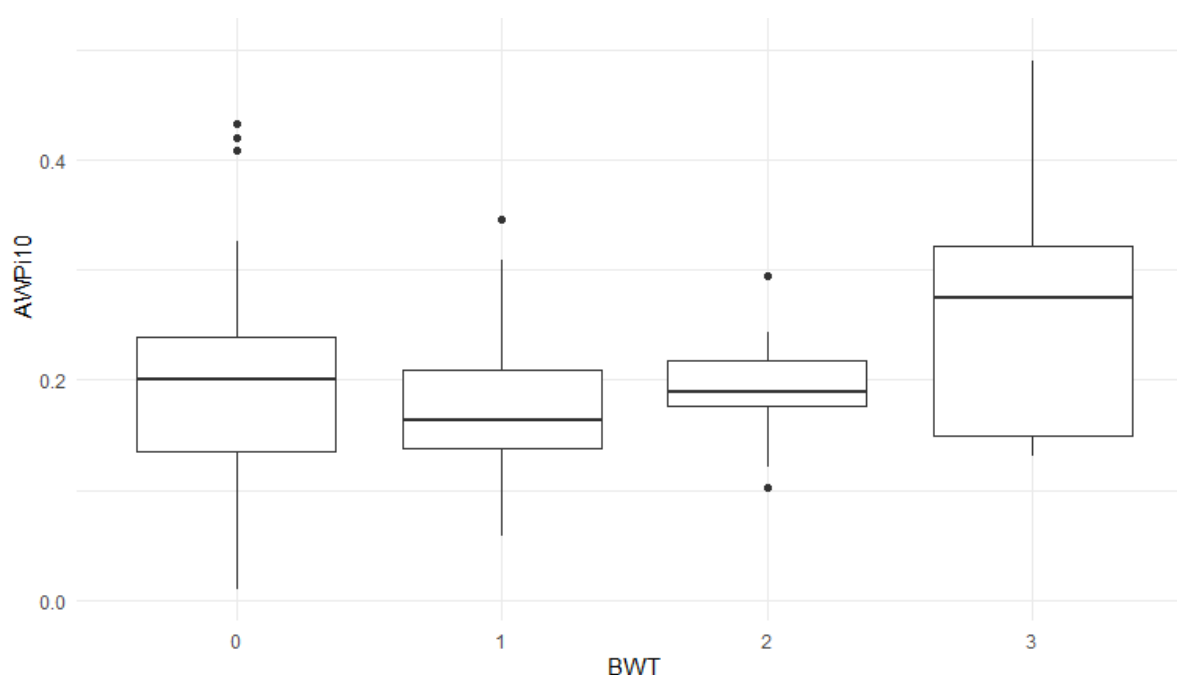
BEI was available for individual lobes in many cases. BEI correlated with visual assessment of bronchiectasis presence or absence on linear regression analysis in the upper and middle lobes, with the largest effect size seen in the right upper lobe (Table 5.8). In the right middle lobe and the lingula, no relationship was observed between visual assessment and BEI.

Table 5.8: BEI association with presence of bronchiectasis in individual lobes on visual assessment by Wilcoxon rank sum test

<i>Lobe</i>	<i>Wilcoxon rank sum estimate</i>	<i>95% CI</i>	<i>p</i>
Left upper	0.90	0.36 – 4.40	<0.001
Lingula	0.24	-0.03 – 2.57	0.08
Left lower	0.38	0.05 – 0.80	0.02
Right upper	1.35	0.67 – 2.30	<0.001
Right middle	0.003	-0.36 – 0.57	0.981
Right lower	0.75	0.31 – 1.17	<0.001

AWPi10 did not correlate with visual bronchial wall thickness score (BWT) (Figure 5.23, $\chi^2 = 3.88$, $p = 0.28$).

Figure 5.23: Boxplot of algorithm-derived airway wall thickness score (AWPi10) compared with visual Bronchial Wall Thickening score (BWT) in AATD patients.

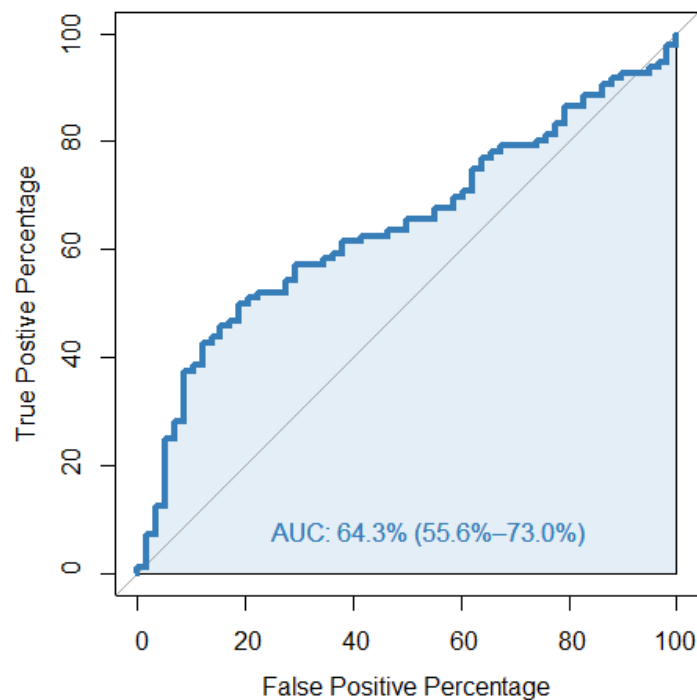


5.3.3 Thresholds for the association of algorithm-derived continuous data with visual assessment of bronchiectasis

Receiver Operator Curve (ROC) analysis was performed for YACTA variables. These variables were selected based on whether they showed correlation with visual parameters in the linear regression analyses above. ROC was also performed for BEI as a measure of visual bronchiectasis of any kind.

Despite not showing an association on linear regression, ROC analysis of BEI and visual diagnosis of bronchiectasis showed an area under the curve (AUC) of greater than 50% (64.3%, 95% CI 55.6% - 73%, Figure 5.24), demonstrating some ability of BEI to discriminate between the classes. Optimum threshold of BEI for the diagnosis of bronchiectasis by Youden's J method was 0.76, with 81.03% sensitivity but only 50% specificity (Youden's J statistic (J) = 0.31).

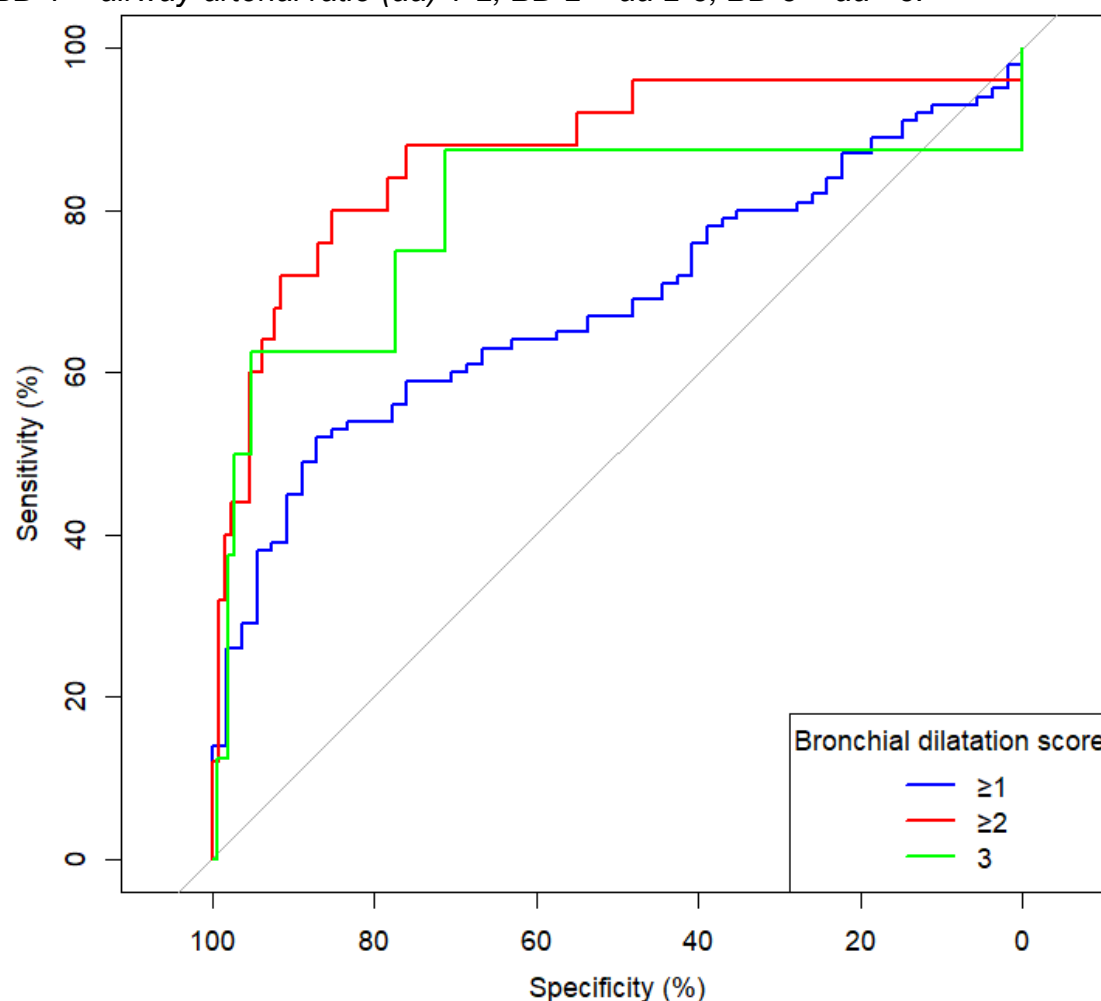
Figure 5.24: ROC curve of algorithm-derived tapering index (Bronchiectasis Index, BEI) vs visual diagnosis of bronchiectasis of any kind in AATD patients. No adequate threshold of sensitivity/specificity was identified.



5.3.3.1 ROC analysis of visual bronchial dilatation score

Visual bronchial dilatation score (BD) was compared with BEI, with the strongest result for the $BD \geq 2$ level (AUC 81.2%, 95% CI 68.9 – 93.5%, Figure 5.25). Optimal threshold for BEI association with $BD \geq 1$ was 0.76, (sensitivity 52%, specificity 87.04%, J 0.39), $BD \geq 2$ optimal threshold was 1.07 (sensitivity 80%, specificity 85.3%, J 0.65), and $BD \geq 3$ optimal threshold was 0.92 (sensitivity 87.5, specificity 71.2, J 0.58).

Figure 5.25: ROC curves for of algorithm-derived tapering index (Bronchiectasis Index, BEI) association with visual Bronchial Dilatation score (BD) in AATD patients. BD 1 = airway-arterial ratio (aa) 1-2; BD 2 = aa 2-3; BD 3 = aa >3.



5.3.3.2 ROC analysis of lobar involvement

For lobar involvement, ROC analysis was performed the association of BEI with 5 binary visual measurements: respectively whether at least 2, 3, 4, 5, or all 6 lobes were affected with bronchiectasis. ROC curves for these 5 visual parameters are shown in Figure 5.26, with AUC values in Table 5.9. Optimal thresholds for each parameter are shown in Table 10. An acceptable level of sensitivity and specificity was achieved for all except the “≥2 lobes” and “≥3 lobes” parameters. A drop in optimal threshold was observed in the “≥5 lobes” category, which is probably anomalous and related to the low number of patients with only 5 lobes affected (Table 5.10).

Figure 5.26: ROC curves comparing algorithm-derived tapering index (Bronchiectasis Index, BEI) to binary measures of visual assessment of lobar involvement (n lobes) in AATD patients. The most sensitive and specific threshold was for involvement of ≥ 5 or 6 lobes.

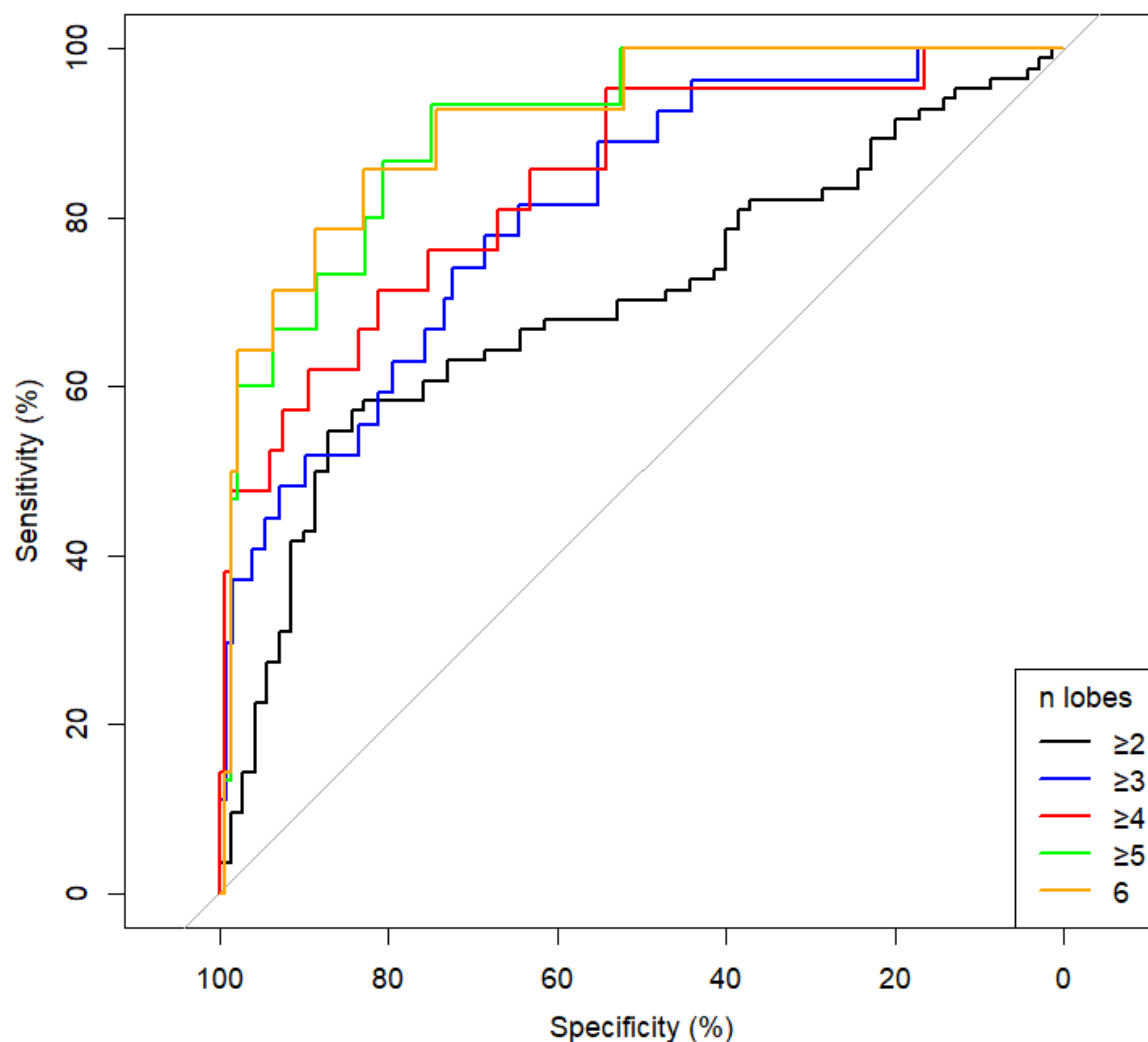


Table 5.9: ROC AUC values for BEI association with visual assessment of bronchiectasis lobar involvement.

n lobes with bronchiectasis	AUC (%)	95% CI (%)
≥ 2	69.8	61.5 – 78.2
≥ 3	80.9	71.0 – 89.9
≥ 4	84.0	74.1 – 93.9
≥ 5	90.6	83.4 – 97.8
6	91.3	83.8 – 98.8

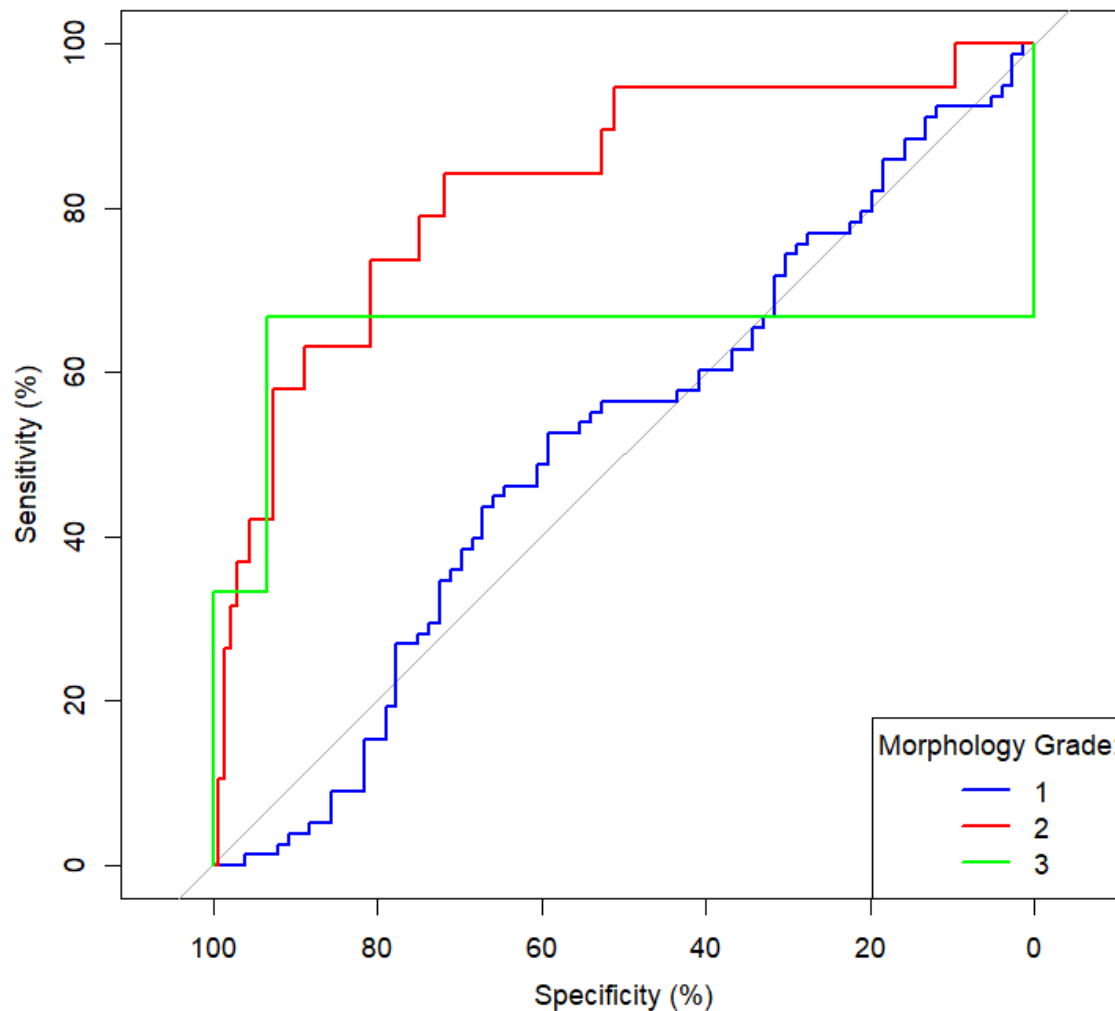
Table 5.10: Optimal thresholds for the association of BEI with lobar involvement, expressed as 5 different binary parameters. N.B. The identical thresholds for both ≥ 2 and ≥ 3 lobes are correct.

<i>Number of lobes with visually assessed bronchiectasis</i>	<i>Optimal BEI threshold</i>	<i>Sensitivity</i>	<i>Specificity</i>	<i>Youden's J statistic</i>
≥ 2	0.81	54.8	87.1	0.42
≥ 3	0.81	74.1	72.4	0.47
≥ 4	1.07	71.4	81.2	0.53
≥ 5	0.92	93.3	74.8	0.68
6	1.10	85.71	82.86	0.69

5.3.3.3 ROC analysis of morphology

ROC analysis was also conducted on the ability of BEI to identify varicose or cystic morphology. These ROC curves are shown in Figures 5.27. 2 optimal thresholds were identified for BEI associating with varicose bronchiectasis: BEI 0.84 had 81.8% sensitivity and 72.7% specificity, and BEI 1.07 had 72.7% sensitivity and 81.8% specificity (J 54.5 for both). Youden's J was not calculated for cystic bronchiectasis as ROC curve analysis was not significant (AUC 64.5%, 95% CI 1.2 - 100).

Figure 5.27: ROC curves for algorithm-derived tapering index (Bronchiectasis Index, BEI) associations with visual morphology score in AATD patients. Morphology grades: 1 = varicose and/or cylindrical; 3 = cystic. The most sensitive and specific BEI level was for grade 2.



5.3.4 Comparison of algorithmic data with clinical outcomes

5.3.4.1 Comparison with lung function

BEI associated with baseline FEV_{1pp} on multivariate analysis, with FEV_{1pp} being 2.78% higher ($p = 0.003$) for every one-unit change in BEI (Table 5.11). AWPi10 also associated with baseline FEV_{1pp}, with each 0.1 increase in AWPi10 being associated with a 5.1% reduction in baseline FEV_{1pp} (95% C -9.57 – -0.64, $p = 0.025$). Smoking

status negatively correlated with baseline FEV_{1pp}, but there was no association with emphysema as measured by PD15.

Table 5.11: Linear regression analysis of YACTA parameter association with baseline FEV1 % predicted value. Age and sex are already accounted for in the GLI method for FEV1pp calculation and are therefore omitted.

Associations	Baseline FEV _{1pp}		<i>p</i>
	Estimates	CI	
BEIndexLung	2.78	0.95 – 4.61	0.003
AWPi10	-51.09	-95.74 – -6.44	0.025
PD15	0.01	-0.00 – 0.02	0.107
Ever smoker [Yes]	-30.69	-38.86 – -22.52	<0.001
Observations	133		
R ² / R ² adjusted	0.444 / 0.427		

However, BEI was not associated with FEV_{1pp} decline (n = 95, estimate -0.03, 95% CI -0.22 – 0.16, p = 0.771), KCOpp (n = 65, estimate -0.10, 95% -0.29 – 0.09, p = 0.290) or TLCOpp (n = 65, est 0.04, 95% CI -0.17 – 0.24, p = 0.708). AWPi10 also did not associate with FEV_{1pp} yearly decline (n = 95, est -4.811, 95% CI 11.15 – 1.5, p = 0.135), KCOpp yearly decline (n = 65, est -6.544, 95% CI -14.08 - 0.99, p = 0.88) or TLCOpp yearly decline (n = 65, est -3.543, 95% CI -11.65 – 4.56, p = 0.386).

Neither BEI and AWPi10 associated with exacerbator status or exacerbation rate (Table 5.12).

Table 5.12: Association of algorithm parameters with annualised exacerbation rate

Annualised exacerbation rate			
Predictors	Incidence Rate Ratios CI		p
Count Model			
BEI	1.04	0.96 – 1.13	0.378
AWPi10	4.65	0.37 – 58.31	0.234
PD15	1.00	1.00 – 1.00	0.737
Age	0.97	0.95 – 1.00	0.017
Sex [Male]	0.83	0.53 – 1.31	0.428
Ever smoker [Yes]	1.20	0.73 – 1.99	0.470
Zero-Inflated Model			
BEI	1.01	0.72 – 1.41	0.976
AWPi10	138.09	0.06 – 307201.71	0.210
PD15	1.00	1.00 – 1.00	0.379
Age	0.93	0.84 – 1.03	0.176
Sex [Male]	0.99	0.21 – 4.71	0.993
Ever smoker [Yes]	1.81	0.18 – 18.32	0.615
Observations	140		
R ² / R ² adjusted	0.038 / -0.014		

On multinomial regression analysis, BEI associated with reduced odds of mMRC scores 1 and 2 relative to score 0, but had no relationship with the other mMRC scores

(Table 5.13). AWPi10 associated with increased odds of all mMRC scores greater than 0 on multivariate multinomial logistic regression analysis, with the highest magnitude of association being for scores of 2 or 3 (Table 5.13).

Table 5.13: Multivariate Multinomial regression analysis for mMRC score compared with AWPi10 score. Covariates age, sex, smoking status and PD15 omitted for clarity. N.B. AWPi10 has mean 0.19 and SD 0.08.

Associations	mode mMRC		p	Response
	Odds	Ratios CI		
BEI	0.76	0.58 – 0.995	0.046	1
AWPi10	604.79	576.98 – 633.95	<0.001	1
BEI	0.34	0.15 – 0.81	0.015	2
AWPi10	25762.15	24115.88 – 27520.79	<0.001	2
BEI	0.82	0.62 – 1.08	0.147	3
AWPi10	776265.45	735044.27 – 819798.30	<0.001	3
BEI	0.52	0.20 – 1.34	0.173	4
AWPi10	865.04	834.33 – 896.87	<0.001	4
Observations	138			
R / R ² adjusted	0.154 / 0.149			

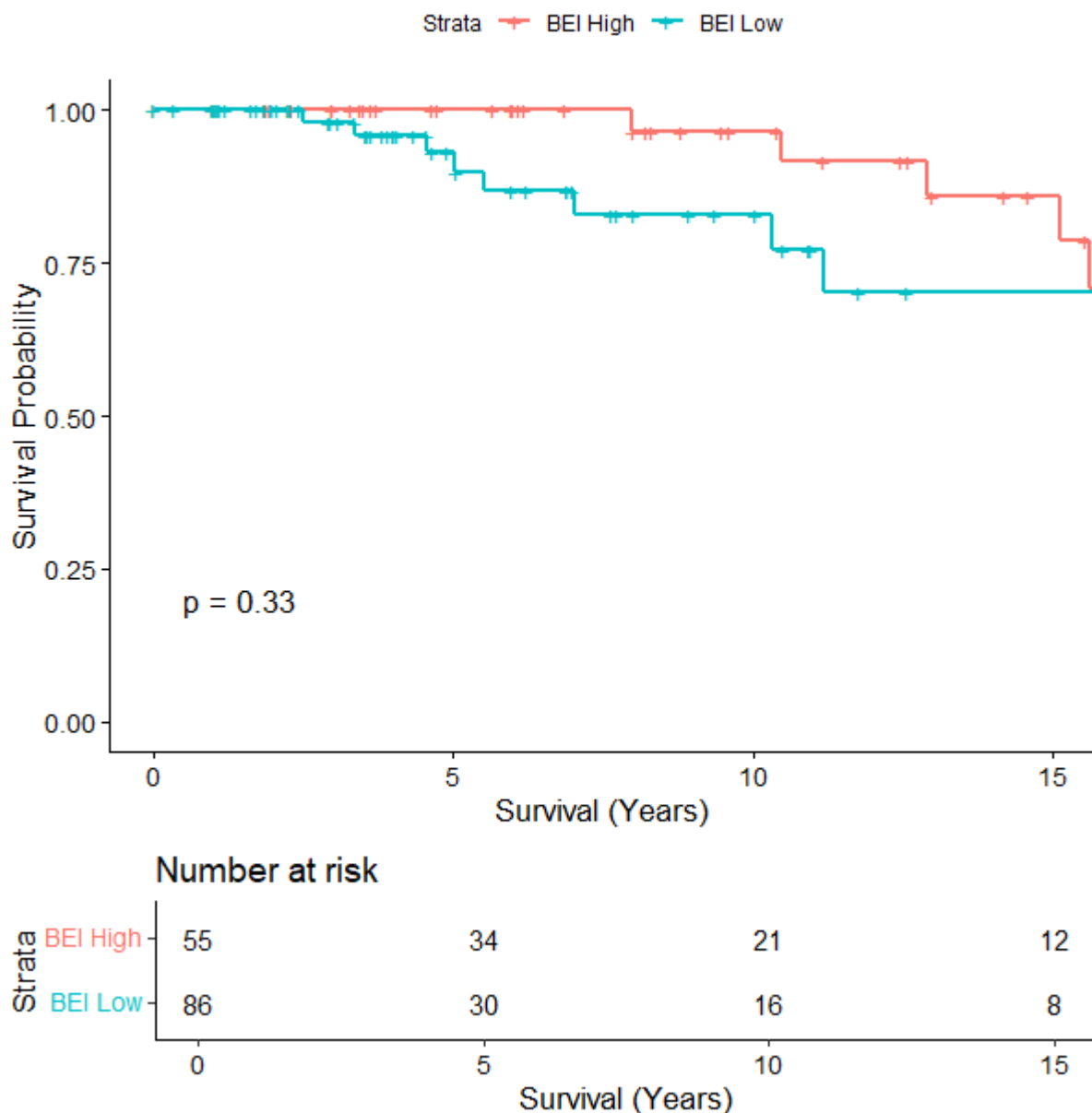
Mean SGRQ score was not associated with BEI (n = 90, estimate 1.36, 95% CI -2.31 – 5.02, p = 0.463) or AWPi10 (n = 90, est 9.43, 95% CI -48.67 to 67.52, p = 0.748).

5.3.4.2 Comparison with mortality

On Cox Proportional Hazard modelling, BEI was not associated with difference in survival from baseline (n = 139, HR 0.92, 95% CI 0.78 – 1.08, p = 0.318); neither was AWPi10 (n = 139, HR 62.78, 95% CI 0.04 – 107010.73, p = 0.276).

Kaplan-Meier Curves were constructed for the association of bronchiectasis dilatation with survival, given the finding in the previous chapter of an association of visual bronchial dilatation score with mortality. The threshold identified in the ROC curve analysis above was used. Results are shown in Figure 5.28: no association with mortality was found (10-year survival 82.8 vs 96.4%, $p = 0.33$).

Figure 5.28: Kaplan-Meier curves demonstrating an apparent initial difference in mortality between those with algorithm-identified significant bronchial dilatation and those without, but which does not reach statistical significance over a 15-year period. BEI: algorithm-derived tapering index (Bronchiectasis Index, BEI). Threshold of BEI high vs BEI low was identified on ROC analysis by comparison with visual assessment of bronchial dilatation.



5.3.4.3 Comparison with bronchiectasis severity index (BSI)

BEI correlated with Bronchiectasis Severity Index (BSI) on multivariate Poisson - regression analysis (Table 11), with a small (6%) increase in BSI for each one-unit increase in BEI (95% CI 2 – 10%, $p = 0.002$). AWPi10 also associated with BSI on (IRR 3.1, 95% CI 1.20 – 7.71, $p = 0.017$), with smoking status also associating (Table 5.14).

Table 5.14: Multivariate Poisson regression analysis of algorithm parameters associations with clinical bronchiectasis severity index (BSI). Age is omitted as already included in BSI.

BSI			
Associations	Incidence Rate Ratios	CI	p
BEIndexLung	1.06	1.02 – 1.10	0.002
AWPi10	3.10	1.20 – 7.71	0.017
PD15	1.00	1.00 – 1.00	0.316
Sex [Male]	1.13	0.97 – 1.33	0.119
Ever smoker	1.42	1.17 – 1.73	<0.001
Observations	138		
R ² Nagelkerke	0.212		

5.4 Discussion

5.4.1 Applicability of the findings to the AATD population

A majority of bronchiectasis patients had CT imaging available. The large number of bronchiectasis patients assessed here is a strength of the work, and represents the largest collection of bronchiectasis scans studied in AATD; more than a 2007 collection of 74 patients ²⁰¹, and slightly more than the 151 patients included in the recently published report of EARCO ³⁵⁵. Additionally, the inclusion of rare genotypes allows for greater validity in the AATD population as a whole. The genotypes included with PiZZ patients in this analysis are known to produce similarly low levels of AAT, therefore their inclusion does not diminish the applicability of the results to PiZZ patients.

The exclusion of those with alternative causes of bronchiectasis from the statistical models improves validity of the results, although only a minority of patients had adequate documentation of investigation for alternative causes of bronchiectasis. Guidance on diagnosis and investigations of bronchiectasis has only been published since 2010³⁸⁷, and many of the patients in the cohort have a baseline visit before this date. Holding these historic cases to the standard of current investigations would not be appropriate, but clearly future cases should be screened appropriately for alternative bronchiectasis causes, and have this documented clearly.

5.4.2 Radiological severity of bronchiectasis in AATD

Bronchiectasis in AATD appears to be mild in the vast majority, involving 2 or fewer lobes in the majority, and only a minority having varicose or cystic morphology. Most also had only the lower grades of bronchial wall thickness and bronchial dilatation scores. Lobar distribution was predominantly lower rather than upper, which is

consistent with the appearance of other causes of bronchiectasis ¹⁹². However, the lack of severity must be interpreted in light of the fact that the cohort is essentially screened for bronchiectasis at a much higher rate than the general population, due to the fact that a large proportion of patients will have CT imaging for the purpose of emphysema assessment, rather than due to a suspicion of bronchiectasis. This will naturally lead to a large number of incidental findings of radiological bronchiectasis, and therefore skew results towards the milder end of the natural history of the disease. Caution should therefore be applied when comparing this data to that in other causes of bronchiectasis.

5.4.3 Inter-rater reliability

The inter-rater reliability assessment in this work was consistently moderate or higher³⁴⁰, though agreement in the lower end of the moderate range was seen in the right middle lobe assessment and assessment of bronchial dilatation. The right middle lobe is anatomically the smallest, and is more complex in its anatomical origin, deriving from a tertiary division of the airways, rather than secondary as in the upper and lower lobes. This may make it harder to identify for the general physician on CT examination, particularly in images with artefact, or which are not fully inspired. In assessment of bronchial dilatation, differences in experience between raters may account for the relative discrepancy.

No attempt to objectively quantify radiological appearances of bronchiectasis has resulted in perfect inter-rater reliability^{180,192,194}: agreement between observers has been found to be moderate for bronchial dilatation, poor for mucous plugging, and particularly poor for bronchial wall thickening assessment ³³⁹. Furthermore, all currently established scoring systems compare the airway diameter to arterial

diameter. However, the ratio between the two can be affected by other factors than simply dilatation of the airway. Bronchial wall thickness and mucous plugging, if severe, could give the appearance of a narrowed internal airway diameter. Stage of inspiration on CT image acquisition can also affect airway diameter, being wider during full inspiration³⁸⁸. Arterial diameter is also greater during full inspiration due to negative pressure within the thorax. Furthermore, prolonged pulmonary hypertension of any cause can be expected to dilate the pulmonary arteries, as can altitude^{389,390}. The converse may happen due to the hypoxic pulmonary vasoconstriction response, whereby the circulation to lung parenchyma is reduced with prolonged injury, or smoking^{177,261}. These factors reduce the reliability of arterial comparison in scoring systems; however, the alternative method of assessing “lack of tapering” is time-consuming, requires a high degree of skill, and is difficult to assess in 2 dimensional images. This supports the use of automated algorithms to process these large volumes of data quickly and objectively, and by assessing 3D models, as we have described.

5.4.4 Relationship of radiological bronchiectasis subtypes with clinical outcomes

Increased lobar involvement, morphology and bronchial dilatation scores were associated with higher baseline FEV_{1pp} values. Morphology score produced a similar, but non-significant result. This appears counter-intuitive at first glance, but must be interpreted in context of the high burden of COPD in AATD: FEV_{1pp} is more useful as a measure of COPD severity than of bronchiectasis severity, as airflow obstruction only appears later in the natural history of bronchiectasis, whereas COPD is defined as chronic airflow obstruction and therefore is present at the point of diagnosis. Most

patients in the cohort have COPD, but a minority will have bronchiectasis as the major cause of their symptoms, and will have been investigated for the more common symptomatic manifestations of bronchiectasis: sputum production and exacerbations. These patients will have comparatively milder, or even absent, COPD when compared with the cohort as a whole, which has a mean baseline FEV₁ in the moderate range for COPD⁸⁸. For a more meaningful assessment of how lobar involvement affects baseline FEV₁, referral bias must be addressed, perhaps by including matched patients identified by family or population screening. However, family and population screening for alpha-1 antitrypsin is limited at present, and lower still in the time period assessed here, so this approach was not achievable for the present work. A large family cohort is included in an Irish research group's database, and collaborative work could use this dataset to establish whether this finding is seen in patients identified by family screening. However, adequate family screening has only been possible in recent years, and therefore non-index cases may not yet have developed airways diseases.

Lung function decline was largely unaffected by the various visual parameters, with the exception of a small effect of cystic bronchiectasis on KCO decline. There was no effect of cystic morphology on TLCO decline, despite KCO and TLCO being intrinsically related. However, since KCO is corrected for alveolar volume, whereas TLCO is not, it is possible that an excess of mucous plugging, not assessed in this analysis, in combination with emphysema in these patients, leads to the discrepancy. Nonetheless, the estimated difference in KCO% predicted between the two groups was less than the minimum clinically important difference for gas transfer ³⁹¹, and therefore is not likely to be useful clinically.

Most of the parameters were not associated with changes in exacerbation rate, with the exception of cystic bronchiectasis associating with higher exacerbation rates among those who have at least 1 exacerbation per year. This is consistent with the association between cystic bronchiectasis and severe exacerbation rates seen in the derivation study for the BSI score¹⁸⁸, which had a much larger sample size.

Bronchial dilatation had an influence on mortality, which is undoubtedly of interest clinically, though the confidence interval was quite wide, and corrections for the multiple-comparisons problem were not made. Bronchial wall thickness score also produced a non-statistically significant difference. The conventional solution to this problem would be to increase sample size, or not to perform so many analyses on the same cohort of patients; however, rare diseases such as AATD are limited by the availability of patients. This work presents results from almost all patients with AATD referred to UK specialist centres until 2014, and the majority of those referred thereafter; therefore, larger sample sizes cannot be generated retrospectively. Consequently, such repeated analyses and relatively less stringent statistical significance thresholds must be used, but interpreted with caution. In the case of the mortality seen here, if interpreting it as a true finding, the clinical application is simply to have low thresholds for the diagnosis and treatment of bronchiectasis with existing treatments. These treatments are beneficial, non-invasive and relatively low risk, as discussed in the literature review; this favours interpreting the finding as true.

Since survival is taken as days survived from baseline visit, the mortality data presented here is open to referral bias, with patients referred at different stages of their disease. However, correcting for age and concurrent COPD diagnosis in the hazards model should increase validity of this method. Measuring survival from birth was considered since genotype-related mortality risk should have been present from birth,

but no precedent was found in the literature for this technique, and it was not attempted.

Interestingly, the only factors to associate with mortality in the established bronchiectasis severity scoring systems were number of lobes involved and cystic morphology, neither of which associated here^{187,188}. However, those studies had far larger sample sizes, such that my study may have been underpowered to detect the relationship, and were not limited to AATD bronchiectasis. It remains possible that the association is absent in AATD.

5.4.5 Validation of the Bronchiectasis Severity Index score

Confirmation that the Bronchiectasis Severity Index (BSI) score is associated with mortality in AATD will be useful clinically. Mortality risk in the 3 severity groups was broadly similar to those in the BSI derivation study, though the “moderate” group in this work had worse mortality than in BSI, as did the “severe” group at 1 year¹⁸⁸. Although this validation has biological plausibility, some degree of caution in interpretation must be taken, as several parameters had to be adjusted for the cross-sectional nature of this work, as discussed in chapter 3 section 5.5. However, since a large-scale prospective validation in AATD is unlikely to be feasible, this data suffices.

Given most patients also have COPD, mortality predictions for both conditions will have to be interpreted, and the more severe communicated to the patient appropriately. For example, the BODE index could also be calculated, since it is already frequently used in COPD to predict 4-year survival, and therefore who may benefit from lung transplant³⁹². Although several parameters of the BODE index and BSI overlap, it would be of great interest to clinicians in AATD, but also in any form of bronchiectasis-COPD overlap syndrome BCOS, to validate a mortality- and morbidity-

predicting score such as BSI or BODE in BCOS. Future research is warranted for this specific aim.

5.4.6 Influence of covariates on clinical outcomes

The influence of the chosen covariates on clinical outcomes largely mirrored the associations seen for the whole cohort in the previous chapter. Ever-smoker status was associated with lower baseline FEV₁% predicted value, exacerbation frequency, and mMRC score. COPD diagnosis was associated with higher mMRC scores, but also was associated with a lower likelihood of exacerbating once or more per year. The covariates in the exacerbation model only encompassed 3.5% of variability in the cohort, limiting the interpretation of this unexpected result.

Phenotype was not reliably correlated with the various outcomes. This is unexpected, since the SZ phenotype has recently been shown not to increase the probability of lung disease⁵³, and therefore would have been expected to be significantly associated with less severe bronchiectasis and less severe clinical outcomes compared with the ZZ phenotype. However, the small numbers of SZ patients in this analysis are probably too low to demonstrate this. Furthermore, if the lack of relationship with phenotype is genuine, this may simply be because the non-severe phenotypes (SZ, MZ, SS) had other causes of bronchiectasis, which were not reliably excluded in our dataset, as we have seen.

The lack of covariate associations in lung function decline is also unexpected, but average yearly change in lung function was small, meaning it may simply be that longer follow-up periods, or more patients, are required to demonstrate an effect. Unfortunately, these strategies are a challenge in rare diseases.

5.4.7 Algorithmic CT bronchiectasis analysis

Although not all CT scans were able to be analysed by the YACTA software, a significant number were, and non-compatibility of some scans is to be expected given the length of time since they were performed, and the advances in CT imaging which have developed in the intervening years. Despite this, a good range of bronchiectasis morphology and severity was included. However, as in the visual assessment, only a small minority had cystic or widespread disease, reducing statistical power of modelling in some cases. A minority of scans were from rare variants: the inclusion of these patients in statistical analysis increases the validity of the data in application to such patients, who are otherwise rarely studied.

5.4.7.1 Correlation of YACTA data with visual assessment of bronchiectasis

The first aim of this analysis was to see how well YACTA parameters correlated with a physician's assessment of bronchiectasis on CT scanning. Bronchiectasis Index (BEI), the measure of lack of tapering on YACTA, correlated with visual assessment of bronchial dilatation, bronchial wall thickness score, morphology, and number of lobes affected. However, caution is required in interpreting these associations, as they were generally weak - the maximum R² value for these models was 0.211, indicating only 21% of the variability in the dataset could be accounted for by the variables used.

When subdivided by lobe, BEI correlated with visual identification of bronchiectasis in only 4 out of 6 lobes, showing no correlation in the right middle and right lower lobes. This discrepancy in the right middle lobe is consistent with the Cohen's κ analysis on visual assessment, indicating that the right middle lobe may be a particularly difficult

lobe to assess visually; however, Cohen's κ data for the right lower lobe showed the strongest level of agreement of any lobe, making the lack of association in this lobe somewhat unexpected. One possible reason is that only 10% of scans were over-read by a radiologist, but a far larger percentage were able to be analysed by YACTA - by chance this 10% may not have included scans with more complex right lower lobe disease.

AWPi10 did not correlate with bronchial wall thickness score, which is consistent with the Cohen's κ analysis in the previous chapter, in which there was only a moderate level of agreement between physician and radiologist. Bronchial wall thickness may therefore be a difficult parameter for a physician to assess by eye, and certainly appears so from available literature ³³⁹. AWPi10 has been validated in other studies, and in this study showed some utility in identification of ever-smokers.

5.4.7.2 Thresholds at which YACTA data correlates with visual assessment of bronchiectasis

Secondly, several thresholds were identified at which BEI and AWPi10 were associated with various visual parameters. For many of these, sensitivity and specificity were too low to be practically useful, although a cutoff for BEI of 1.07 seemed to identify bronchial dilatation of 2x the arterial diameter, 4 or more lobe involvement, and varicose/cystic bronchiectasis, suggesting 1.07 may be the most useful threshold to use in practice. Sensitivity of this value is more important than specificity, as this will identify a greater proportion of patients, and any false positives could be addressed by visual assessment by a radiologist.

Furthermore, the abilities of BEI to associate with the visual parameters of bronchial dilatation score of ≥ 2 , ≥ 4 lobe involvement, and the presence of varicose or cystic bronchiectasis, achieved adequate sensitivity and specificity according to published principles for high-quality clinical testing ³⁹³, and at a similar standard to lung cancer screening in the majority of studies in that field ³⁹⁴. It is known that bronchiectasis involving multiple lobes, or of cystic morphology, is associated with hospitalisation and mortality ^{187,188}. With refinement, the thresholds identified here could conceivably lead to efficiencies in radiology reporting, by identifying patients who are likely to have these most clinically relevant radiological patterns. Only a small number of our patients had exactly 3 lobes affected, which may have reduced the ability of the models and ROC analysis to identify an optimal threshold for 3 or more lobes, as Chalmers et al did ¹⁸⁸, but studies of larger numbers of CT scans may be able to improve on this. Indeed, published validation data for YACTA shows that certain parameters may have clinical relevance: airway wall thickness and bronchial diameter in the intermediate airways was negatively correlated with FEV₁pp in adults with cystic fibrosis in one small study ¹⁹⁹, and wall thickness has also been negatively correlated with FEV₁pp in COPD patients ³⁹⁵, although wall thickness is only a non-specific sign of bronchiectasis and is also present in chronic bronchitis¹⁷⁸.

5.4.7.3 Correlation of YACTA data with clinical outcomes

Thirdly, the relationship of YACTA parameters with clinical outcomes was assessed. The correlation of BEI with baseline FEV₁pp was statistically significant, but is unlikely to be clinically relevant according to current consensus on minimum clinically

important differences for spirometry, where 5-10% change is the range of thresholds used for drug development ³⁹⁶, and 6% variability is seen in the majority of individuals ³⁹¹. Additionally, the direction of the association is unexpected: patients with bronchiectasis appear to have slightly better baseline FEV_{1pp}. This could be explained by the degree of airways obstruction on referral to tertiary services: those with bronchiectasis, or bronchiectasis-COPD overlap, are likely to have less severe airways obstruction at referral. Airways obstruction only develops later in the natural history of bronchiectasis, and instead the core symptoms of bronchiectasis, such as exacerbations or phlegm production, are likely to prompt referral in these cases. AWPi10 correlated more strongly with baseline FEV_{1pp}, and with a negative relationship. Although it did not correlate with lung function decline, it did increase the odds of higher mMRC scores. However, as a measure of airway thickness, AWPi10 is not specific as a marker of bronchiectasis, and may represent chronic bronchitis in many cases. As a constituent part of COPD, chronic bronchitis will have a direct relationship with baseline FEV_{1pp} and mMRC in these cases.

The threshold of BEI used to identify bronchial dilatation was not correlated with mortality; this is unexpected, since the visual parameter of bronchial dilatation was significantly correlated with a small increase in mortality risk. However, as described in chapter 3 and section 3.3 of this chapter, visual and algorithmic assessment of dilatation are very different: visual assessment relied on relationship to arterial diameter, which can be influenced by pulmonary hypertension among other things, but BEI is more objective by quantifying tapering through the entire airway. As the more objective measure found no association with mortality, the association of visual bronchial dilatation with mortality must be interpreted with caution.

BEI also correlated with BSI score, despite not correlating with mortality or exacerbations. This may simply be because of the relationship between BEI and number of lobes affected, and with morphology, both of which feature in the radiology section of the BSI.

5.4.8 Strengths and weaknesses

Weaknesses of all models in this analysis include the correlation coefficients, which were at best only moderate, and in some cases rather weak. This suggests variance within the model is due to variables which are not accounted for in the analysis, a common finding in clinical datasets. Random variation will account for some of the remaining variability, but other contributing factors may include early childhood exposures³⁹⁷, occupation and exercise habits, which are not stored reliably in the database.

Strengths include level of detail available from YACTA, which is rare among radiological algorithms in being able to create a 3-dimensional model of the bronchial tree. Automatic algorithms are increasingly used in research of lung diseases, but their main validated utility has so far been the assessment of lung density rather than the airways³⁹⁸. Perossi et al published work in 2022 demonstrating the association of algorithm-derived luminal area and AWPi10 with poorer spirometry, impulse oscillometer measurements and bronchiectasis severity index scores³⁵¹. However, they did not use the bronchiectasis index used here, which requires further validity study.

A further great strength of the YACTA approach is its objective assessment of bronchial dilatation by analysis of bronchial tapering, which is distinct from other algorithms which have used airway-artery ratio, as is common in visual assessment^{179,399}. As mentioned in the previous chapter, assessment of bronchiectasis relative to the accompanying artery is open to bias based on dilatation or constriction of the artery. Use of automated analysis of tapering such as YACTA means these biases are avoided, and the previously time-consuming tapering analysis can be performed at scale.

5.5 Conclusions

Overall, this chapter has demonstrated that bronchiectasis in alpha-1 antitrypsin deficiency is usually mild and of a lower lobe preponderance. However, it bears clinical relevance, as degree of bronchial dilatation is associated with mortality, and exacerbation rate is increased in those with cystic bronchiectasis who exacerbate. Degree of bronchial dilatation appears to be one of the more difficult visual parameters for observers to agree on, which supports the use of objective or automatic measures of airways being used for analysis.

6 Conclusions and future directions

6.1 Summary of the thesis

This thesis has provided a wide-ranging exposition of bronchiectasis in Alpha-1 Antitrypsin Deficiency, with several key findings and relevance for clinical practice.

Firstly, in chapter 2 the pre-existing literature was confirmed to be lacking in subcategorisation of bronchiectasis. This strengthened the case for the rest of the thesis.

Secondly, in chapter 3, bronchiectasis was found to be independently associated with serum alpha-1 antitrypsin, but not with COPD, thus clarifying a longstanding point of uncertainty. However, due to the retrospective nature of this analysis it is not appropriate to recommend a change in bronchiectasis screening practice, though in cases of bronchiectasis and emphysema the use of alpha-1 antitrypsin deficiency screening would be appropriate. This satisfies research objective 1 (section 1.5.1).

Thirdly, relating to research objective 5 (section 1.5.5), microbiological findings were found to correlate broadly with evidence in usual bronchiectasis, thus providing confidence in treating *pseudomonas* colonised patients with AATD-bronchiectasis according to existing guidelines.

Fourthly, the association of CRP with clinical outcomes supports the use of this biomarker in routine blood tests in AATD, and is easily integrated into clinical practice given the availability of the test in NHS laboratories. The correlation of eosinophils and platelets with clinical outcomes also suggests clinicians should pay close attention to these routine markers. Although a more comprehensive network analysis of

inflammatory biomarkers could not be completed within the timeframe of this work, such an analysis could reveal further associations.

Fifthly, bronchiectasis has been assessed visually and found to be predominantly mild and to affect the lower lobes. Extent and morphology of bronchiectasis in AATD has been shown to associate with clinical outcomes including exacerbation rate and gas transfer, as they have in other causes of bronchiectasis. Bronchiectasis in AATD is usually mild, but its presence and severity are associated with an increase in mortality. This satisfies research objective 2 (section 1.5.2). Furthermore, the most commonly used predictive tool in all-cause bronchiectasis, namely BSI, has been validated in AATD-bronchiectasis. These findings suggest that, within an AATD population, bronchiectasis constitutes a distinct phenotype which may be used to select patients for trials of emerging drugs, or to guide day to day therapy towards those already recommended in bronchiectasis of other aetiologies. However, since most AATD patients will also have COPD, trials of new drugs for AATD-bronchiectasis specifically is too small a niche.

Finally, relating to research objective 4 (section 1.5.4), an automated algorithm has been used to confirm a physician's visual assessment of bronchiectasis, with corresponding thresholds, which may ultimately allow automated analysis of bronchiectasis in clinical practice and reduce clinicians' workload.

6.2 Clinical implications

This work has provided evidence to support the inclusion of patients with AATD, bronchiectasis, but no COPD, in clinical trials of emerging drugs. As seen in chapter 4, a minority (11.5%) of PiZZ patients with a clinical diagnosis of bronchiectasis did not also have COPD. Whilst this is a small number in our cohort, when extrapolating to

the wider populations needed for approval of a new drug, and to the increased numbers expected to be identified with increased awareness and testing for AATD, such patients form a not insignificant minority of PiZZ AATD patients, who should not be excluded from the research available to those with concurrent COPD.

The validation of the BSI in patients with AATD is among the most clinically important findings of this thesis. As a rare disease with overlap with a very common disease (usual-COPD), AATD relies on extrapolation from data from usual-COPD and usual-bronchiectasis to inform its management; however, as discussed in section 1.1.2.2, there are many differences between AATD and these conditions⁴⁰⁰. As such, the findings presented in section 5.2 provide valuable clinical confidence in giving prognostic information to patients with AATD and bronchiectasis.

6.2.1 Screening for AATD in idiopathic bronchiectasis

In section 4.1, evidence was found, discussed in section 4.4, to suggest that there may be an independent relationship between AATD and bronchiectasis. Several of the classical criteria for adequate screening tests by Wilson and Jungner⁴⁰¹ have been met by this work: the natural history of the disease is now better understood; an accurate, cheap and non-invasive test is available (serum AAT and genotyping); there is a recognisable early stage (i.e. confirmed genotype before lung disease develops); and in many countries a treatment is available, with many more becoming available in the near future. However, in the UK at least, disease-specific treatment is currently not available, it remains difficult to determine which patients will deteriorate, and as a rare disease the yield from screening all cases of idiopathic bronchiectasis is low⁴⁰², meaning it may not constitute an important health problem for a nationwide health service. Furthermore, since this work has used largely retrospective data, the evidence

threshold to support widespread screening for bronchiectasis in AATD has not been met.

6.3 Endotyping bronchiectasis in AATD

This findings of this thesis have also contributed to the concept of endotyping in bronchiectasis associated with alpha-1 antitrypsin deficiency. Endotyping involves stratifying patients into biologically distinct subgroups based on underlying pathophysiological mechanisms. In this cohort, the association of *Pseudomonas* growth and elevated eosinophil counts, and lack of association with other species or inflammatory markers, suggests an inflammatory and microbial profile which helps to distinguish bronchiectasis-AATD from other phenotypes of AATD. However, the strength of these relationships was modest, and no significant differences were observed in other inflammatory markers, including IL-6, IL-8, TNF- α , platelets, or CRP, nor in the prevalence of other bacterial species. These results imply that while certain features, such as eosinophilia and *Pseudomonas* growth, may contribute to bronchiectasis in AATD, this does not appear to reflect a broader inflammatory dysregulation or microbiome imbalance. These findings are therefore not sufficient to advocate for specific targeted therapies, but the identification of even subtle patterns in inflammatory or microbial markers may offer insights into the mechanisms driving bronchiectasis in AATD and thereby guide hypotheses in future research, as discussed in section 6.6 below.

6.4 Strengths of the thesis

This work has included the largest population of patients with AATD-Bronchiectasis available at present; studies with larger samples would only be possible with international collaboration. This has increased the knowledge of this less common AATD manifestation. Additionally, the thesis has included rare genotypes, unlike many similar studies. This means that patients with such rare genotypes, and the physicians who treat them, can be more confident in applying the results of this work, rather than relying on findings in more common genotypes and assuming the results will apply to them.

The use of an algorithmic software to objectively describe airway dilatation is little used in the literature to date, and as such provides important evidence to support ongoing development of a clinically useful algorithm. Finally, there is now clear evidence to support the use of the BSI in AATD, giving patients and their clinicians more confidence in giving accurate estimates of prognosis. This is of great importance, as hitherto both patients and their clinicians may have had doubts in applying this score, due to the differences in underlying aetiology between the original BSI cohort and AATD.

6.5 Limitations

The main limitation of this thesis is its retrospective nature. This results in multiple limitations, but the most important is the relatively low proportion of patients who were appropriately investigated for other causes of bronchiectasis. As previously discussed, this was unavoidable, since many patients' baseline appointments were before the point at which clear guidance for bronchiectasis investigations was available. This limitation was mitigated by statistical adjustment for the presence of bronchiectasis, and by finding the prevalence of bronchiectasis in AATD to be higher compared with

all-cause bronchiectasis in the general population. This is still a useful finding, but would be a more robust conclusion if all patients had been appropriately screened for alternative bronchiectasis causes as per BTS guidance. Additionally, bronchiectasis was identified in several patients not previously given a clinical diagnosis (Table 4.3), which introduces an element of classification bias to the models demonstrating no relationship between bronchiectasis and COPD shown in Figure 4.2 and 4.3. However, as shown in Table 4.3, such diagnostic inaccuracy was low, meaning these models remain valid.

Furthermore, radiology, microbiology and serum measurements were taken at variable times, and compared to lung function tests which may not have been taken at the same time. This limitation is a product of the geographically widespread nature of the cohort, with some patients sending in sputum from many miles away, or having CT scans remotely, whilst attending for lung function tests on different days. The discrepancy between the recorded lung function and the actual lung function on the day of the CT scan, or sputum production, may account for some of the variability not encompassed by the statistical models in this work. However, in a rare disease with a limited patient population spread over a wide geographic area, such variability is almost unavoidable in a retrospective study.

New and retrospective results were pooled in order to generate the largest samples sizes possible; however, these results are taken from a wide timespan and laboratory techniques may have varied across the years. Older results may therefore be less accurate, although the microbiological techniques used for the new sputum samples sent in are well established. CT scan technique in particular has developed significantly since the oldest scans included here were performed, therefore the visual assessment of the older scans could have been less sensitive than the new ones.

Concordance between visual and algorithmic assessment of bronchiectasis stratified by age of scan was not studied here, but could be an interesting subject of future research to ensure conclusions of research based on older CT scan data are valid.

Not all originally envisaged methods were available within the timeframe of this work. The proteomic analysis was not able to be performed, leading to reliance on historical data, which only gave a limited view of the inflammatory cascade. In particular, CRP results relied on clinical data, which, as discussed in section 4.3.4.7, meant that values under 5mg/L could not be accurately reported. Furthermore, the laboratory used for the microbiology data could not safely study *Mycobacteriae*, and lacked the necessary equipment for the study of the microbiome by PCR, both of which are known to be important for the study of pathogenesis and disease progression in bronchiectasis^{214,403}. The study of the microbiome specifically could have provided a more comprehensive assessment of sputum bacterial colonisation, although without differentiating between living and dead organisms, meaning combination with the culture techniques as presented in chapter 3 would still have been necessary. Additionally, neither the visual nor algorithmic assessment of radiological bronchiectasis could analyse for mucous plugging in this work. This is discussed further in section 6.7.2 in the context of recommendation for future research.

Analyses were limited by being conducted by a physician with limited microbiology and radiology training compared to specialists in those fields. However, these factors were addressed by good inter-rater reliability and concordance with an automated algorithm in chapter 5, and the aid of microbiology specialists in chapter 4.

6.6 Publications and presentations

Citations for publications and presentation arising from this thesis are given in Appendix section 7. Work from chapter 1 summarising emerging treatments in AATD has been published in *Expert Opinion on Emerging Drugs*⁴⁰⁴. Work from chapter 2 was presented at the European Respiratory Society congress 2023⁴⁰⁵. Work from chapter 4 section 1 has been published in *Journal of COPD*⁴⁰⁶, and incorporates the basic visual assessment data from chapter 5. Work from chapter 4 section 3 is intended to be combined with the delayed full biomarker panel data for publication in a more extensive network analysis of proteomic data and Z polymer levels, but could include the eosinophil and platelet data. Work from chapter 5 on clinical outcomes of the visual assessment parameters has been presented at the winter meeting of the British Thoracic Society 2024. Combining this data with the YACTA algorithm data will result in another manuscript intended for publication over the coming months.

6.7 Future research

6.7.1 Potential for prospective validation

Since a major limitation of this thesis is much of the data's retrospective nature, it is reasonable to suggest equivalent prospective work. This could build on the BSI score data to assess whether it may also be predictive of hospital admissions in AATD-bronchiectasis, as the derivation study found¹⁸⁸. The findings of this thesis could be compared with matched patients with other causes of bronchiectasis, to confirm whether or not there are any specific features to AATD-bronchiectasis. Using a single cohort may not result in enough events (admissions) to achieve enough statistical

power, so collaborative work with EARCO would be a better method to increase sample size.

6.7.2 Further radiological techniques

One component of clinically significant bronchiectasis is mucous plugging. This is associated with all-cause mortality in COPD^{407,408}, and has been associated with poorer outcomes in some causes of bronchiectasis: poorer lung function and exacerbation rate in ABPA⁴⁰⁹, along with predicting progression in cystic fibrosis⁴¹⁰. Furthermore, recent developments show automated analysis of mucous plugging in COPD to independently associate with mortality and GOLD severity^{411,412}. Unfortunately, mucus plugging was not able to be assessed as part of this thesis, as findings on visual CT assessment are ambiguous even among trained radiologists (see section 5.3.3), and the algorithm used did not have the capability to assess this at the time of writing. However, given the importance of mucus clearance to all causes of bronchiectasis, it would be of great interest to assess whether the degree of mucus impaction on CT correlates with disease severity or progression in AATD lung disease. Other software is available that can assess mucus plugging, including LungQ⁴¹², but its use would require extra funding to cover licensing that software.

6.7.3 Further microbiological techniques

The microbiology work presented here relied on bacterial culture, but this has long been known to be inadequate in fully representing the complex ecosystem of the respiratory tract. Microbiomics analysis is now capable of this, using 16S ribosomal RNA sequencing, which is distinct to each species of microorganism due to the uniqueness of their genetics. This technique has already revealed new findings in COPD, with significance of organisms which would be dismissed as “mixed normal

flora” in a culture-based approach, such as *Veillonella*⁴¹³. However, this comes with its own limitations, in not being able to distinguish between dead or living bacteria, and not being able to accurately represent bacterial load⁴¹⁴. Fully standardised protocols for 16S sequencing, and ensuing computational analysis have also not yet been established⁴¹⁵. Nonetheless, a full understanding of the microbiome in bronchiectasis may reveal as yet unknown treatable traits to guide development of novel treatments. Microbiome studies outside AATD have been informative⁴¹⁶, highlighting the importance of reducing bacterial diversity in bronchiectasis: conducting similar studies in AATD may reveal equivalent endotypes of patients. Therapeutic targeting of the respiratory microbiome in general is understudied⁴¹⁷, and no novel therapeutic strategies for the treatment of bacterial diversity have been suggested to date, but the concept is a frequently identified area of research priority⁴⁰³, and would rely on a detailed understanding of such endotypes.

6.7.3.1 Difficulty in obtaining sputum samples

All research concerned with respiratory microbiology is limited by the fact that not all patients will be able to produce sputum, and still fewer will be able to provide more than one sample. However, invasive strategies to obtain samples, such as bronchoscopy or induced sputum, have potentially unacceptable risks in a research setting, including dilutional issues or bronchospasm. It is therefore of great importance to establish whether a non-invasive surrogate marker of the lung microbiome could be clinically useful. Oropharyngeal swabs are a candidate: some evidence exists that the lung microbiome correlates with that of the oropharynx in healthy individuals^{418,419}, but this would need validation in bronchiectasis and COPD.

6.7.4 Further serum biomarker techniques

This thesis was only able to examine limited biomarkers, due to logistical delays. Consequently, there remains scope for more complex network analyses of inflammatory biomarkers, to assess whether single or clusters of biomarkers may associate with clinical outcomes. This gives the advantage of an unbiased, exploratory approach which can potentially reveal new inflammatory pathways of relevance. Furthermore, given the instability of these measurements over time, measures of longitudinal change may be appropriate, and given the number of tests this is likely to involve, proteomics data would be the best way of assessing this. Proteomics refers to the reporting of all proteins within a sample, and has already been used to correlate certain serum proteins or groups of proteins with FEV₁ changes⁴²⁰, gas transfer decline⁴²¹, mortality⁴²², and exacerbation status⁴²³. Although sputum proteomics in bronchiectasis have been studied, finding an association with *P. aeruginosa* infection⁴²⁴, serum proteomics are less well studied. However, it is important to find out whether serum biomarkers correlate with clinical outcomes, and not simply rely on findings from sputum biomarkers, as one group have found that the two do not necessarily correlate⁴²⁵. The work scheduled within the Birmingham AATD registry will use SomaScan®, a platform capable of identifying over 5000 proteins, and which has already been shown to be of value in identifying imaging outcomes in patients with cystic fibrosis⁴²⁶. Such vast quantities of data may require the use of statistical methods not used here, including principal component analysis⁴²⁷. Such analysis has previously been used to identify five endotypes of the bronchiectasis-COPD overlap syndrome²³⁰. I plan to be involved in the statistical analysis of this project, which will also be submitted for publication.

6.7.5 Comparison with other causes of bronchiectasis

There is also scope to compare this data to that in usual-COPD-bronchiectasis overlap. This would be helpful to clarify whether AATD-bronchiectasis differs from other causes of bronchiectasis sufficiently to require differing treatment strategies. However, such an endotype-based approach is not always used even in the more common phenotype of AATD-emphysema: evidence suggests that lung volume reduction surgery (LVRS) is beneficial for upper lobe emphysema, but benefits for lower lobe emphysema (as seen in AATD, see introduction section 1.1.2.2) are less clear⁴²⁸, yet patients with AATD-emphysema are frequently still treated with LVRS.

Potential collaborations include with the European Multicentre Bronchiectasis Audit and Research Collaboration (EMBARC), which maintains a pan-European registry of patients with non-cystic fibrosis bronchiectasis. Requests have been sent to the team to collaborate using the data presented in this work.

6.7.6 The importance of bronchiectasis in co-existing COPD

Whilst this thesis has controlled for the presence of COPD in its statistical models, there remains insufficient detail in the literature base to guide clinicians faced with bronchiectasis-COPD overlap syndrome (BCOS). In such circumstances, experienced clinicians will rely on their judgement to decide whether to base prognostic estimates on COPD severity (as per GOLD⁷⁹ etc) or bronchiectasis severity (as per BSI¹⁸⁸ or FACED¹⁸⁷). The inclusion of emphysema in the BRICS¹⁸⁰ score can be seen as an acknowledgement of a demand for a severity index combining both diseases. This is an important area of future research, and one which should include AATD patients as a cohort in which the two diseases often coexist.

6.8 Final conclusion

To address the final research objective (section 1.5.6), bronchiectasis in alpha-1 antitrypsin deficiency occurs independently of COPD, but is usually mild. However, its presence and severity are correlated with mortality, as it is in other causes. It therefore constitutes an important clinical phenotype of alpha-1 antitrypsin deficiency, and may be used as a distinct phenotype in clinical trials of emerging therapies. However, since the majority of patients will also have COPD, further research in BCOS is required to clarify treatment strategies in this overlap.

7 References

1. Brantly ML, Wittes JT, Vogelmeier CF, Hubbard RC, Fells GA, Crystal RG. Use of a highly purified alpha 1-antitrypsin standard to establish ranges for the common normal and deficient alpha 1-antitrypsin phenotypes. *Chest*. Sep 1991;100(3):703-8. doi:10.1378/chest.100.3.703
2. Ryu SE, Choi HJ, Kwon KS, Lee KN, Yu MH. The native strains in the hydrophobic core and flexible reactive loop of a serine protease inhibitor: crystal structure of an uncleaved alpha1-antitrypsin at 2.7 Å. *Structure*. Oct 15 1996;4(10):1181-92. doi:10.1016/s0969-2126(96)00126-8
3. Elliott PR, Lomas DA, Carrell RW, Abrahams JP. Inhibitory conformation of the reactive loop of alpha 1-antitrypsin. *Nat Struct Biol*. Aug 1996;3(8):676-81. doi:10.1038/nsb0896-676
4. Dementiev A, Dobo J, Gettins PG. Active site distortion is sufficient for proteinase inhibition by serpins: structure of the covalent complex of alpha1-proteinase inhibitor with porcine pancreatic elastase. *J Biol Chem*. Feb 10 2006;281(6):3452-7. doi:10.1074/jbc.M510564200
5. Huntington JA, Read RJ, Carrell RW. Structure of a serpin-protease complex shows inhibition by deformation. *Nature*. Oct 19 2000;407(6806):923-6. doi:10.1038/35038119
6. Yamasaki M, Sendall TJ, Pearce MC, Whisstock JC, Huntington JA. Molecular basis of alpha1-antitrypsin deficiency revealed by the structure of a domain-swapped trimer. *EMBO Rep*. Sep 30 2011;12(10):1011-7. doi:10.1038/embor.2011.171
7. Lomas DA, Evans DL, Stone SR, Chang WS, Carrell RW. Effect of the Z mutation on the physical and inhibitory properties of alpha 1-antitrypsin. *Biochemistry*. Jan 19 1993;32(2):500-8. doi:10.1021/bi00053a014
8. Lomas DA, Evans DL, Finch JT, Carrell RW. The mechanism of Z alpha 1-antitrypsin accumulation in the liver. *Nature*. Jun 18 1992;357(6379):605-7. doi:10.1038/357605a0
9. Schroeder WT, Miller MF, Woo SL, Saunders GF. Chromosomal localization of the human alpha 1-antitrypsin gene (PI) to 14q31-32. *Am J Hum Genet*. Sep 1985;37(5):868-72.
10. Sinden NJ, Koura F, Stockley RA. The significance of the F variant of alpha-1-antitrypsin and unique case report of a PiFF homozygote. *BMC Pulm Med*. Aug 7 2014;14:132. doi:10.1186/1471-2466-14-132
11. Cook L, Burdon J, Brenton S, Janus ED, Knight K. Alpha-1-antitrypsin P^{Lowell}: a normally functioning variant present in low concentration. *Aust N Z J Med*. Dec 1995;25(6):695-7. doi:10.1111/j.1445-5994.1995.tb02855.x
12. Figueira Goncalves JM, Martinez Bugallo F, Diaz Perez D, Martin Martinez MD, Garcia-Talavera I, Pitti Perez R. Clinical manifestations of the Mmalton alpha-1 antitrypsin deficiency variant. *Pulmonology*. Dec 12 2017;doi:10.1016/j.rppnen.2017.11.004
13. Blanco I, Fernandez E, Bustillo EF. Alpha-1-antitrypsin PI phenotypes S and Z in Europe: an analysis of the published surveys. *Clin Genet*. Jul 2001;60(1):31-41. doi:10.1034/j.1399-0004.2001.600105.x
14. de Serres FJ, Blanco I. Prevalence of α1-antitrypsin deficiency alleles PI*S and PI*Z worldwide and effective screening for each of the five phenotypic classes PI*MS,

- PI*MZ, PI*SS, PI*SZ, and PI*ZZ: a comprehensive review. *Therapeutic Advances in Respiratory Disease*. 2012;6(5):277-295. doi:10.1177/1753465812457113
15. Haq I, Irving JA, Saleh AD, et al. Deficiency Mutations of Alpha-1 Antitrypsin. Effects on Folding, Function, and Polymerization. *Am J Respir Cell Mol Biol*. Jan 2016;54(1):71-80. doi:10.1165/rcmb.2015-0154OC
 16. Silverman EK, Miletich JP, Pierce JA, et al. Alpha-1-antitrypsin deficiency. High prevalence in the St. Louis area determined by direct population screening. *Am Rev Respir Dis*. Oct 1989;140(4):961-6. doi:10.1164/ajrccm/140.4.961
 17. Stoller JK, Sandhaus RA, Turino G, Dickson R, Rodgers K, Strange C. Delay in diagnosis of alpha1-antitrypsin deficiency: a continuing problem. *Chest*. Oct 2005;128(4):1989-94. doi:10.1378/chest.128.4.1989
 18. Stoller JK, Brantly M. The challenge of detecting alpha-1 antitrypsin deficiency. *COPD*. Mar 2013;10 Suppl 1:26-34. doi:10.3109/15412555.2013.763782
 19. Blanco I, Bueno P, Diego I, et al. Alpha-1 antitrypsin Pi*SZ genotype: estimated prevalence and number of SZ subjects worldwide. *Int J Chron Obstruct Pulmon Dis*. 2017;12:1683-1694. doi:10.2147/COPD.S137852
 20. Blanco I, Bueno P, Diego I, et al. Alpha-1 antitrypsin Pi*Z gene frequency and Pi*ZZ genotype numbers worldwide: an update. *Int J Chron Obstruct Pulmon Dis*. 2017;12:561-569. doi:10.2147/COPD.S125389
 21. Kaplan PD, Kuhn C, Pierce JA. The induction of emphysema with elastase. I. The evolution of the lesion and the influence of serum. *J Lab Clin Med*. Sep 1973;82(3):349-56.
 22. Lucey EC, Stone PJ, Christensen TG, Breuer R, Snider GL. An 18-month study of the effects on hamster lungs of intratracheally administered human neutrophil elastase. *Exp Lung Res*. 1988;14(5):671-86. doi:10.3109/01902148809087836
 23. Borel F, Sun H, Zieger M, et al. Editing out five Serpina1 paralogs to create a mouse model of genetic emphysema. *Proc Natl Acad Sci U S A*. Mar 13 2018;115(11):2788-2793. doi:10.1073/pnas.1713689115
 24. Guttman O, Baranovski BM, Schuster R, et al. Acute-phase protein alpha1-anti-trypsin: diverting injurious innate and adaptive immune responses from non-authentic threats. *Clin Exp Immunol*. Feb 2015;179(2):161-72. doi:10.1111/cei.12476
 25. Sanders CL, Ponte A, Kueppers F. The Effects of Inflammation on Alpha 1 Antitrypsin Levels in a National Screening Cohort. *COPD*. Feb 2018;15(1):10-16. doi:10.1080/15412555.2017.1401600
 26. Eriksson S. Pulmonary Emphysema and Alpha1-Antitrypsin Deficiency. *Acta Med Scand*. Feb 1964;175:197-205. doi:10.1111/j.0954-6820.1964.tb00567.x
 27. Anthonisen NR, Manfreda J, Warren CP, Hershfield ES, Harding GK, Nelson NA. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. *Ann Intern Med*. Feb 1987;106(2):196-204. doi:10.7326/0003-4819-106-2-196
 28. Dowson LJ, Guest PJ, Stockley RA. Longitudinal changes in physiological, radiological, and health status measurements in alpha(1)-antitrypsin deficiency and factors associated with decline. *Am J Respir Crit Care Med*. Nov 15 2001;164(10 Pt 1):1805-9. doi:10.1164/ajrccm.164.10.2106036
 29. Seemungal TA, Donaldson GC, Paul EA, Bestall JC, Jeffries DJ, Wedzicha JA. Effect of exacerbation on quality of life in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. May 1998;157(5 Pt 1):1418-22. doi:10.1164/ajrccm.157.5.9709032
 30. Donaldson GC, Seemungal TA, Bhowmik A, Wedzicha JA. Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. *Thorax*. Oct 2002;57(10):847-52. doi:10.1136/thorax.57.10.847

31. Strnad P, Brantly ML, Bals R. *α 1-Antitrypsin Deficiency*. 2019:208.
32. Wencker M, Marx A, Konietzko N, Schaefer B, Campbell EJ. Screening for alpha1-Pi deficiency in patients with lung diseases. *Eur Respir J*. Aug 2002;20(2):319-24. doi:10.1183/09031936.02.02012001
33. Tomashefski JF, Jr., Crystal RG, Wiedemann HP, Mascha E, Stoller JK, Alpha 1-Antitrypsin Deficiency Registry Study G. The bronchopulmonary pathology of alpha-1 antitrypsin (AAT) deficiency: findings of the Death Review Committee of the national registry for individuals with Severe Deficiency of Alpha-1 Antitrypsin. *Hum Pathol*. Dec 2004;35(12):1452-61. doi:10.1016/j.humpath.2004.08.013
34. Stavngaard T, Shaker SB, Bach KS, Stoel BC, Dirksen A. Quantitative assessment of regional emphysema distribution in patients with chronic obstructive pulmonary disease (COPD). *Acta Radiol*. Nov 2006;47(9):914-21. doi:10.1080/02841850600917170
35. Nakano Y, Coxson HO, Bosan S, et al. Core to rind distribution of severe emphysema predicts outcome of lung volume reduction surgery. *Am J Respir Crit Care Med*. Dec 15 2001;164(12):2195-9. doi:10.1164/ajrccm.164.12.2012140
36. Fishman A, Martinez F, Naunheim K, et al. A randomized trial comparing lung-volume-reduction surgery with medical therapy for severe emphysema. *N Engl J Med*. May 22 2003;348(21):2059-73. doi:10.1056/NEJMoa030287
37. Piras B, Ferrarotti I, Lara B, et al. Clinical phenotypes of Italian and Spanish patients with alpha1-antitrypsin deficiency. *Eur Respir J*. Jul 2013;42(1):54-64. doi:10.1183/09031936.00104712
38. National Institute for Health and Care Excellence: Guidelines. *Chronic obstructive pulmonary disease in over 16s: diagnosis and management*. National Institute for Health and Care Excellence (NICE) Copyright © NICE 2018.; 2019.
39. Seemungal TA, Donaldson GC, Bhowmik A, Jeffries DJ, Wedzicha JA. Time course and recovery of exacerbations in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. May 2000;161(5):1608-13. doi:10.1164/ajrccm.161.5.9908022
40. Hiller AM, Piitulainen E, Jøhansson L, Tanash H. Decline in FEV(1) and hospitalized exacerbations in individuals with severe alpha-1 antitrypsin deficiency. *Int J Chron Obstruct Pulmon Dis*. 2019;14:1075-1083. doi:10.2147/COPD.S195847
41. Fahndrich S, Biertz F, Karch A, et al. Cardiovascular risk in patients with alpha-1-antitrypsin deficiency. *Respir Res*. Sep 15 2017;18(1):171. doi:10.1186/s12931-017-0655-1
42. Holme J, Stockley JA, Stockley RA. Age related development of respiratory abnormalities in non-index alpha-1 antitrypsin deficient studies. *Respir Med*. Mar 2013;107(3):387-93. doi:10.1016/j.rmed.2012.12.003
43. Buist AS, Burrows B, Eriksson S, Mittman C, Wu M. The natural history of air-flow obstruction in PiZ emphysema. Report of an NHLBI workshop. *Am Rev Respir Dis*. Feb 1983;127(2):S43-5. doi:10.1164/arrd.1983.127.2P2.S43
44. Brantly ML, Paul LD, Miller BH, Falk RT, Wu M, Crystal RG. Clinical features and history of the destructive lung disease associated with alpha-1-antitrypsin deficiency of adults with pulmonary symptoms. *Am Rev Respir Dis*. Aug 1988;138(2):327-36. doi:10.1164/ajrccm/138.2.327
45. Gadek JE, Fells GA, Crystal RG. Cigarette smoking induces functional antiprotease deficiency in the lower respiratory tract of humans. *Science*. Dec 14 1979;206(4424):1315-6. doi:10.1126/science.316188
46. McElvaney NG, Stoller JK, Buist AS, et al. Baseline characteristics of enrollees in the National Heart, Lung and Blood Institute Registry of alpha 1-antitrypsin

- deficiency. Alpha 1-Antitrypsin Deficiency Registry Study Group. *Chest*. Feb 1997;111(2):394-403. doi:10.1378/chest.111.2.394
47. Tanash HA, Ekstrom M, Ronmark E, Lindberg A, Piitulainen E. Survival in individuals with severe alpha 1-antitrypsin deficiency (PiZZ) in comparison to a general population with known smoking habits. *Eur Respir J*. Sep 2017;50(3)doi:10.1183/13993003.00198-2017
 48. Fahndrich S, Herr C, Greulich T, et al. Sex differences in alpha-1-antitrypsin deficiency lung disease-analysis from the German registry. *COPD*. May 2015;12 Suppl 1:58-62. doi:10.3109/15412555.2015.1023785
 49. Dahl M, Hersh CP, Ly NP, Berkey CS, Silverman EK, Nordestgaard BG. The protease inhibitor Pi*S allele and COPD: a meta-analysis. *Eur Respir J*. Jul 2005;26(1):67-76. doi:10.1183/09031936.05.00135704
 50. Dahl M, Tybjaerg-Hansen A, Lange P, Vestbo J, Nordestgaard BG. Change in lung function and morbidity from chronic obstructive pulmonary disease in alpha1-antitrypsin MZ heterozygotes: A longitudinal study of the general population. *Ann Intern Med*. Feb 19 2002;136(4):270-9. doi:10.7326/0003-4819-136-4-200202190-00006
 51. Molloy K, Hersh CP, Morris VB, et al. Clarification of the risk of chronic obstructive pulmonary disease in alpha1-antitrypsin deficiency PiMZ heterozygotes. *Am J Respir Crit Care Med*. Feb 15 2014;189(4):419-27. doi:10.1164/rccm.201311-1984OC
 52. Thun GA, Ferrarotti I, Imboden M, et al. SERPINA1 PiZ and PiS heterozygotes and lung function decline in the SAPALDIA cohort. *PLoS One*. 2012;7(8):e42728. doi:10.1371/journal.pone.0042728
 53. Franciosi AN, Hobbs BD, McElvaney OJ, et al. Clarifying the Risk of Lung Disease in SZ Alpha-1 Antitrypsin Deficiency. *Am J Respir Crit Care Med*. Jul 1 2020;202(1):73-82. doi:10.1164/rccm.202002-0262OC
 54. Parr DG, Stoel BC, Stolk J, Stockley RA. Pattern of emphysema distribution in alpha1-antitrypsin deficiency influences lung function impairment. *Am J Respir Crit Care Med*. Dec 1 2004;170(11):1172-8. doi:10.1164/rccm.200406-761OC
 55. Marsh SE, Travers J, Weatherall M, et al. Proportional classifications of COPD phenotypes. *Thorax*. Sep 2008;63(9):761-7. doi:10.1136/thx.2007.089193
 56. Piitulainen E, Mostafavi B, Tanash HA. Health status and lung function in the Swedish alpha 1-antitrypsin deficient cohort, identified by neonatal screening, at the age of 37-40 years. *Int J Chron Obstruct Pulmon Dis*. 2017;12:495-500. doi:10.2147/COPD.S120241
 57. Needham M, Stockley RA. Exacerbations in alpha1-antitrypsin deficiency. *Eur Respir J*. Jun 2005;25(6):992-1000. doi:10.1183/09031936.05.00074704
 58. Stoller JK, Smith P, Yang P, Spray J. Physical and social impact of alpha 1-antitrypsin deficiency: results of a survey. *Cleve Clin J Med*. Nov-Dec 1994;61(6):461-7. doi:10.3949/ccjm.61.6.461
 59. Anzueto A. Alpha-1 Antitrypsin Deficiency-Associated Chronic Obstructive Pulmonary Disease: A Family Perspective. *COPD*. Aug 2015;12(4):462-7. doi:10.3109/15412555.2014.974746
 60. Klitzman R. The impact of social contexts in testing for alpha-1 antitrypsin deficiency: the roles of physicians and others. *Genet Test Mol Biomarkers*. Apr 2009;13(2):269-76. doi:10.1089/gtmb.2008.0106
 61. Strange C, Wienke S, Walker D, et al. Social impact identified in and by the alpha-1 antitrypsin deficiency community. *Rare Diseases and Orphan Drugs*. 2014;1(3):76-82.

62. Patel D, Teckman JH. Alpha-1-Antitrypsin Deficiency Liver Disease. *Clin Liver Dis.* Nov 2018;22(4):643-655. doi:10.1016/j.cld.2018.06.010
63. Lindblad D, Blomenkamp K, Teckman J. Alpha-1-antitrypsin mutant Z protein content in individual hepatocytes correlates with cell death in a mouse model. *Hepatology.* Oct 2007;46(4):1228-35. doi:10.1002/hep.21822
64. Marcus NY, Brunt EM, Blomenkamp K, et al. Characteristics of hepatocellular carcinoma in a murine model of alpha-1-antitrypsin deficiency. *Hepatol Res.* Jun 2010;40(6):641-53. doi:10.1111/j.1872-034X.2010.00663.x
65. Giovannoni I, Callea F, Stefanelli M, Mariani R, Santorelli FM, Francalanci P. Alpha-1-antitrypsin deficiency: from genome to liver disease. PiZ mouse as model for the development of liver pathology in human. *Liver Int.* Jan 2015;35(1):198-206. doi:10.1111/liv.12504
66. Dawwas MF, Davies SE, Griffiths WJ, Lomas DA, Alexander GJ. Prevalence and risk factors for liver involvement in individuals with PiZZ-related lung disease. *Am J Respir Crit Care Med.* Mar 1 2013;187(5):502-8. doi:10.1164/rccm.201204-0739OC
67. Eriksson S, Carlson J, Velez R. Risk of cirrhosis and primary liver cancer in alpha 1-antitrypsin deficiency. *N Engl J Med.* Mar 20 1986;314(12):736-9. doi:10.1056/NEJM198603203141202
68. Townsend SA, Edgar RG, Ellis PR, Kantas D, Newsome PN, Turner AM. Systematic review: the natural history of alpha-1 antitrypsin deficiency, and associated liver disease. *Aliment Pharmacol Ther.* Apr 2018;47(7):877-885. doi:10.1111/apt.14537
69. Tanash HA, Piitulainen E. Liver disease in adults with severe alpha-1-antitrypsin deficiency. *J Gastroenterol.* Jun 2019;54(6):541-548. doi:10.1007/s00535-019-01548-y
70. Schaefer B, Mandorfer M, Viveiros A, et al. Heterozygosity for the alpha-1-antitrypsin Z allele in cirrhosis is associated with more advanced disease. *Liver Transpl.* Jun 2018;24(6):744-751. doi:10.1002/lt.25057
71. Turino GM, Barker AF, Brantly ML, et al. Clinical features of individuals with PI*SZ phenotype of alpha 1-antitrypsin deficiency. alpha 1-Antitrypsin Deficiency Registry Study Group. *Am J Respir Crit Care Med.* Dec 1996;154(6 Pt 1):1718-25. doi:10.1164/ajrccm.154.6.8970361
72. Piitulainen E, Carlson J, Ohlsson K, Sveger T. Alpha1-antitrypsin deficiency in 26-year-old subjects: lung, liver, and protease/protease inhibitor studies. *Chest.* Oct 2005;128(4):2076-81. doi:10.1378/chest.128.4.2076
73. Blanco I, Lipsker D, Lara B, Janciauskiene S. Neutrophilic panniculitis associated with alpha-1-antitrypsin deficiency: an update. *Br J Dermatol.* Apr 2016;174(4):753-62. doi:10.1111/bjd.14309
74. Furey NL, Golden RS, Potts SR. Treatment of alpha-1-antitrypsin deficiency, massive edema, and panniculitis with alpha-1 protease inhibitor. *Ann Intern Med.* Oct 15 1996;125(8):699. doi:10.7326/0003-4819-125-8-199610150-00027
75. Chowdhury MM, Williams EJ, Morris JS, et al. Severe panniculitis caused by homozygous ZZ alpha1-antitrypsin deficiency treated successfully with human purified enzyme (Prolastin). *Br J Dermatol.* Dec 2002;147(6):1258-61. doi:10.1046/j.1365-2133.2002.05095.x
76. O'Riordan K, Blei A, Rao MS, Abecassis M. alpha 1-antitrypsin deficiency-associated panniculitis: resolution with intravenous alpha 1-antitrypsin administration and liver transplantation. *Transplantation.* Feb 15 1997;63(3):480-2. doi:10.1097/00007890-199702150-00028

77. Deshayes S, Martin Silva N, Grandhomme F, et al. Clinical Effect of Alpha-1 Antitrypsin Deficiency in Antineutrophil Cytoplasmic Antibody-associated Vasculitis: Results from a French Retrospective Monocentric Cohort. *J Rheumatol*. Nov 2019;46(11):1502-1508. doi:10.3899/jrheum.180591
78. Lyons PA, Rayner TF, Trivedi S, et al. Genetically distinct subsets within ANCA-associated vasculitis. *N Engl J Med*. Jul 19 2012;367(3):214-23. doi:10.1056/NEJMoa1108735
79. Agusti A, Celli BR, Criner GJ, et al. Global Initiative for Chronic Obstructive Lung Disease 2023 Report: GOLD Executive Summary. *Eur Respir J*. Mar 3 2023;doi:10.1183/13993003.00239-2023
80. Piitulainen E, Tanash HA. The Clinical Profile of Subjects Included in the Swedish National Register on Individuals with Severe Alpha 1-Antitrypsin deficiency. *COPD*. May 2015;12 Suppl 1:36-41. doi:10.3109/15412555.2015.1021909
81. Ward H, Turner AM, Stockley RA. Spirometric and gas transfer discordance in a alpha1 -antitrypsin deficiency. patient characteristics and progression. *Chest*. Jun 2014;145(6):1316-1324. doi:10.1378/chest.13-1886
82. Fahndrich S, Bernhard N, Lepper PM, et al. Exacerbations and duration of smoking abstinence are associated with the annual loss of FEV(1) in individuals with PiZZ alpha-1-antitrypsin deficiency. *Respir Med*. Aug 2017;129:8-15. doi:10.1016/j.rmed.2017.05.011
83. Bernhard N, Lepper PM, Vogelmeier C, et al. Intensive smoking diminishes the differences in quality of life and exacerbation frequency between the alpha-1-antitrypsin deficiency genotypes PiZZ and PiSZ. *Respir Med*. Sep 2017;130:1-8. doi:10.1016/j.rmed.2017.07.004
84. Campos MA, Alazemi S, Zhang G, et al. Exacerbations in subjects with alpha-1 antitrypsin deficiency receiving augmentation therapy. *Respir Med*. Oct 2009;103(10):1532-9. doi:10.1016/j.rmed.2009.04.008
85. Dawkins PA, Dawkins CL, Wood AM, Nightingale PG, Stockley JA, Stockley RA. Rate of progression of lung function impairment in alpha1-antitrypsin deficiency. *Eur Respir J*. Jun 2009;33(6):1338-44. doi:10.1183/09031936.00061208
86. Celli BR, Thomas NE, Anderson JA, et al. Effect of pharmacotherapy on rate of decline of lung function in chronic obstructive pulmonary disease: results from the TORCH study. *Am J Respir Crit Care Med*. Aug 15 2008;178(4):332-8. doi:10.1164/rccm.200712-1869OC
87. Dransfield MT, Kunisaki KM, Strand MJ, et al. Acute Exacerbations and Lung Function Loss in Smokers with and without Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*. Feb 1 2017;195(3):324-330. doi:10.1164/rccm.201605-1014OC
88. Venkatesan P. GOLD COPD report: 2023 update. *Lancet Respir Med*. Jan 2023;11(1):18. doi:10.1016/S2213-2600(22)00494-5
89. Soler-Cataluna JJ, Martinez-Garcia MA, Roman Sanchez P, Salcedo E, Navarro M, Ochando R. Severe acute exacerbations and mortality in patients with chronic obstructive pulmonary disease. *Thorax*. Nov 2005;60(11):925-31. doi:10.1136/thx.2005.040527
90. Donaldson GC, Seemungal TA, Patel IS, Lloyd-Owen SJ, Wilkinson TM, Wedzicha JA. Longitudinal changes in the nature, severity and frequency of COPD exacerbations. *Eur Respir J*. Dec 2003;22(6):931-6. doi:10.1183/09031936.03.00038303

91. Hurst JR, Vestbo J, Anzueto A, et al. Susceptibility to exacerbation in chronic obstructive pulmonary disease. *N Engl J Med*. Sep 16 2010;363(12):1128-38. doi:10.1056/NEJMoa0909883
92. Han MK, Quibrera PM, Carretta EE, et al. Frequency of exacerbations in patients with chronic obstructive pulmonary disease: an analysis of the SPIROMICS cohort. *Lancet Respir Med*. Aug 2017;5(8):619-626. doi:10.1016/S2213-2600(17)30207-2
93. Krachunov I, Kyuchukov N, Ivanova Z, et al. Stability of Frequent Exacerbator Phenotype in Patients with Chronic Obstructive Pulmonary Disease. *Folia Med (Plovdiv)*. Dec 1 2018;60(4):536-545. doi:10.2478/foimed-2018-0023
94. Sadatsafavi M, McCormack J, Petkau J, Lynd LD, Lee TY, Sin DD. Should the number of acute exacerbations in the previous year be used to guide treatments in COPD? *Eur Respir J*. Feb 2021;57(2)doi:10.1183/13993003.02122-2020
95. Santos NCD, Miravittles M, Camelier AA, Almeida VDC, Maciel R, Camelier FWR. Prevalence and Impact of Comorbidities in Individuals with Chronic Obstructive Pulmonary Disease: A Systematic Review. *Tuberc Respir Dis (Seoul)*. Jul 2022;85(3):205-220. doi:10.4046/trd.2021.0179
96. Shaker SB, Maltbaek N, Brand P, Haeussermann S, Dirksen A. Quantitative computed tomography and aerosol morphometry in COPD and alpha1-antitrypsin deficiency. *Eur Respir J*. Jan 2005;25(1):23-30. doi:10.1183/09031936.04.00075304
97. Hill AT, Sullivan AL, Chalmers JD, et al. British Thoracic Society Guideline for bronchiectasis in adults. *Thorax*. Jan 2019;74(Suppl 1):1-69. doi:10.1136/thoraxjnl-2018-212463
98. Crossley D, Stockley J, Bolton CE, et al. Relationship of CT densitometry to lung physiological parameters and health status in alpha-1 antitrypsin deficiency: initial report of a centralised database of the NIHR rare diseases translational research collaborative. *BMJ Open*. Jun 30 2020;10(6):e036045. doi:10.1136/bmjopen-2019-036045
99. Fromme M, Rademacher L, Amzou S, et al. Association of circulating Z-polymer with adverse clinical outcomes and liver fibrosis in adults with alpha-1 antitrypsin deficiency. *United European Gastroenterol J*. Jul 18 2024;doi:10.1002/ueg2.12629
100. Elliott PR, Bilton D, Lomas DA. Lung polymers in Z alpha1-antitrypsin deficiency-related emphysema. *Am J Respir Cell Mol Biol*. May 1998;18(5):670-4. doi:10.1165/ajrcmb.18.5.3065
101. Mulgrew AT, Taggart CC, Lawless MW, et al. Z alpha1-antitrypsin polymerizes in the lung and acts as a neutrophil chemoattractant. *Chest*. May 2004;125(5):1952-7. doi:10.1378/chest.125.5.1952
102. Mahadeva R, Atkinson C, Li Z, et al. Polymers of Z alpha1-antitrypsin co-localize with neutrophils in emphysematous alveoli and are chemotactic in vivo. *Am J Pathol*. Feb 2005;166(2):377-86. doi:10.1016/s0002-9440(10)62261-4
103. Parmar JS, Mahadeva R, Reed BJ, et al. Polymers of alpha(1)-antitrypsin are chemotactic for human neutrophils: a new paradigm for the pathogenesis of emphysema. *Am J Respir Cell Mol Biol*. Jun 2002;26(6):723-30. doi:10.1165/ajrcmb.26.6.4739
104. Sark AD, Fromme M, Olejnicka B, et al. The Relationship between Plasma Alpha-1-Antitrypsin Polymers and Lung or Liver Function in ZZ Alpha-1-Antitrypsin-Deficient Patients. *Biomolecules*. Feb 28 2022;12(3)doi:10.3390/biom12030380
105. Nunez A, Belmonte I, Miranda E, et al. Association between circulating alpha-1 antitrypsin polymers and lung and liver disease. *Respir Res*. Sep 15 2021;22(1):244. doi:10.1186/s12931-021-01842-5

106. Pini L, Tiberio L, Arici M, et al. Z-alpha1-antitrypsin polymers and small airways disease: a new paradigm in alfa-1 anti-trypsin deficiency-related COPD development? *Monaldi Arch Chest Dis*. Jun 8 2021;91(4)doi:10.4081/monaldi.2021.1883
107. Griggs RC, Batshaw M, Dunkle M, et al. Clinical research for rare disease: opportunities, challenges, and solutions. *Mol Genet Metab*. Jan 2009;96(1):20-6. doi:10.1016/j.ymgme.2008.10.003
108. Wastfelt M, Fadeel B, Henter JL. A journey of hope: lessons learned from studies on rare diseases and orphan drugs. *J Intern Med*. Jul 2006;260(1):1-10. doi:10.1111/j.1365-2796.2006.01666.x
109. Ragni MV, Moore CG, Bias V, et al. Challenges of rare disease research: limited patients and competing priorities. *Haemophilia*. May 2012;18(3):e192-4. doi:10.1111/j.1365-2516.2011.02646.x
110. Overview of Rare Diseases Research and Product Development: Challenges and Opportunities. In: Field MJ, Boat TF, eds. *Rare Diseases and Orphan Products: Accelerating Research and Development*. 2010:19-21. *The National Academies Collection: Reports funded by National Institutes of Health*.
111. Gadek JE, Fells GA, Zimmerman RL, Rennard SI, Crystal RG. Antielastases of the human alveolar structures. Implications for the protease-antiprotease theory of emphysema. *J Clin Invest*. Oct 1981;68(4):889-98. doi:10.1172/jci110344
112. Wewers MD, Casolaro MA, Sellers SE, et al. Replacement therapy for alpha 1-antitrypsin deficiency associated with emphysema. *N Engl J Med*. Apr 23 1987;316(17):1055-62. doi:10.1056/NEJM198704233161704
113. CSLBehring. Respreeza® Summary of Product Characteristics. 2015.
114. Soy D, de la Roza C, Lara B, Esquinas C, Torres A, Miravittles M. Alpha-1-antitrypsin deficiency: optimal therapeutic regimen based on population pharmacokinetics. *Thorax*. Dec 2006;61(12):1059-64. doi:10.1136/thx.2005.057943
115. Zamora NP, Pla RV, Del Rio PG, Margaleff RJ, Frias FR, Ronsano JB. Intravenous human plasma-derived augmentation therapy in alpha 1-antitrypsin deficiency: from pharmacokinetic analysis to individualizing therapy. *Ann Pharmacother*. May 2008;42(5):640-6. doi:10.1345/aph.1K505
116. Chapman KR, Burdon JG, Piitulainen E, et al. Intravenous augmentation treatment and lung density in severe alpha1 antitrypsin deficiency (RAPID): a randomised, double-blind, placebo-controlled trial. *Lancet*. Jul 25 2015;386(9991):360-8. doi:10.1016/S0140-6736(15)60860-1
117. McElvaney NG, Burdon J, Holmes M, et al. Long-term efficacy and safety of alpha1 proteinase inhibitor treatment for emphysema caused by severe alpha1 antitrypsin deficiency: an open-label extension trial (RAPID-OLE). *Lancet Respir Med*. Jan 2017;5(1):51-60. doi:10.1016/S2213-2600(16)30430-1
118. Dirksen A, Piitulainen E, Parr DG, et al. Exploring the role of CT densitometry: a randomised study of augmentation therapy in alpha1-antitrypsin deficiency. *Eur Respir J*. Jun 2009;33(6):1345-53. doi:10.1183/09031936.00159408
119. Green CE, Parr DG, Edgar RG, Stockley RA, Turner AM. Lung density associates with survival in alpha 1 antitrypsin deficient patients. *Respir Med*. Mar 2016;112:81-7. doi:10.1016/j.rmed.2016.01.007
120. Crossley D, Renton M, Khan M, Low EV, Turner AM. CT densitometry in emphysema: a systematic review of its clinical utility. *Int J Chron Obstruct Pulmon Dis*. 2018;13:547-563. doi:10.2147/COPD.S143066
121. Miravittles M, Dirksen A, Ferrarotti I, et al. European Respiratory Society statement: diagnosis and treatment of pulmonary disease in alpha(1)-antitrypsin deficiency. *Eur Respir J*. Nov 2017;50(5)doi:10.1183/13993003.00610-2017

122. Sandhaus RA, Turino G, Brantly ML, et al. The Diagnosis and Management of Alpha-1 Antitrypsin Deficiency in the Adult. *Chronic Obstr Pulm Dis*. Jun 6 2016;3(3):668-682. doi:10.15326/jcopdf.3.3.2015.0182
123. Edgar RG, Patel M, Bayliss S, Crossley D, Sapey E, Turner AM. Treatment of lung disease in alpha-1 antitrypsin deficiency: a systematic review. *Int J Chron Obstruct Pulmon Dis*. 2017;12:1295-1308. doi:10.2147/COPD.S130440
124. Piitulainen E, Bernspang E, Bjorkman S, Berntorp E. Tailored pharmacokinetic dosing allows self-administration and reduces the cost of IV augmentation therapy with human alpha(1)-antitrypsin. *Eur J Clin Pharmacol*. Jun 2003;59(2):151-6. doi:10.1007/s00228-003-0589-z
125. Sieluk J, Slejko JF, Silverman H, Perfetto E, Mullins CD. Medical costs of Alpha-1 antitrypsin deficiency-associated COPD in the United States. *Orphanet J Rare Dis*. Sep 23 2020;15(1):260. doi:10.1186/s13023-020-01523-4
126. Kropp J, Wencker M, Hotze A, et al. Inhalation of [123I]alpha1-protease inhibitor: toward a new therapeutic concept of alpha1-protease inhibitor deficiency? *J Nucl Med*. May 2001;42(5):744-51.
127. Stocks JM, Brantly ML, Wang-Smith L, et al. Pharmacokinetic comparability of Prolastin(R)-C to Prolastin(R) in alpha(1)-antitrypsin deficiency: a randomized study. *BMC Clin Pharmacol*. Sep 30 2010;10:13. doi:10.1186/1472-6904-10-13
128. Inhibrx I. Study of INBRX-101 Compared to Plasma-derived A1PI Therapy in Adults With AATD Emphysema. <https://classic.clinicaltrials.gov/show/NCT05856331>; 2023.
129. Corp. HB. Study of OsrhAAT or Placebo in Healthy Volunteers. <https://classic.clinicaltrials.gov/show/NCT05315921>; 2022.
130. Petrache I, Hajjar J, Campos M. Safety and efficacy of alpha-1-antitrypsin augmentation therapy in the treatment of patients with alpha-1-antitrypsin deficiency. *Biologics*. 2009;3:193-204. doi:10.2147/btt.2009.3088
131. Kamada L. Phase II, Safety and Efficacy Study of Kamada-alpha-1-antitrypsin (AAT) for Inhalation". <https://classic.clinicaltrials.gov/show/NCT02001688>; 2014.
132. Kamada L, Health S. Evaluate Efficacy and Safety of "Kamada-AAT for Inhalation" in Patients With AATD. <https://classic.clinicaltrials.gov/show/NCT04204252>; 2019.
133. Rabbie S, Black I, Inshaw J. Analysis From Phase II Clinical Trial, Alvelestat, NE (Neutrophil Elastase) Inhibitor in AATD-LD: Correlation Between Biomarker Response (Desmosine and Aa-val^{³⁶⁰}) and Clinical Outcome (SGRQ). *B22 NEW TREATMENTS AND NOVEL TARGETS FOR COPD AND ALPHA-1*. A2844-A2844.
134. Birmingham UoAa, Health Nlo, BioPharma M. Alvelestat (MPH966) for the Treatment of ALpha-1 ANTitypsin Deficiency. <https://classic.clinicaltrials.gov/show/NCT03679598>; 2019.
135. Watz H, Nagelschmitz J, Kirsten A, et al. Safety and efficacy of the human neutrophil elastase inhibitor BAY 85-8501 for the treatment of non-cystic fibrosis bronchiectasis: A randomized controlled trial. *Pulmonary Pharmacology & Therapeutics*. 2019/06/01/ 2019;56:86-93. doi:<https://doi.org/10.1016/j.pupt.2019.03.009>
136. A Study of Safety, Tolerability, and Pharmacokinetics of Single Doses of PHP-303 in Healthy Subjects. <https://classic.clinicaltrials.gov/show/NCT03627845>.
137. Incorporated VP. A Phase 1, First-in-human Study of VX-634. <https://classic.clinicaltrials.gov/show/NCT05579431>; 2022.

138. Limited ZF, Research HM, plc CP. A Single Ascending and Repeated Dose Study of Oral ZF874 in Healthy Volunteers and PiXZ Subjects. <https://classic.clinicaltrials.gov/show/NCT04443192>; 2020.
139. Incorporated VP. A Study to Evaluate Efficacy and Safety of VX-864 in Participants With the PiZZ Genotype. <https://classic.clinicaltrials.gov/show/NCT05643495>; 2023.
140. Incorporated VP. Evaluation of the Efficacy and Safety of VX-814 in Subjects With the PiZZ Genotype. <https://classic.clinicaltrials.gov/show/NCT04167345>; 2020.
141. Incorporated VP. Evaluation of the Efficacy and Safety of VX-864 in Subjects With the PiZZ Genotype. <https://classic.clinicaltrials.gov/show/NCT04474197>; 2020.
142. Adverum Biotechnologies I. Safety Dose Finding Study of ADVN-043 Gene Therapy to Treat Alpha-1 Antitrypsin (A1AT) Deficiency. <https://classic.clinicaltrials.gov/show/NCT02168686>; 2017.
143. Adverum Biotechnologies I. Long-Term Follow-up Study of ADVN-043. <https://classic.clinicaltrials.gov/show/NCT03804021>; 2018.
144. Beam Therapeutics Presents Preclinical Data Highlighting Utility of BEAM-302 to Correct an Alpha-1 Antitrypsin (AAT) Deficiency Disease-Causing Mutation. 2023. <https://investors.beamtx.com/node/8731/pdf>
145. Intellia. Intellia Therapeutics Announces Second Quarter 2023 Financial Results and Highlights Recent Company Progress. 2023;
146. Wave Life Sciences Announces Submission of First Clinical Trial Application for WVE-006, the First-ever RNA Editing Clinical Candidate, and Plans for Upcoming Virtual “R&D Day”. 2023. <https://ir.wavelifesciences.com/news-releases/news-release-details/wave-life-sciences-announces-submission-first-clinical-trial>
147. Strnad P, Mandorfer M, Choudhury G, et al. Fazirsiran for Liver Disease Associated with Alpha(1)-Antitrypsin Deficiency. *N Engl J Med*. Aug 11 2022;387(6):514-524. doi:10.1056/NEJMoa2205416
148. Dicerna Pharmaceuticals I, a Novo Nordisk company. A Study of Belcesiran in Patients With AATLD. <https://classic.clinicaltrials.gov/show/NCT04764448>; 2021.
149. Jenkins CR, Jones PW, Calverley PM, et al. Efficacy of salmeterol/fluticasone propionate by GOLD stage of chronic obstructive pulmonary disease: analysis from the randomised, placebo-controlled TORCH study. *Respir Res*. Jun 30 2009;10(1):59. doi:10.1186/1465-9921-10-59
150. Zeng Z, Yang D, Huang X, Xiao Z. Effect of carbocysteine on patients with COPD: a systematic review and meta-analysis. *Int J Chron Obstruct Pulmon Dis*. 2017;12:2277-2283. doi:10.2147/COPD.S140603
151. Nakamura K, Fujita Y, Chen H, et al. The Effectiveness and Safety of Long-Term Macrolide Therapy for COPD in Stable Status: A Systematic Review and Meta-Analysis. *Diseases*. Oct 27 2023;11(4)doi:10.3390/diseases11040152
152. Esposito A, Valentino MR, Bruzzese D, et al. Effect of CARbocysteine in Prevention of exacerBation of chronic obstructive pulmonary disease (CAPRI study): An observational study. *Pulm Pharmacol Ther*. Apr 2016;37:85-8. doi:10.1016/j.pupt.2016.03.003
153. Ram FS, Jones PW, Castro AA, et al. Oral theophylline for chronic obstructive pulmonary disease. *Cochrane Database Syst Rev*. 2002;2002(4):CD003902. doi:10.1002/14651858.CD003902
154. AstraZeneca. Efficacy and Safety of Benralizumab in Moderate to Very Severe Chronic Obstructive Pulmonary Disease (COPD) With a History of Frequent Exacerbations. <https://classic.clinicaltrials.gov/show/NCT04053634>; 2019.

155. Halpin DM, Miravittles M, Metzdorf N, Celli B. Impact and prevention of severe exacerbations of COPD: a review of the evidence. *Int J Chron Obstruct Pulmon Dis*. 2017;12:2891-2908. doi:10.2147/COPD.S139470
156. Poole PJ, Chacko E, Wood-Baker RW, Cates CJ. Influenza vaccine for patients with chronic obstructive pulmonary disease. *Cochrane Database Syst Rev*. Jan 25 2006;(1):CD002733. doi:10.1002/14651858.CD002733.pub2
157. Campos MA, Alazemi S, Zhang G, Sandhaus RA, Wanner A. Influenza vaccination in subjects with alpha1-antitrypsin deficiency. *Chest*. Jan 2008;133(1):49-55. doi:10.1378/chest.07-1482
158. Tanash HA, Nilsson PM, Nilsson JA, Piitulainen E. Clinical course and prognosis of never-smokers with severe alpha-1-antitrypsin deficiency (PiZZ). *Thorax*. Dec 2008;63(12):1091-5. doi:10.1136/thx.2008.095497
159. Jarosch I, Hitzl W, Koczulla AR, et al. Comparison of exercise training responses in COPD patients with and without Alpha-1 antitrypsin deficiency. *Respir Med*. Sep 2017;130:98-101. doi:10.1016/j.rmed.2017.07.009
160. Everaerts S, Hartman JE, Van Dijk M, Koster TD, Slebos DJ, Klooster K. Bronchoscopic Lung Volume Reduction in Patients with Emphysema due to Alpha-1 Antitrypsin Deficiency. *Respiration*. 2023;102(2):134-142. doi:10.1159/000528182
161. Hogarth DK, Delage A, Zgoda MA, Nsiah-Dosu S, Himes D, Reed MF. Efficacy and safety of the Spiration Valve System for the treatment of severe emphysema in patients with Alpha-1 antitrypsin deficiency (EMPROVE). *Respir Med*. Apr 2024;224:107565. doi:10.1016/j.rmed.2024.107565
162. Perch M, Hayes D, Jr., Cherikh WS, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-ninth adult lung transplantation report-2022; focus on lung transplant recipients with chronic obstructive pulmonary disease. *J Heart Lung Transplant*. Oct 2022;41(10):1335-1347. doi:10.1016/j.healun.2022.08.007
163. Transplant NBa. *Annual Report on Lung Transplantation*. 2024. <https://nhsbtldb.blob.core.windows.net/umbraco-assets-corp/34299/nhsbt-lung-transplantation-report-2324.pdf>
164. Stone HM, Edgar RG, Thompson RD, Stockley RA. Lung Transplantation in Alpha-1-Antitrypsin Deficiency. *COPD*. 2016;13(2):146-52. doi:10.3109/15412555.2015.1048850
165. Tanash HA, Riise GC, Hansson L, Nilsson PM, Piitulainen E. Survival benefit of lung transplantation in individuals with severe alpha1-anti-trypsin deficiency (PiZZ) and emphysema. *J Heart Lung Transplant*. Dec 2011;30(12):1342-7. doi:10.1016/j.healun.2011.07.003
166. Chalmers JD, Polverino E, Aliberti S. *Bronchiectasis*. European Respiratory Society; 2018:424.
167. Chandrasekaran R, Mac Aogain M, Chalmers JD, Elborn SJ, Chotirmall SH. Geographic variation in the aetiology, epidemiology and microbiology of bronchiectasis. *BMC Pulm Med*. May 22 2018;18(1):83. doi:10.1186/s12890-018-0638-0
168. Cole PJ. Inflammation: a two-edged sword--the model of bronchiectasis. *Eur J Respir Dis Suppl*. 1986;147:6-15.
169. Flume PA, Chalmers JD, Olivier KN. Advances in bronchiectasis: endotyping, genetics, microbiome, and disease heterogeneity. *Lancet*. Sep 8 2018;392(10150):880-890. doi:10.1016/S0140-6736(18)31767-7

170. Rita Boaventura, AMelia Shoemark, Chalmers JD. Pathophysiology. In: James D. Chalmers, Eva Polverino, Aliberti S, eds. *Bronchiectasis (ERS Monograph)*. European Respiratory Society; 2018:8-28.
171. Chalmers JD, Moffitt KL, Suarez-Cuartin G, et al. Neutrophil Elastase Activity Is Associated with Exacerbations and Lung Function Decline in Bronchiectasis. *Am J Respir Crit Care Med*. May 15 2017;195(10):1384-1393. doi:10.1164/rccm.201605-1027OC
172. Middelhoven PJ, Van Buul JD, Hordijk PL, Roos D. Different proteolytic mechanisms involved in Fc gamma RIIIb shedding from human neutrophils. *Clin Exp Immunol*. Jul 2001;125(1):169-75. doi:10.1046/j.1365-2249.2001.01548.x
173. Aliberti S, Lonni S, Dore S, et al. Clinical phenotypes in adult patients with bronchiectasis. *Eur Respir J*. Apr 2016;47(4):1113-22. doi:10.1183/13993003.01899-2015
174. Guan WJ, Oscullo G, He MZ, Xu DY, Gomez-Olivas JD, Martinez-Garcia MA. Significance and Potential Role of Eosinophils in Non-Cystic Fibrosis Bronchiectasis. *J Allergy Clin Immunol Pract*. Apr 2023;11(4):1089-1099. doi:10.1016/j.jaip.2022.10.027
175. Shoemark A, Shteinberg M, De Soyza A, et al. Characterization of Eosinophilic Bronchiectasis: A European Multicohort Study. *Am J Respir Crit Care Med*. Apr 15 2022;205(8):894-902. doi:10.1164/rccm.202108-1889OC
176. Rademacher J, Konwert S, Fuge J, Dettmer S, Welte T, Ringshausen FC. Anti-IL5 and anti-IL5Ralpha therapy for clinically significant bronchiectasis with eosinophilic endotype: a case series. *Eur Respir J*. Jan 2020;55(1)doi:10.1183/13993003.01333-2019
177. Matsuoka S, Uchiyama K, Shima H, Ueno N, Oish S, Nojiri Y. Bronchoarterial ratio and bronchial wall thickness on high-resolution CT in asymptomatic subjects: correlation with age and smoking. *AJR Am J Roentgenol*. Feb 2003;180(2):513-8. doi:10.2214/ajr.180.2.1800513
178. Meerburg JJ, Veerman GDM, Aliberti S, Tiddens H. Diagnosis and quantification of bronchiectasis using computed tomography or magnetic resonance imaging: A systematic review. *Respir Med*. Aug-Sep 2020;170:105954. doi:10.1016/j.rmed.2020.105954
179. Aliberti S, Goeminne PC, O'Donnell AE, et al. Criteria and definitions for the radiological and clinical diagnosis of bronchiectasis in adults for use in clinical trials: international consensus recommendations. *Lancet Respir Med*. Mar 2022;10(3):298-306. doi:10.1016/S2213-2600(21)00277-0
180. Bedi P, Chalmers JD, Goeminne PC, et al. The BRICS (Bronchiectasis Radiologically Indexed CT Score): A Multicenter Study Score for Use in Idiopathic and Postinfective Bronchiectasis. *Chest*. May 2018;153(5):1177-1186. doi:10.1016/j.chest.2017.11.033
181. Gao YH, Guan WJ, Liu SX, et al. Aetiology of bronchiectasis in adults: A systematic literature review. *Respirology*. Nov 2016;21(8):1376-1383. doi:10.1111/resp.12832
182. Pasteur MC, Helliwell SM, Houghton SJ, et al. An investigation into causative factors in patients with bronchiectasis. *Am J Respir Crit Care Med*. Oct 2000;162(4 Pt 1):1277-84. doi:10.1164/ajrccm.162.4.9906120
183. Zoumot Z, Boutou AK, Gill SS, et al. Mycobacterium avium complex infection in non-cystic fibrosis bronchiectasis. *Respirology*. Jul 2014;19(5):714-22. doi:10.1111/resp.12287

184. Baird T.M., R. T. Diagnosis, classification and epidemiology of pulmonary nontuberculous mycobacterial disease. In: Chalmers JD, Polverino E, Aliberti S, eds. *Bronchiectasis (ERS Monograph)*. European Respiratory Society; 2018:204-221.
185. McDonnell MJ, Aliberti S, Goeminne PC, et al. Comorbidities and the risk of mortality in patients with bronchiectasis: an international multicentre cohort study. *Lancet Respir Med*. Dec 2016;4(12):969-979. doi:10.1016/S2213-2600(16)30320-4
186. Roberts HR, Wells AU, Milne DG, et al. Airflow obstruction in bronchiectasis: correlation between computed tomography features and pulmonary function tests. *Thorax*. Mar 2000;55(3):198-204. doi:10.1136/thorax.55.3.198
187. Martinez-Garcia MA, de Gracia J, Vendrell Relat M, et al. Multidimensional approach to non-cystic fibrosis bronchiectasis: the FACED score. *Eur Respir J*. May 2014;43(5):1357-67. doi:10.1183/09031936.00026313
188. Chalmers JD, Goeminne P, Aliberti S, et al. The bronchiectasis severity index. An international derivation and validation study. *Am J Respir Crit Care Med*. Mar 1 2014;189(5):576-85. doi:10.1164/rccm.201309-1575OC
189. Chalmers JD, Aliberti S, Filonenko A, et al. Characterization of the "Frequent Exacerbator Phenotype" in Bronchiectasis. *Am J Respir Crit Care Med*. Jun 1 2018;197(11):1410-1420. doi:10.1164/rccm.201711-2202OC
190. Ellis HC, Cowman S, Fernandes M, Wilson R, Loebinger MR. Predicting mortality in bronchiectasis using bronchiectasis severity index and FACED scores: a 19-year cohort study. *Eur Respir J*. Feb 2016;47(2):482-9. doi:10.1183/13993003.01312-2015
191. Rosales-Mayor E, Polverino E, Raguer L, et al. Comparison of two prognostic scores (BSI and FACED) in a Spanish cohort of adult patients with bronchiectasis and improvement of the FACED predictive capacity for exacerbations. *PLoS One*. 2017;12(4):e0175171. doi:10.1371/journal.pone.0175171
192. Reiff DB, Wells AU, Carr DH, Cole PJ, Hansell DM. CT findings in bronchiectasis: limited value in distinguishing between idiopathic and specific types. *AJR Am J Roentgenol*. Aug 1995;165(2):261-7. doi:10.2214/ajr.165.2.7618537
193. Reid LM. Reduction in bronchial subdivision in bronchiectasis. *Thorax*. Sep 1950;5(3):233-47. doi:10.1136/thx.5.3.233
194. Bhalla M, Turcios N, Aponte V, et al. Cystic fibrosis: scoring system with thin-section CT. *Radiology*. Jun 1991;179(3):783-8. doi:10.1148/radiology.179.3.2027992
195. Vizza CD, Hoeper MM, Huscher D, et al. Pulmonary Hypertension in Patients With COPD: Results From the Comparative, Prospective Registry of Newly Initiated Therapies for Pulmonary Hypertension (COMPERA). *Chest*. Aug 2021;160(2):678-689. doi:10.1016/j.chest.2021.02.012
196. Alzeer AH, Al-Mobeirek AF, Al-Otair HA, Elzamzamy UA, Joherjy IA, Shaffi AS. Right and left ventricular function and pulmonary artery pressure in patients with bronchiectasis. *Chest*. Feb 2008;133(2):468-73. doi:10.1378/chest.07-1639
197. Nathan SD, Barnett SD, King CS, et al. Impact of the new definition for pulmonary hypertension in patients with lung disease: an analysis of the United Network for Organ Sharing database. *Pulm Circ*. Apr-Jun 2021;11(2):2045894021999960. doi:10.1177/2045894021999960
198. Quan K, Tanno R, Shipley RJ, et al. Reproducibility of an airway tapering measurement in computed tomography with application to bronchiectasis. *J Med Imaging (Bellingham)*. Jul 2019;6(3):034003. doi:10.1117/1.JMI.6.3.034003
199. Wielputz MO, Eichinger M, Weinheimer O, et al. Automatic airway analysis on multidetector computed tomography in cystic fibrosis: correlation with pulmonary

- function testing. *J Thorac Imaging*. Mar 2013;28(2):104-13. doi:10.1097/RTI.0b013e3182765785
200. Weinheimer O, Wielpütz MO, Konietzke P, et al. Fully automated lobe-based airway taper index calculation in a low dose MDCT CF study over 4 time-points. *Proc Spie*. 2017;10133doi:Unsp 101330u 10.1117/12.2254387
201. Parr DG, Guest PG, Reynolds JH, Dowson LJ, Stockley RA. Prevalence and impact of bronchiectasis in alpha1-antitrypsin deficiency. *Am J Respir Crit Care Med*. Dec 15 2007;176(12):1215-21. doi:10.1164/rccm.200703-489OC
202. Altenburg J, Goeminne PC. Defining severe bronchiectasis. In: Chalmers JD, Polverino E, Aliberti S, eds. *Bronchiectasis (ERS Monograph)*. European Respiratory Society; 2018:62-81.
203. Woo TE, Lim R, Heirali AA, et al. A longitudinal characterization of the Non-Cystic Fibrosis Bronchiectasis airway microbiome. *Sci Rep*. May 3 2019;9(1):6871. doi:10.1038/s41598-019-42862-y
204. Guan WJ, Yuan JJ, Li HM, et al. Proteobacteria community compositions correlate with bronchiectasis severity. *Int J Tuberc Lung Dis*. Sep 1 2018;22(9):1095-1105. doi:10.5588/ijtld.18.0037
205. Rogers GB, van der Gast CJ, Cuthbertson L, et al. Clinical measures of disease in adult non-CF bronchiectasis correlate with airway microbiota composition. *Thorax*. Aug 2013;68(8):731-7. doi:10.1136/thoraxjnl-2012-203105
206. Araujo D, Shteinberg M, Aliberti S, et al. The independent contribution of *Pseudomonas aeruginosa* infection to long-term clinical outcomes in bronchiectasis. *Eur Respir J*. Feb 2018;51(2)doi:10.1183/13993003.01953-2017
207. Martinez-Garcia MA, Oscullo G, Posadas T, et al. *Pseudomonas aeruginosa* and lung function decline in patients with bronchiectasis. *Clin Microbiol Infect*. Mar 2021;27(3):428-434. doi:10.1016/j.cmi.2020.04.007
208. Guan WJ, Gao YH, Xu G, et al. Effect of airway *Pseudomonas aeruginosa* isolation and infection on steady-state bronchiectasis in Guangzhou, China. *J Thorac Dis*. Apr 2015;7(4):625-36. doi:10.3978/j.issn.2072-1439.2015.04.04
209. Keir HR, Shoemark A, Dicker AJ, et al. Neutrophil extracellular traps, disease severity, and antibiotic response in bronchiectasis: an international, observational, multicohort study. *Lancet Respir Med*. Aug 2021;9(8):873-884. doi:10.1016/S2213-2600(20)30504-X
210. Duan K, Dammel C, Stein J, Rabin H, Surette MG. Modulation of *Pseudomonas aeruginosa* gene expression by host microflora through interspecies communication. *Mol Microbiol*. Dec 2003;50(5):1477-91. doi:10.1046/j.1365-2958.2003.03803.x
211. Coyte KZ, Schluter J, Foster KR. The ecology of the microbiome: Networks, competition, and stability. *Science*. Nov 6 2015;350(6261):663-6. doi:10.1126/science.aad2602
212. Chalmers JD, Polverino E, Crichton ML, et al. Bronchiectasis in Europe: data on disease characteristics from the European Bronchiectasis registry (EMBARC). *Lancet Respir Med*. Jul 2023;11(7):637-649. doi:10.1016/S2213-2600(23)00093-0
213. Fowler SJ, French J, Screaton NJ, et al. Nontuberculous mycobacteria in bronchiectasis: Prevalence and patient characteristics. *Eur Respir J*. Dec 2006;28(6):1204-10. doi:10.1183/09031936.06.00149805
214. Faverio P, Stainer A, Bonaiti G, et al. Characterizing Non-Tuberculous Mycobacteria Infection in Bronchiectasis. *Int J Mol Sci*. Nov 16 2016;17(11)doi:10.3390/ijms17111913

215. Maiz L, Giron R, Oliveira C, Vendrell M, Nieto R, Martinez-Garcia MA. Prevalence and factors associated with nontuberculous mycobacteria in non-cystic fibrosis bronchiectasis: a multicenter observational study. *BMC Infect Dis.* Aug 22 2016;16(1):437. doi:10.1186/s12879-016-1774-x
216. Shteinberg M, Stein N, Adir Y, et al. Prevalence, risk factors and prognosis of nontuberculous mycobacterial infection among people with bronchiectasis: a population survey. *Eur Respir J.* May 2018;51(5)doi:10.1183/13993003.02469-2017
217. Mac Aogain M, Chandrasekaran R, Lim AYH, et al. Immunological corollary of the pulmonary mycobioime in bronchiectasis: the CAMEB study. *Eur Respir J.* Jul 2018;52(1)doi:10.1183/13993003.00766-2018
218. Green H, Newton P, Jones AM. Fungal lung disease. In: Chalmers J, Polverino E, Aliberti S, eds. *Bronchiectasis*. European Respiratory Society; 2018:186-203.
219. Metersky ML, Choate R, Aksamit TR, et al. *Stenotrophomonas maltophilia* in patients with bronchiectasis: An analysis of the US bronchiectasis and NTM Research Registry. *Respir Med.* Mar 2022;193:106746. doi:10.1016/j.rmed.2022.106746
220. Marra R, Sgalla G, Richeldi L, Conte EG, Hill AT. Role of *Stenotrophomonas maltophilia* isolation in patients with non-CF bronchiectasis. *QJM.* Oct 1 2020;113(10):726-730. doi:10.1093/qjmed/hcaa120
221. Anwar GA, McDonnell MJ, Worthy SA, et al. Phenotyping adults with non-cystic fibrosis bronchiectasis: a prospective observational cohort study. *Respir Med.* Jul 2013;107(7):1001-7. doi:10.1016/j.rmed.2013.04.013
222. Tsang KW, Chan K, Ho P, et al. Sputum elastase in steady-state bronchiectasis. *Chest.* Feb 2000;117(2):420-6. doi:10.1378/chest.117.2.420
223. Stockley R, De Soyza A, Gunawardena K, et al. Phase II study of a neutrophil elastase inhibitor (AZD9668) in patients with bronchiectasis. *Respir Med.* Apr 2013;107(4):524-33. doi:10.1016/j.rmed.2012.12.009
224. Campos MA, Geraghty P, Holt G, et al. The Biological Effects of Double-Dose Alpha-1 Antitrypsin Augmentation Therapy. A Pilot Clinical Trial. *Am J Respir Crit Care Med.* Aug 1 2019;200(3):318-326. doi:10.1164/rccm.201901-0010OC
225. Shoemark A, Cant E, Carreto L, et al. A point-of-care neutrophil elastase activity assay identifies bronchiectasis severity, airway infection and risk of exacerbation. *Eur Respir J.* Jun 2019;53(6)doi:10.1183/13993003.00303-2019
226. Guan WJ, Gao YH, Xu G, et al. Sputum matrix metalloproteinase-8 and -9 and tissue inhibitor of metalloproteinase-1 in bronchiectasis: clinical correlates and prognostic implications. *Respirology.* Oct 2015;20(7):1073-81. doi:10.1111/resp.12582
227. Taylor SL, Rogers GB, Chen AC, Burr LD, McGuckin MA, Serisier DJ. Matrix metalloproteinases vary with airway microbiota composition and lung function in non-cystic fibrosis bronchiectasis. *Ann Am Thorac Soc.* May 2015;12(5):701-7. doi:10.1513/AnnalsATS.201411-513OC
228. Tsikrika S, Dimakou K, Papaioannou AI, et al. The role of non-invasive modalities for assessing inflammation in patients with non-cystic fibrosis bronchiectasis. *Cytokine.* Nov 2017;99:281-286. doi:10.1016/j.cyto.2017.08.005
229. Sepper R, Kontinen YT, Ingman T, Sorsa T. Presence, activities, and molecular forms of cathepsin G, elastase, alpha 1-antitrypsin, and alpha 1-antichymotrypsin in bronchiectasis. *J Clin Immunol.* Jan 1995;15(1):27-34. doi:10.1007/BF01489487
230. Huang JT, Cant E, Keir HR, et al. Endotyping Chronic Obstructive Pulmonary Disease, Bronchiectasis, and the "Chronic Obstructive Pulmonary Disease-

- Bronchiectasis Association". *Am J Respir Crit Care Med*. Aug 15 2022;206(4):417-426. doi:10.1164/rccm.202108-1943OC
231. Saleh AD, Chalmers JD, De Soyza A, et al. The heterogeneity of systemic inflammation in bronchiectasis. *Respir Med*. Jun 2017;127:33-39. doi:10.1016/j.rmed.2017.04.009
232. Oriano M, Gramegna A, Amati F, et al. T2-High Endotype and Response to Biological Treatments in Patients with Bronchiectasis. *Biomedicines*. Jul 2021;9(7)doi:10.3390/biomedicines9070772
233. Wang X, Villa C, Dobarganes Y, et al. Phenotypic Clustering in Non-Cystic Fibrosis Bronchiectasis Patients: The Role of Eosinophils in Disease Severity. *Int J Environ Res Public Health*. Aug 10 2021;18(16)doi:10.3390/ijerph18168431
234. Aliberti S, Sotgiu G, Gramegna A, et al. Thrombocytosis during Stable State Predicts Mortality in Bronchiectasis. *Ann Am Thorac Soc*. Aug 2021;18(8):1316-1325. doi:10.1513/AnnalsATS.202002-094OC
235. Martinez-Garcia MA, Perpina-Tordera M, Roman-Sanchez P, et al. [The association between bronchiectasis, systemic inflammation, and tumor necrosis factor alpha]. *Arch Bronconeumol*. Jan 2008;44(1):8-14. Bronquiectasias, inflamacion sistémica y factor de necrosis tumoral alfa: factores asociados. doi:10.1016/s1579-2129(08)60003-8
236. Chalmers JD, Smith MP, McHugh BJ, Doherty C, Govan JR, Hill AT. Short- and long-term antibiotic treatment reduces airway and systemic inflammation in non-cystic fibrosis bronchiectasis. *Am J Respir Crit Care Med*. Oct 1 2012;186(7):657-65. doi:10.1164/rccm.201203-0487OC
237. Posadas T, Oscullo G, Zaldivar E, et al. C-Reactive Protein Concentration in Steady-State Bronchiectasis: Prognostic Value of Future Severe Exacerbations. Data From the Spanish Registry of Bronchiectasis (RIBRON). *Arch Bronconeumol (Engl Ed)*. Jan 2021;57(1):21-27. doi:10.1016/j.arbres.2019.12.017
238. Soyza AD, Smith G, Killick H, et al. S51 Heterogeneity of sputum and systemic inflammatory mediator profiles in bronchiectasis. *Thorax*. 2022;77(Suppl 1):A34. doi:10.1136/thorax-2022-BTSabstracts.57
239. Wang X, Villa C, Dobarganes Y, et al. Systemic Inflammatory Biomarkers Define Specific Clusters in Patients with Bronchiectasis: A Large-Cohort Study. *Biomedicines*. Jan 21 2022;10(2)doi:10.3390/biomedicines10020225
240. Murray MP, Pentland JL, Hill AT. A randomised crossover trial of chest physiotherapy in non-cystic fibrosis bronchiectasis. *Eur Respir J*. Nov 2009;34(5):1086-92. doi:10.1183/09031936.00055509
241. Mutalithas K, Watkin G, Willig B, Wardlaw A, Pavord ID, Birring SS. Improvement in health status following bronchopulmonary hygiene physical therapy in patients with bronchiectasis. *Respir Med*. Aug 2008;102(8):1140-4. doi:10.1016/j.rmed.2008.03.011
242. McCullough AR, Tunney MM, Stuart Elborn J, Bradley JM, Hughes CM. Predictors of adherence to treatment in bronchiectasis. *Respir Med*. Jul 2015;109(7):838-45. doi:10.1016/j.rmed.2015.04.011
243. Lee AL, Hill CJ, Cecins N, et al. The short and long term effects of exercise training in non-cystic fibrosis bronchiectasis--a randomised controlled trial. *Respir Res*. Apr 15 2014;15(1):44. doi:10.1186/1465-9921-15-44
244. Kapur N, Bell S, Kolbe J, Chang AB. Inhaled steroids for bronchiectasis. *Cochrane Database Syst Rev*. Jan 21 2009;(1):CD000996. doi:10.1002/14651858.CD000996.pub2

245. Holme J, Tomlinson JW, Stockley RA, Stewart PM, Barlow N, Sullivan AL. Adrenal suppression in bronchiectasis and the impact of inhaled corticosteroids. *Eur Respir J*. Oct 2008;32(4):1047-52. doi:10.1183/09031936.00016908
246. Nicolson CH, Stirling RG, Borg BM, Button BM, Wilson JW, Holland AE. The long term effect of inhaled hypertonic saline 6% in non-cystic fibrosis bronchiectasis. *Respir Med*. May 2012;106(5):661-7. doi:10.1016/j.rmed.2011.12.021
247. Bradley JM, Anand R, O'Neill B, et al. A 2 x 2 factorial, randomised, open-label trial to determine the clinical and cost-effectiveness of hypertonic saline (HTS 6%) and carbocysteine for airway clearance versus usual care over 52 weeks in adults with bronchiectasis: a protocol for the CLEAR clinical trial. *Trials*. Dec 19 2019;20(1):747. doi:10.1186/s13063-019-3766-9
248. Gao YH, Guan WJ, Xu G, et al. Macrolide therapy in adults and children with non-cystic fibrosis bronchiectasis: a systematic review and meta-analysis. *PLoS One*. 2014;9(3):e90047. doi:10.1371/journal.pone.0090047
249. Chalmers JD, Usansky H, Rubino CM, et al. Pharmacokinetic/Pharmacodynamic Evaluation of the Dipeptidyl Peptidase 1 Inhibitor Brensocatib for Non-cystic Fibrosis Bronchiectasis. *Clin Pharmacokinet*. Oct 2022;61(10):1457-1469. doi:10.1007/s40262-022-01147-w
250. O'Brien C, Guest PJ, Hill SL, Stockley RA. Physiological and radiological characterisation of patients diagnosed with chronic obstructive pulmonary disease in primary care. *Thorax*. Aug 2000;55(8):635-42. doi:10.1136/thorax.55.8.635
251. Gatheral T, Kumar N, Sansom B, et al. COPD-related bronchiectasis; independent impact on disease course and outcomes. *COPD*. Dec 2014;11(6):605-14. doi:10.3109/15412555.2014.922174
252. Bafadhel M, Umar I, Gupta S, et al. The role of CT scanning in multidimensional phenotyping of COPD. *Chest*. Sep 2011;140(3):634-642. doi:10.1378/chest.10-3007
253. Dou S, Zheng C, Cui L, et al. High prevalence of bronchiectasis in emphysema-predominant COPD patients. *Int J Chron Obstruct Pulmon Dis*. 2018;13:2041-2047. doi:10.2147/COPD.S163243
254. Agustí A, Calverley PM, Celli B, et al. Characterisation of COPD heterogeneity in the ECLIPSE cohort. *Respir Res*. Sep 10 2010;11(1):122. doi:10.1186/1465-9921-11-122
255. Martinez-Garcia MA, Soler-Cataluna JJ, Donat Sanz Y, et al. Factors associated with bronchiectasis in patients with COPD. *Chest*. Nov 2011;140(5):1130-1137. doi:10.1378/chest.10-1758
256. Garcia-Vidal C, Almagro P, Romani V, et al. *Pseudomonas aeruginosa* in patients hospitalised for COPD exacerbation: a prospective study. *Eur Respir J*. Nov 2009;34(5):1072-8. doi:10.1183/09031936.00003309
257. Miravittles M, Espinosa C, Fernandez-Laso E, Martos JA, Maldonado JA, Gallego M. Relationship between bacterial flora in sputum and functional impairment in patients with acute exacerbations of COPD. Study Group of Bacterial Infection in COPD. *Chest*. Jul 1999;116(1):40-6. doi:10.1378/chest.116.1.40
258. Rodrigo-Troyano A, Melo V, Marcos PJ, et al. *Pseudomonas aeruginosa* in Chronic Obstructive Pulmonary Disease Patients with Frequent Hospitalized Exacerbations: A Prospective Multicentre Study. *Respiration*. 2018;96(5):417-424. doi:10.1159/000490190
259. Sobala R, De Soyza A. Bronchiectasis and Chronic Obstructive Pulmonary Disease Overlap Syndrome. *Clin Chest Med*. Mar 2022;43(1):61-70. doi:10.1016/j.ccm.2021.11.005

260. Traversi L, Miravittles M, Martinez-Garcia MA, et al. ROSE: radiology, obstruction, symptoms and exposure - a Delphi consensus definition of the association of COPD and bronchiectasis by the EMBARC Airways Working Group. *ERJ Open Res.* Oct 2021;7(4)doi:10.1183/23120541.00399-2021
261. Diaz AA, Young TP, Maselli DJ, et al. Quantitative CT Measures of Bronchiectasis in Smokers. *Chest.* Jun 2017;151(6):1255-1262. doi:10.1016/j.chest.2016.11.024
262. Diaz AA, Young TP, Maselli DJ, et al. Bronchoarterial ratio in never-smokers adults: Implications for bronchial dilation definition. *Respirology.* Jan 2017;22(1):108-113. doi:10.1111/resp.12875
263. Martinez-Garcia MA, de la Rosa Carrillo D, Soler-Cataluna JJ, et al. Prognostic value of bronchiectasis in patients with moderate-to-severe chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* Apr 15 2013;187(8):823-31. doi:10.1164/rccm.201208-1518OC
264. Patel IS, Vlahos I, Wilkinson TM, et al. Bronchiectasis, exacerbation indices, and inflammation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* Aug 15 2004;170(4):400-7. doi:10.1164/rccm.200305-648OC
265. Gursel G. Does coexistence with bronchiectasis influence intensive care unit outcome in patients with chronic obstructive pulmonary disease? *Heart Lung.* Jan-Feb 2006;35(1):58-65. doi:10.1016/j.hrtlng.2005.04.003
266. Sahin H, Naz I, Susam S, Erbaycu AE, Olcay S. The effect of the presence and severity of bronchiectasis on the respiratory functions, exercise capacity, dyspnea perception, and quality of life in patients with chronic obstructive pulmonary disease. *Ann Thorac Med.* Jan-Mar 2020;15(1):26-32. doi:10.4103/atm.ATM_198_19
267. Du Q, Jin J, Liu X, Sun Y. Bronchiectasis as a Comorbidity of Chronic Obstructive Pulmonary Disease: A Systematic Review and Meta-Analysis. *PLoS One.* 2016;11(3):e0150532. doi:10.1371/journal.pone.0150532
268. Crisafulli E, Guerrero M, Ielpo A, et al. Impact of bronchiectasis on outcomes of hospitalized patients with acute exacerbation of chronic obstructive pulmonary disease: A propensity matched analysis. *Sci Rep.* Jun 18 2018;8(1):9236. doi:10.1038/s41598-018-27680-y
269. NICE. Chronic obstructive pulmonary disease in over 16s: diagnosis and management NICE; 2018.
270. Singh S, Loke YK. Risk of pneumonia associated with long-term use of inhaled corticosteroids in chronic obstructive pulmonary disease: a critical review and update. *Curr Opin Pulm Med.* Mar 2010;16(2):118-22. doi:10.1097/MCP.0b013e328334c085
271. *Controversies in COPD.* 2015:333.
272. Hill AB. The Environment and Disease: Association or Causation? *Proc R Soc Med.* May 1965;58(5):295-300.
273. Lu HY, Liao KM. The incidence of bronchiectasis in chronic obstructive pulmonary disease. *Open Med (Wars).* 2022;17(1):1927-1934. doi:10.1515/med-2022-0599
274. Eden E, Choate R, Barker A, et al. The Clinical Features of Bronchiectasis Associated with Alpha-1 Antitrypsin Deficiency, Common Variable Immunodeficiency and Primary Ciliary Dyskinesia--Results from the U.S. Bronchiectasis Research Registry. *Chronic Obstr Pulm Dis.* Apr 9 2019;6(2):145-153. doi:10.15326/jcopdf.6.2.2018.0156
275. Araújo D, Sucena M. Association between alpha 1 antitrypsin and bronchiectasis. *European Respiratory Journal.* 2015;46(suppl 59):PA1248. doi:10.1183/13993003.congress-2015.PA1248

276. Dandurand R, Carroll T, Estépar RSJ, Bourbeau J, Eidelman D. Bronchiectasis but not emphysema is more prevalent in non-PiZ alpha-1 antitrypsin deficiency (AATD) COPD than in usual COPD. *European Respiratory Journal*. 2014;44(Suppl 58):P4780.
277. Lonni S, Chalmers JD, Goeminne PC, et al. Etiology of Non-Cystic Fibrosis Bronchiectasis in Adults and Its Correlation to Disease Severity. *Ann Am Thorac Soc*. Dec 2015;12(12):1764-70. doi:10.1513/AnnalsATS.201507-472OC
278. Cuvelier A, Muir JF, Hellot MF, et al. Distribution of alpha(1)-antitrypsin alleles in patients with bronchiectasis. *Chest*. Feb 2000;117(2):415-9. doi:10.1378/chest.117.2.415
279. Polverino E, Goeminne PC, McDonnell MJ, et al. European Respiratory Society guidelines for the management of adult bronchiectasis. *Eur Respir J*. Sep 2017;50(3)doi:10.1183/13993003.00629-2017
280. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst Rev*. Dec 5 2016;5(1):210. doi:10.1186/s13643-016-0384-4
281. Google I. Google Translate. Accessed 30/11/2022, <https://translate.google.com/>
282. JBI. Critical Appraisal Tools. Joanna Briggs Institute. Accessed 30/11/2022, 2022. <https://jbi.global/critical-appraisal-tools>
283. Aggarwal N, Delgado BM, Salipalli S, et al. Why do some adults with PiMZ α 1-antitrypsin develop bronchiectasis? [corrected]. *ERJ Open Res*. Apr 2016;2(2)doi:10.1183/23120541.00021-2016
284. Carpagnano GE, Santacroce R, Palmiotti GA, et al. A New SERPINA-1 Missense Mutation Associated with Alpha-1 Antitrypsin Deficiency and Bronchiectasis. *Lung*. 2017/10/01 2017;195(5):679-682. doi:10.1007/s00408-017-0033-2
285. Fahim A, Wilmot R, Hart SP. Emphysema and bronchiectasis secondary to alpha-1 antitrypsin deficiency. *J Coll Physicians Surg Pak*. Mar 2013;23(3):224-5.
286. Milger K, Holdt LM, Teupser D, Huber RM, Behr J, Kneidinger N. Identification of a novel SERPINA-1 mutation causing alpha-1 antitrypsin deficiency in a patient with severe bronchiectasis and pulmonary embolism. *Int J Chron Obstruct Pulmon Dis*. 2015;10:891-7. doi:10.2147/COPD.S80173
287. Mosella M, Accardo M, Molino A, Maniscalco M, Zamparelli A. Description of a new rare alpha-1 antitrypsin mutation in Naples (Italy): PI*M S-Napoli. Case Report. *Annals of Thoracic Medicine*. January 1, 2018 2018;13(1):59-61. doi:10.4103/atm.ATM_234_17
288. Rodriguez-Cintron W, Guntupalli K, Fraire AE. Bronchiectasis and homozygous (P1ZZ) alpha 1-antitrypsin deficiency in a young man. *Thorax*. 1995;50(4):424-425. doi:10.1136/thx.50.4.424
289. Scott JH, Anderson CL, Shankar PS, Stavrides A. Alpha₁-Antitrypsin Deficiency with Diffuse Bronchiectasis and Cirrhosis of the Liver. *CHEST*. 1977;71(4):535-538. doi:10.1378/chest.71.4.535
290. Simpson S, Kueppers F, Steiner RM. Images in COPD: Alpha-1 Antitrypsin Deficiency and Bronchiectasis. *Chronic Obstr Pulm Dis*. Apr 7 2016;3(2):601-604. doi:10.15326/jcopdf.3.2.2016.0143
291. Veloso S, Aguilar X, Paniagua MJ, Vidal F, Richart C. [PiSZ-phenotype alpha 1-antitrypsin deficiency: a rare cause of bronchiectasis]. *Arch Bronconeumol*. May 2002;38(5):249-50. Deficit de alpha-1-antitripsina PiSZ. Una rara causa de bronquiectasias. doi:10.1016/s0300-2896(02)75204-6

292. Hersh CP, Campbell EJ, Scott LR, Raby BA. Alpha-1 Antitrypsin Deficiency as an Incidental Finding in Clinical Genetic Testing. *American Journal of Respiratory and Critical Care Medicine*. 2019;199(2):246-248. doi:10.1164/rccm.201809-1679LE
293. Jouhadi Z, Odou MF, Zerimech F, et al. Alpha1 antitrypsin deficiency due to an homozygous PI* Null Q0Cairo mutation: Early onset of pulmonary manifestations and variability of clinical expression. *Respir Med Case Rep*. 2018;24:58-62. doi:10.1016/j.rmcr.2018.04.005
294. Shin MS, Ho K-J. Bronchiectasis in Patients With α 1-Antitrypsin Deficiency: A Rare Occurrence? *CHEST*. 1993;104(5):1384-1386. doi:10.1378/chest.104.5.1384
295. Miravittles M, de Gracia J, Rodrigo MJ, et al. Specific antibody response against the 23-valent pneumococcal vaccine in patients with alpha(1)-antitrypsin deficiency with and without bronchiectasis. *Chest*. Oct 1999;116(4):946-52. doi:10.1378/chest.116.4.946
296. O'Brien ME, Pennycooke K, Carroll TP, et al. The impact of smoke exposure on the clinical phenotype of alpha-1 antitrypsin deficiency in Ireland: exploiting a national registry to understand a rare disease. *COPD*. May 2015;12 Suppl 1:2-9. doi:10.3109/15412555.2015.1021913
297. Wood AM, Bassford C, Webster D, et al. Vitamin D-binding protein contributes to COPD by activation of alveolar macrophages. *Thorax*. Mar 2011;66(3):205-10. doi:10.1136/thx.2010.140921
298. Guyatt GH, Sackett DL, Sinclair JC, Hayward R, Cook DJ, Cook RJ. Users' guides to the medical literature. IX. A method for grading health care recommendations. Evidence-Based Medicine Working Group. *JAMA*. Dec 13 1995;274(22):1800-4. doi:10.1001/jama.274.22.1800
299. Standards Unit NIS, Public Health England. UK Standards for Microbiology Investigations. Investigations of specimens for Mycobacterium species. London: NHS; 2020.
300. Kim YW, Jin KN, Heo EY, Park SS, Chung HS, Kim DK. The association between combined non-cystic fibrosis bronchiectasis and lung cancer in patients with chronic obstructive lung disease. *Int J Chron Obstruct Pulmon Dis*. 2015;10:873-9. doi:10.2147/COPD.S80439
301. Tiew PY, Lim AYH, Keir HR, et al. High Frequency of Allergic Bronchopulmonary Aspergillosis in Bronchiectasis-COPD Overlap. *Chest*. Jan 2022;161(1):40-53. doi:10.1016/j.chest.2021.07.2165
302. Bak SH, Kim S, Hong Y, Heo J, Lim MN, Kim WJ. Quantitative computed tomography features and clinical manifestations associated with the extent of bronchiectasis in patients with moderate-to-severe COPD. *Int J Chron Obstruct Pulmon Dis*. 2018;13:1421-1431. doi:10.2147/COPD.S157953
303. Tufvesson E, Markstad H, Bozovic G, Ekberg M, Bjermer L. Inflammation and chronic colonization of Haemophilus influenzae in sputum in COPD patients related to the degree of emphysema and bronchiectasis in high-resolution computed tomography. *Int J Chron Obstruct Pulmon Dis*. 2017;12:3211-3219. doi:10.2147/COPD.S137578
304. Xie C, Wen Y, Zhao Y, et al. Clinical Features of Patients with Bronchiectasis with Comorbid Chronic Obstructive Pulmonary Disease in China. *Med Sci Monit*. Sep 10 2019;25:6805-6811. doi:10.12659/MSM.917034
305. RStudio: Integrated Development for R. Version 2023.03.0. RStudio; 2020. <http://www.rstudio.com/>

306. Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J*. Dec 2012;40(6):1324-43. doi:10.1183/09031936.00080312
307. Stanojevic S, Graham BL, Cooper BG, et al. Official ERS technical standards: Global Lung Function Initiative reference values for the carbon monoxide transfer factor for Caucasians. *Eur Respir J*. Sep 2017;50(3)doi:10.1183/13993003.00010-2017
308. Ferrarotti I, Wencker M, Chorostowska-Wynimko J. Rare variants in alpha 1 antitrypsin deficiency: a systematic literature review. *Orphanet J Rare Dis*. Feb 22 2024;19(1):82. doi:10.1186/s13023-024-03069-1
309. Lomas DA, Irving JA, Gooptu B. Serpinopathies. In: Strnad P, Brantly M, Bals R, eds. *α 1-Antitrypsin Deficiency (ERS Monograph)*. European Respiratory Society; 2019:6-26.
310. Mahler DA, Wells CK. Evaluation of clinical methods for rating dyspnea. *Chest*. Mar 1988;93(3):580-6. doi:10.1378/chest.93.3.580
311. Lüdtke D, Ben-Shachar M, Patil I, Waggoner P, Makowski D. performance: An R Package for Assessment, Comparison and Testing of Statistical Models. *Journal of Open Source Software*. 2021;6(60)doi:10.21105/joss.03139
312. Venables W, Ripley B. *Modern Applied Statistics with S*. 4th ed. Springer; 2002.
313. Zeileis A, Kleiber C, Jackman S. Regression Models for Count Data in R. *Journal of Statistical Software*. 07/29 2008;27(8):1 - 25. doi:10.18637/jss.v027.i08
314. *A Package for Survival Analysis in R*. 2024. <https://CRAN.R-project.org/package=survival>
315. Adibi A, Sin DD, Safari A, et al. The Acute COPD Exacerbation Prediction Tool (ACCEPT): a modelling study. *Lancet Respir Med*. Oct 2020;8(10):1013-1021. doi:10.1016/S2213-2600(19)30397-2
316. Sandberg J, Engstrom G, Ekstrom M. Breathlessness and incidence of COPD, cardiac events and all-cause mortality: A 44-year follow-up from middle age throughout life. *PLoS One*. 2019;14(3):e0214083. doi:10.1371/journal.pone.0214083
317. de Torres JP, Casanova C, Montejó de Garcini A, Aguirre-Jaime A, Celli BR. Gender and respiratory factors associated with dyspnea in chronic obstructive pulmonary disease. *Respir Res*. Mar 6 2007;8(1):18. doi:10.1186/1465-9921-8-18
318. Choate R, Holm KE, Sandhaus RA, Mannino DM, Strange C. Long-Term SGRQ Stability in a Cohort of Individuals with Alpha-1 Antitrypsin Deficiency-Associated Lung Disease. *Int J Chron Obstruct Pulmon Dis*. 2024;19:889-900. doi:10.2147/COPD.S443183
319. Thomas ET, Guppy M, Straus SE, Bell KJL, Glasziou P. Rate of normal lung function decline in ageing adults: a systematic review of prospective cohort studies. *BMJ Open*. Jun 27 2019;9(6):e028150. doi:10.1136/bmjopen-2018-028150
320. Nielsen AO, Lange P, Hilberg O, Farver-Vestergaard I, Ibsen R, Lokke A. COPD and Smoking Status - It Does Matter: Characteristics and Prognosis of COPD According to Smoking Status. *Chronic Obstr Pulm Dis*. Jan 25 2024;11(1):56-67. doi:10.15326/jcopdf.2023.0433
321. Finklea JD, Khan G, Thomas S, Song J, Myers D, Arroliga AC. Predictors of mortality in hospitalized patients with acute exacerbation of bronchiectasis. *Respir Med*. Jun 2010;104(6):816-21. doi:10.1016/j.rmed.2009.11.021
322. Doll R, Peto R, Boreham J, Sutherland I. Mortality in relation to smoking: 50 years' observations on male British doctors. *BMJ*. Jun 26 2004;328(7455):1519. doi:10.1136/bmj.38142.554479.AE

323. Mortality GBD, Causes of Death C. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. Jan 10 2015;385(9963):117-71. doi:10.1016/S0140-6736(14)61682-2
324. Bouzid D, Hingrat QL, Salipante F, et al. Agreement of respiratory viruses' detection between nasopharyngeal swab and bronchoalveolar lavage in adults admitted for pneumonia: a retrospective study. *Clin Microbiol Infect*. Jul 2023;29(7):942 e1-942 e6. doi:10.1016/j.cmi.2022.12.024
325. Hare KM, Chang AB, Smith-Vaughan HC, et al. Do combined upper airway cultures identify lower airway infections in children with chronic cough? *Pediatr Pulmonol*. Jun 2019;54(6):907-913. doi:10.1002/ppul.24336
326. Pye A, Hill SL, Bharadwa P, Stockley RA. Effect of storage and postage on recovery and quantitation of bacteria in sputum samples. *J Clin Pathol*. Mar 2008;61(3):352-4. doi:10.1136/jcp.2007.051490
327. Pabreja K, Gibson P, Lochrin AJ, Wood L, Baines KJ, Simpson JL. Sputum colour can identify patients with neutrophilic inflammation in asthma. *BMJ Open Respir Res*. 2017;4(1):e000236. doi:10.1136/bmjresp-2017-000236
328. Channa S, Gale N, Lai E, Hall L, Quinn M, Turner AM. Colour vision deficiency and sputum colour charts in COPD patients: an exploratory mixed-method study. *NPJ Prim Care Respir Med*. Mar 4 2021;31(1):13. doi:10.1038/s41533-021-00225-z
329. Kelly MG, Murphy S, Elborn JS. Bronchiectasis in secondary care: a comprehensive profile of a neglected disease. *Eur J Intern Med*. Dec 2003;14(8):488-492. doi:10.1016/j.ejim.2003.10.002
330. Goeminne PC, Nawrot TS, Ruttens D, Seys S, Dupont LJ. Mortality in non-cystic fibrosis bronchiectasis: a prospective cohort analysis. *Respir Med*. Feb 2014;108(2):287-96. doi:10.1016/j.rmed.2013.12.015
331. Loebinger MR, Wells AU, Hansell DM, et al. Mortality in bronchiectasis: a long-term study assessing the factors influencing survival. *Eur Respir J*. Oct 2009;34(4):843-9. doi:10.1183/09031936.00003709
332. Wilson CB, Jones PW, O'Leary CJ, Hansell DM, Cole PJ, Wilson R. Effect of sputum bacteriology on the quality of life of patients with bronchiectasis. *Eur Respir J*. Aug 1997;10(8):1754-60. doi:10.1183/09031936.97.10081754
333. Evans SA, Turner SM, Bosch BJ, Hardy CC, Woodhead MA. Lung function in bronchiectasis: the influence of *Pseudomonas aeruginosa*. *Eur Respir J*. Aug 1996;9(8):1601-4. doi:10.1183/09031936.96.09081601
334. Martinez-Garcia MA, Faner R, Oscullo G, et al. Chronic Bronchial Infection Is Associated with More Rapid Lung Function Decline in Chronic Obstructive Pulmonary Disease. *Ann Am Thorac Soc*. Nov 2022;19(11):1842-1847. doi:10.1513/AnnalsATS.202108-974OC
335. Davis CP. Normal Flora. In: Baron S, ed. *Medical Microbiology*. 4th ed. 1996.
336. InflammationMAP® v. 1.1. Q2 Solutions: Rules-Based Medicine. 2024. <https://rbm.q2labsolutions.com/products-services/humanmap-services/inflammationmap/>
337. *pheatmap: Pretty Heatmaps*. 2018. <https://github.com/raivokolde/pheatmap>
338. *Carestream Vue Motion*. Version 12.2.1.600164. Carestream Health; 2014.
339. de Brito MC, Ota MK, Leitao Filho FS, Meirelles GS. Radiologist agreement on the quantification of bronchiectasis by high-resolution computed tomography. *Radiol Bras*. Jan-Feb 2017;50(1):26-31. doi:10.1590/0100-3984.2015.0146
340. Cohen J. A Coefficient of Agreement for Nominal Scales. *Educational and Psychological Measurement*. 1960;20(1):37-46. doi:10.1177/001316446002000104

341. Nakata H, Kimoto T, Nakayama T, Kido M, Miyazaki N, Harada S. Diffuse peripheral lung disease: evaluation by high-resolution computed tomography. *Radiology*. Oct 1985;157(1):181-5. doi:10.1148/radiology.157.1.4034963
342. Ueno J, Murase T, Yoneda K, Tsujikawa T, Sakiyama S, Kondoh K. Three-dimensional imaging of thoracic diseases with multi-detector row CT. *J Med Invest*. Aug 2004;51(3-4):163-70. doi:10.2152/jmi.51.163
343. Weinheimer O, Achenbach T, Buschsiewke C, Heussel CP, Uthmann T, Kauczor H-U. Quantification and Characterization of Pulmonary Emphysema in Multislice-CT. Springer Berlin Heidelberg; 2003:75-82.
344. Weinheimer O, Achenbach T, Bletz C, Duber C, Kauczor HU, Heussel CP. About objective 3-d analysis of airway geometry in computerized tomography. *IEEE Trans Med Imaging*. Jan 2008;27(1):64-74. doi:10.1109/TMI.2007.902798
345. Gu S, Wang Z, Siegfried JM, Wilson D, Bigbee WL, Pu J. Automated lobe-based airway labeling. *Int J Biomed Imaging*. 2012;2012:382806. doi:10.1155/2012/382806
346. Kang EY, Miller RR, Muller NL. Bronchiectasis: comparison of preoperative thin-section CT and pathologic findings in resected specimens. *Radiology*. Jun 1995;195(3):649-54. doi:10.1148/radiology.195.3.7753989
347. Hansell DM. Thin-section CT of the lungs: the Hinterland of normal. *Radiology*. Sep 2010;256(3):695-711. doi:10.1148/radiol.10092307
348. Cheplygina V, Perez-Rovira A, Kuo WY, Tiddens HAWM, de Bruijne M. Early Experiences with Crowdsourcing Airway Annotations in Chest CT. *Lect Notes Comput Sc*. 2016;10008:209-218. doi:10.1007/978-3-319-46976-8_22
349. Bodenberger AL, Konietzke P, Weinheimer O, et al. Quantification of airway wall contrast enhancement on virtual monoenergetic images from spectral computed tomography. *Eur Radiol*. Aug 2023;33(8):5557-5567. doi:10.1007/s00330-023-09514-2
350. Nardelli P, Lanng MB, Moller CB, et al. Accurate Measurement of Airway Morphology on Chest CT Images. *Image Anal Mov Organ Breast Thorac Images (2018)*. Sep 2018;11040:335-347. doi:10.1007/978-3-030-00946-5_34
351. Perossi J, Koenigkam-Santos M, Perossi L, et al. Correlation among clinical, functional and morphological indexes of the respiratory system in non-cystic fibrosis bronchiectasis patients. *PLoS One*. 2022;17(7):e0269897. doi:10.1371/journal.pone.0269897
352. Shaker SB, Dirksen A, Laursen LC, et al. Short-term reproducibility of computed tomography-based lung density measurements in alpha-1 antitrypsin deficiency and smokers with emphysema. *Acta Radiol*. Jul 2004;45(4):424-30. doi:10.1080/02841850410005642
353. Strange C, McElvaney NG, Vogelmeier CF, et al. The effect of exacerbations on lung density in alpha(1)-antitrypsin deficiency. *ERJ Open Res*. Mar 2023;9(2)doi:10.1183/23120541.00457-2022
354. Ronit A, Kristensen T, Colak Y, et al. Validation of lung density indices by cardiac CT for quantification of lung emphysema. *Int J Chron Obstruct Pulmon Dis*. 2018;13:3321-3330. doi:10.2147/COPD.S172695
355. Stockley RA, Pye A, De Soyza J, Turner AM, Miravittles M, investigators Es. The prevalence of bronchiectasis in patients with alpha-1 antitrypsin deficiency: initial report of EARCO. *Orphanet J Rare Dis*. Aug 12 2023;18(1):243. doi:10.1186/s13023-023-02830-2

356. Snell N, Gibson J, Jarrold I, Quint JK. Epidemiology of bronchiectasis in the UK: Findings from the British lung foundation's 'Respiratory health of the nation' project. *Respir Med*. Oct-Nov 2019;158:21-23. doi:10.1016/j.rmed.2019.09.012
357. Nash A. *National Population Projections: 2012-based Statistical Bulletin*. 2013:5.
358. Mao B, Lu HW, Li MH, et al. The existence of bronchiectasis predicts worse prognosis in patients with COPD. *Sci Rep*. Jun 16 2015;5:10961. doi:10.1038/srep10961
359. Ferraz MBD, Oliveira T, Ramos B, et al. Bronchiectasis as a prognostic factor in patients with alpha 1 antitrypsin deficiency? *European Respiratory Journal*. 2023;62(suppl 67):PA389. doi:10.1183/13993003.congress-2023.PA389
360. Spittle D, Pye A, Stockley RA, Turner AM. Bacterial Colonisation Doubles the Risk of Exacerbation in Subjects with Alpha-1 Antitrypsin Deficiency (AATD)-related COPD. *American Journal of Respiratory and Critical Care Medicine*. 2024;209:A2925-A2925. doi:10.1164/ajrccm-conference.2024.209.1_MeetingAbstracts.A2925
361. Stone H, McNab G, Wood AM, Stockley RA, Sapey E. Variability of sputum inflammatory mediators in COPD and alpha1-antitrypsin deficiency. *Eur Respir J*. Sep 2012;40(3):561-9. doi:10.1183/09031936.00162811
362. Malerba M, Ricciardolo F, Radaeli A, et al. Neutrophilic inflammation and IL-8 levels in induced sputum of alpha-1-antitrypsin PiMZ subjects. *Thorax*. Feb 2006;61(2):129-33. doi:10.1136/thx.2005.043471
363. Ramsey BW, Pepe MS, Quan JM, et al. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. Cystic Fibrosis Inhaled Tobramycin Study Group. *N Engl J Med*. Jan 7 1999;340(1):23-30. doi:10.1056/NEJM199901073400104
364. de Farias CF, Amorim MM, Dracoulakis M, Caetano LB, Santoro IL, Fernandes AL. Nasal lavage, blood or sputum: Which is best for phenotyping asthma? *Respirology*. May 2017;22(4):671-677. doi:10.1111/resp.12958
365. Savage HR, Rickman HM, Burke RM, et al. Accuracy of upper respiratory tract samples to diagnose Mycobacterium tuberculosis: a systematic review and meta-analysis. *Lancet Microbe*. Oct 2023;4(10):e811-e821. doi:10.1016/S2666-5247(23)00190-8
366. Aliberti S, Ringshausen FC, Dhar R, et al. Objective sputum colour assessment and clinical outcomes in bronchiectasis: data from the European Bronchiectasis Registry (EMBARC). *Eur Respir J*. Apr 2024;63(4)doi:10.1183/13993003.01554-2023
367. Stockley RA, Bayley D, Hill SL, Hill AT, Crooks S, Campbell EJ. Assessment of airway neutrophils by sputum colour: correlation with airways inflammation. *Thorax*. May 2001;56(5):366-72. doi:10.1136/thorax.56.5.366
368. Daniels JM, de Graaff CS, Vlaspoolder F, Snijders D, Jansen HM, Boersma WG. Sputum colour reported by patients is not a reliable marker of the presence of bacteria in acute exacerbations of chronic obstructive pulmonary disease. *Clin Microbiol Infect*. Jun 2010;16(6):583-8. doi:10.1111/j.1469-0691.2009.02892.x
369. Miravittles M, Marin A, Monso E, et al. Colour of sputum is a marker for bacterial colonisation in chronic obstructive pulmonary disease. *Respir Res*. May 14 2010;11(1):58. doi:10.1186/1465-9921-11-58
370. Wilkinson TM, Patel IS, Wilks M, Donaldson GC, Wedzicha JA. Airway bacterial load and FEV1 decline in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. Apr 15 2003;167(8):1090-5. doi:10.1164/rccm.200210-1179OC
371. Sibila O, Laserna E, Shoemark A, et al. Airway Bacterial Load and Inhaled Antibiotic Response in Bronchiectasis. *Am J Respir Crit Care Med*. Jul 1 2019;200(1):33-41. doi:10.1164/rccm.201809-1651OC

372. Yang SH, Song MJ, Kim YW, et al. Understanding the effects of *Haemophilus influenzae* colonization on bronchiectasis: a retrospective cohort study. *BMC Pulm Med*. Jan 2 2024;24(1):7. doi:10.1186/s12890-023-02823-8
373. Tufvesson E, Bjermer L, Ekberg M. Patients with chronic obstructive pulmonary disease and chronically colonized with *Haemophilus influenzae* during stable disease phase have increased airway inflammation. *Int J Chron Obstruct Pulmon Dis*. 2015;10:881-9. doi:10.2147/COPD.S78748
374. O'Neill K, Einarsson GG, Rowan S, et al. Composition of airway bacterial community correlates with chest HRCT in adults with bronchiectasis. *Respirology*. Jan 2020;25(1):64-70. doi:10.1111/resp.13653
375. Millares L, Pascual S, Monton C, et al. Relationship between the respiratory microbiome and the severity of airflow limitation, history of exacerbations and circulating eosinophils in COPD patients. *BMC Pulm Med*. Jun 24 2019;19(1):112. doi:10.1186/s12890-019-0867-x
376. Pini L, Tiberio L, Venkatesan N, et al. The role of bronchial epithelial cells in the pathogenesis of COPD in α -1 antitrypsin deficiency. *Respir Res*. Sep 14 2014;15(1):112. doi:10.1186/s12931-014-0112-3
377. Fawzy A, Anderson JA, Cowans NJ, et al. Association of platelet count with all-cause mortality and risk of cardiovascular and respiratory morbidity in stable COPD. *Respir Res*. May 8 2019;20(1):86. doi:10.1186/s12931-019-1059-1
378. Ellis P, Bailey E, Choate R, et al. Prevalence of Cardiovascular Disease and Rate of Major Adverse Cardiovascular Events in Severe Alpha-1 Antitrypsin Deficiency COPD. *Int J Chron Obstruct Pulmon Dis*. 2024;19:149-159. doi:10.2147/COPD.S419846
379. Biljak VR, Pancirov D, Cepelak I, Popovic-Grle S, Stjepanovic G, Grubisic TZ. Platelet count, mean platelet volume and smoking status in stable chronic obstructive pulmonary disease. *Platelets*. 2011;22(6):466-70. doi:10.3109/09537104.2011.573887
380. Wang RT, Li JY, Cao ZG, Li Y. Mean platelet volume is decreased during an acute exacerbation of chronic obstructive pulmonary disease. *Respirology*. Nov 2013;18(8):1244-8. doi:10.1111/resp.12143
381. Rahimi-Rad MH, Soltani S, Rabieepour M, Rahimirad S. Thrombocytopenia as a marker of outcome in patients with acute exacerbation of chronic obstructive pulmonary disease. *Pneumonol Alergol Pol*. 2015;83(5):348-51. doi:10.5603/PiAP.2015.0056
382. Doganci M, Eraslan Doganay G. Prognostic Significance of Thrombocytopenia and Mean Platelet Volume in COPD Patients with Acute Exacerbations in ICU Settings. *Med Sci Monit*. Jul 9 2024;30:e944946. doi:10.12659/MSM.944946
383. Nording HM, Seizer P, Langer HF. Platelets in inflammation and atherogenesis. *Front Immunol*. 2015;6:98. doi:10.3389/fimmu.2015.00098
384. Levine RF, Eldor A, Shoff PK, Kirwin S, Tenza D, Cramer EM. Circulating megakaryocytes: delivery of large numbers of intact, mature megakaryocytes to the lungs. *Eur J Haematol*. Oct 1993;51(4):233-46. doi:10.1111/j.1600-0609.1993.tb00637.x
385. Sever ZK, Bircan HA, Sirin FB, Evrimler S, Celik S, Merd N. Serum biomarkers in patients with stable and exacerbated COPD-bronchiectasis overlap syndrome. *Clin Respir J*. Nov 2020;14(11):1032-1039. doi:10.1111/crj.13238
386. de Camargo AA, de Castro RAS, Vieira RP, et al. Systemic Inflammation and Oxidative Stress in Adults with Bronchiectasis: Association with Clinical and Functional Features. *Clinics (Sao Paulo)*. 2021;76:e2474. doi:10.6061/clinics/2021/e2474

387. Pasteur MC, Bilton D, Hill AT, British Thoracic Society Bronchiectasis non CFGG. British Thoracic Society guideline for non-CF bronchiectasis. *Thorax*. Jul 2010;65 Suppl 1:i1-58. doi:10.1136/thx.2010.136119
388. Mott LS, Graniel KG, Park J, et al. Assessment of early bronchiectasis in young children with cystic fibrosis is dependent on lung volume. *Chest*. Oct 2013;144(4):1193-1198. doi:10.1378/chest.12-2589
389. Kim JS, Muller NL, Park CS, et al. Bronchoarterial ratio on thin section CT: comparison between high altitude and sea level. *J Comput Assist Tomogr*. Mar-Apr 1997;21(2):306-11. doi:10.1097/00004728-199703000-00028
390. Jacob J, Bartholmai BJ, Rajagopalan S, et al. Mortality prediction in idiopathic pulmonary fibrosis: evaluation of computer-based CT analysis with conventional severity measures. *Eur Respir J*. Jan 2017;49(1)doi:10.1183/13993003.01011-2016
391. Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. *Eur Respir J*. Nov 2005;26(5):948-68. doi:10.1183/09031936.05.00035205
392. Celli BR, Cote CG, Marin JM, et al. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. *N Engl J Med*. Mar 4 2004;350(10):1005-12. doi:10.1056/NEJMoa021322
393. Power M, Fell G, Wright M. Principles for high-quality, high-value testing. *Evid Based Med*. Feb 2013;18(1):5-10. doi:10.1136/eb-2012-100645
394. Jonas DE, Reuland DS, Reddy SM, et al. Screening for Lung Cancer With Low-Dose Computed Tomography: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. *JAMA*. Mar 9 2021;325(10):971-987. doi:10.1001/jama.2021.0377
395. Achenbach T, Weinheimer O, Biedermann A, et al. MDCT assessment of airway wall thickness in COPD patients using a new method: correlations with pulmonary function tests. *Eur Radiol*. Dec 2008;18(12):2731-8. doi:10.1007/s00330-008-1089-4
396. Cazzola M, MacNee W, Martinez FJ, et al. Outcomes for COPD pharmacological trials: from lung function to biomarkers. *Eur Respir J*. Feb 2008;31(2):416-69. doi:10.1183/09031936.00099306
397. Collaro AJ, McElrea MS, Marchant JM, et al. The effect of early childhood respiratory infections and pneumonia on lifelong lung function: a systematic review. *Lancet Child Adolesc Health*. Jun 2023;7(6):429-440. doi:10.1016/S2352-4642(23)00030-5
398. Ash SY, San Jose Estepar R, Fain SB, et al. Relationship between Emphysema Progression at CT and Mortality in Ever-Smokers: Results from the COPDGene and ECLIPSE Cohorts. *Radiology*. Apr 2021;299(1):222-231. doi:10.1148/radiol.2021203531
399. Perez-Rovira A, Kuo W, Petersen J, Tiddens HA, de Bruijne M. Automatic airway-artery analysis on lung CT to quantify airway wall thickening and bronchiectasis. *Med Phys*. Oct 2016;43(10):5736. doi:10.1118/1.4963214
400. De Soyza J, Chien HY, Onasanya AA, Turner AM. Is It Time Alpha-1 Antitrypsin Deficiency Had a Specific Patient Reported Outcome Measure? A Review. *Patient Relat Outcome Meas*. 2025;16:23-35. doi:10.2147/PROM.S490849
401. Wilson JMG, Jungner G, World Health O. Principles and practice of screening for disease. Geneva: World Health Organization; 1968.
402. Carreto L, Morrison M, Donovan J, et al. Utility of routine screening for alpha-1 antitrypsin deficiency in patients with bronchiectasis. *Thorax*. Jul 2020;75(7):592-593. doi:10.1136/thoraxjnl-2019-214195

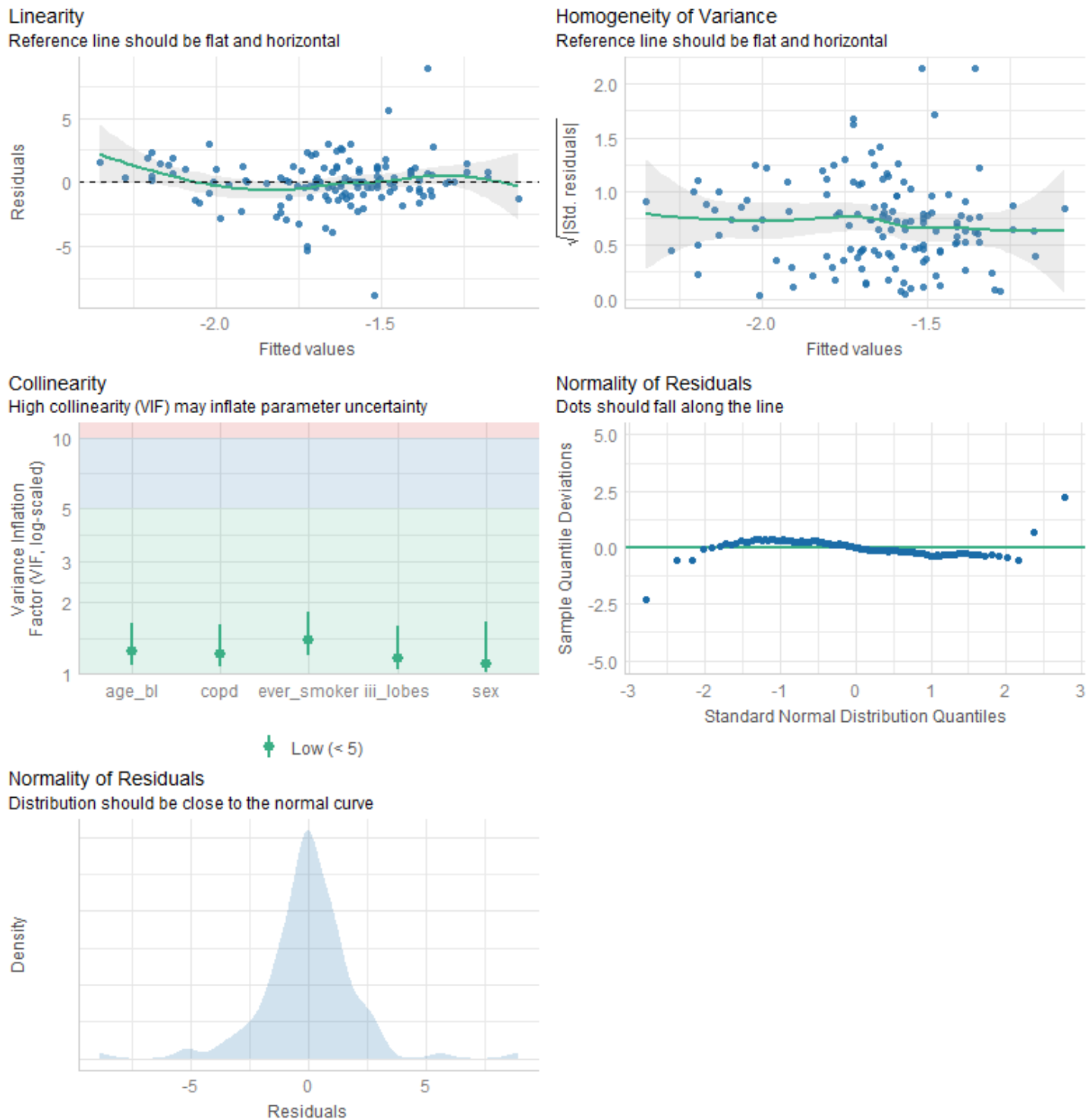
403. Mac Aogain M, Dicker AJ, Mertsch P, Chotirmall SH. Infection and the microbiome in bronchiectasis. *Eur Respir Rev.* Jul 2024;33(173)doi:10.1183/16000617.0038-2024
404. De Soyza J, Pye A, Turner AM. Are clinical trials into emerging drugs for the treatment of alpha-1 antitrypsin deficiency providing promising results? *Expert Opin Emerg Drugs.* Dec 2023;28(4):227-231. doi:10.1080/14728214.2023.2296088
405. De Soyza J, Pye A, Spittle DA, Turner AM. What are the radiological, microbiological and biochemical characteristics of bronchiectasis in alpha-1 antitrypsin deficiency? A systematic review. *European Respiratory Journal.* 2023;62(suppl 67):PA394. doi:10.1183/13993003.congress-2023.PA394
406. De Soyza J, Ellis P, Newnham M, Rickard L, Turner AM. Bronchiectasis Occurs Independently of Chronic Obstructive Pulmonary Disease in Alpha-1 Antitrypsin Deficiency. *Chronic Obstr Pulm Dis.* Sep 27 2024;11(5):507-514. doi:10.15326/jcopdf.2024.0526
407. Diaz AA, Orejas JL, Grumley S, et al. Airway-Occluding Mucus Plugs and Mortality in Patients With Chronic Obstructive Pulmonary Disease. *JAMA.* Jun 6 2023;329(21):1832-1839. doi:10.1001/jama.2023.2065
408. Dunican EM, Elicker BM, Henry T, et al. Mucus Plugs and Emphysema in the Pathophysiology of Airflow Obstruction and Hypoxemia in Smokers. *Am J Respir Crit Care Med.* Apr 15 2021;203(8):957-968. doi:10.1164/rccm.202006-2248OC
409. Lu HW, Mao B, Wei P, et al. The clinical characteristics and prognosis of ABPA are closely related to the mucus plugs in central bronchiectasis. *Clin Respir J.* Feb 2020;14(2):140-147. doi:10.1111/crj.13111
410. Robinson TE, Goris ML, Moss RB, et al. Mucus plugging, air trapping, and bronchiectasis are important outcome measures in assessing progressive childhood cystic fibrosis lung disease. *Pediatr Pulmonol.* Apr 2020;55(4):929-938. doi:10.1002/ppul.24646
411. Tiddens H, Van der Veer T, Charbonnier J-P, Latisenko R, Andrinopoulou E, Lynch DA. Fully Automated Mucus Plug Quantification on Chest CTs and Its Correlation With All-cause Mortality (abstract). *Am J Respir Crit Care Med.* 2024;209(A2778)doi:10.1164/ajrccm-conference.2024.209.1_MeetingAbstracts.A2778
412. Tiddens H, Van der Veer T, Charbonnier J-P, Latisenko R, Andrinopoulou E, Lynch DA. Cross-sectional Analysis of Automated Mucus Plug Quantification and Airway Wall Thickness in COPD Using AI-based Detection (abstract). *Am J Respir Crit Care Med.* 2024;209(A2779)doi:10.1164/ajrccm-conference.2024.209.1_MeetingAbstracts.A2779
413. Leitao Filho FS, Alotaibi NM, Ngan D, et al. Sputum Microbiome Is Associated with 1-Year Mortality after Chronic Obstructive Pulmonary Disease Hospitalizations. *Am J Respir Crit Care Med.* May 15 2019;199(10):1205-1213. doi:10.1164/rccm.201806-1135OC
414. Alsayed AR, Abed A, Khader HA, et al. Molecular Accounting and Profiling of Human Respiratory Microbial Communities: Toward Precision Medicine by Targeting the Respiratory Microbiome for Disease Diagnosis and Treatment. *Int J Mol Sci.* Feb 17 2023;24(4)doi:10.3390/ijms24044086
415. Bharti R, Grimm DG. Current challenges and best-practice protocols for microbiome analysis. *Brief Bioinform.* Jan 18 2021;22(1):178-193. doi:10.1093/bib/bbz155

416. Dicker AJ, Lonergan M, Keir HR, et al. The sputum microbiome and clinical outcomes in patients with bronchiectasis: a prospective observational study. *Lancet Respir Med*. Aug 2021;9(8):885-896. doi:10.1016/S2213-2600(20)30557-9
417. Chotirmall SH, Bogaert D, Chalmers JD, et al. Therapeutic Targeting of the Respiratory Microbiome. *Am J Respir Crit Care Med*. Sep 1 2022;206(5):535-544. doi:10.1164/rccm.202112-2704PP
418. Bassis CM, Erb-Downward JR, Dickson RP, et al. Analysis of the upper respiratory tract microbiotas as the source of the lung and gastric microbiotas in healthy individuals. *mBio*. Mar 3 2015;6(2):e00037. doi:10.1128/mBio.00037-15
419. Yagi K, Huffnagle GB, Lukacs NW, Asai N. The Lung Microbiome during Health and Disease. *Int J Mol Sci*. Oct 8 2021;22(19)doi:10.3390/ijms221910872
420. Ruvuna L, Hijazi K, Guzman DE, et al. Dynamic and prognostic proteomic associations with FEV(1) decline in chronic obstructive pulmonary disease. *medRxiv*. Aug 8 2024;doi:10.1101/2024.08.07.24311507
421. Spittle DA, Mansfield A, Pye A, Turner AM, Newnham M. Predicting Lung Function Using Biomarkers in Alpha-1 Antitrypsin Deficiency. *Biomedicines*. Jul 15 2023;11(7)doi:10.3390/biomedicines11072001
422. Enriquez-Rodriguez CJ, Casadevall C, Faner R, et al. A Pilot Study on Proteomic Predictors of Mortality in Stable COPD. *Cells*. Aug 14 2024;13(16)doi:10.3390/cells13161351
423. Enriquez-Rodriguez CJ, Casadevall C, Faner R, et al. COPD: systemic proteomic profiles in frequent and infrequent exacerbators. *ERJ Open Res*. Mar 2024;10(2)doi:10.1183/23120541.00004-2024
424. Finch S, Shoemark A, Dicker AJ, et al. Pregnancy Zone Protein Is Associated with Airway Infection, Neutrophil Extracellular Trap Formation, and Disease Severity in Bronchiectasis. *Am J Respir Crit Care Med*. Oct 15 2019;200(8):992-1001. doi:10.1164/rccm.201812-2351OC
425. De Soyza A, Smith G, Killick H, et al. S51 Heterogeneity of sputum and systemic inflammatory mediator profiles in bronchiectasis. *Thorax*. 2022;77(Suppl 1):A34-A34. doi:10.1136/thorax-2022-BTSabstracts.57
426. DeBoer EM, Kroehl ME, Wagner BD, et al. Proteomic profiling identifies novel circulating markers associated with bronchiectasis in cystic fibrosis. *Proteomics Clin Appl*. Sep 2017;11(9-10)doi:10.1002/prca.201600147
427. Pearson K. LIII. On lines and planes of closest fit to systems of points in space. *The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science*. 1901/11/01 1901;2(11):559-572. doi:10.1080/14786440109462720
428. Criner GJ, Cordova F, Sternberg AL, Martinez FJ. The National Emphysema Treatment Trial (NETT) Part II: Lessons learned about lung volume reduction surgery. *Am J Respir Crit Care Med*. Oct 15 2011;184(8):881-93. doi:10.1164/rccm.201103-0455CI

8 Appendix

8.1 Example of checking model assumptions

Figure A1: Testing of model assumptions using “performance” package in R, for the association of KCO % predicted annual change with ≥ 3 lobe involvement, with covariates of age, sex, smoking status (ever vs never) and COPD diagnosis.



8.2 Multivariate multinomial regression models

Multivariate multinomial regression models which did not find significant correlations with the variables of interest are presented here to avoid an overabundance of large complex tables in the main thesis. In many cases, there was insufficient variance of variables in each mMRC score category; this is noted in each case.

8.2.1 Sputum colour and mMRC

Figure A2.35: Associations of mMRC score with sputum colour, whole cohort

mode mMRC				
Associations	Odds Ratios	CI	p	mMRC score
colour	1.68	0.91 – 3.12	0.099	1
age bl	1.11	1.04 – 1.18	0.003	1
sex [Male]	2.46	0.63 – 9.61	0.194	1
ever smoker [Yes]	0.99	0.28 – 3.52	0.985	1
COPD [Yes]	1.21	0.25 – 5.73	0.813	1
colour	1.09	0.66 – 1.81	0.728	2
age bl	1.10	1.04 – 1.17	0.001	2
sex [Male]	0.87	0.31 – 2.45	0.787	2
ever smoker [Yes]	2.39	0.77 – 7.37	0.130	2
COPD [Yes]	Insufficient variance	Insufficient variance	Insufficient variance	2
colour	1.22	0.74 – 2.01	0.443	3
age bl	1.11	1.05 – 1.18	<0.001	3
sex [Male]	0.61	0.22 – 1.67	0.331	3
ever smoker [Yes]	8.70	2.58 – 29.32	0.001	3
COPD [Yes]	28.20	2.77 – 287.50	0.005	3
colour	1.48	0.85 – 2.57	0.161	4
age bl	1.02	0.96 – 1.08	0.532	4
sex [Male]	1.28	0.42 – 3.92	0.663	4
ever smoker [Yes]	7.06	1.59 – 31.42	0.010	4
COPD [Yes]	9.18	0.96 – 87.27	0.054	4
Observations	256			
R ² / R ² adjusted	0.242 / 0.239			

Figure 36: Associations of sputum colour and mMRC score, bronchiectasis only

mode mMRC				
<i>Associations</i>	<i>Odds Ratios</i>	<i>CI</i>	<i>p</i>	<i>mMRC score</i>
colour	1.08	0.39 – 2.96	0.885	1
age bl	1.06	0.94 – 1.20	0.331	1
sex [Male]	3.78	0.47 – 30.61	0.209	1
ever smoker [Yes]	0.64	0.07 – 5.55	0.681	1
COPD [Yes]	0.95	0.07 – 12.20	0.969	1
colour	0.74	0.28 – 1.94	0.536	2
age bl	1.00	0.89 – 1.13	0.956	2
sex [Male]	3.59	0.43 – 29.96	0.233	2
ever smoker [Yes]	0.32	0.04 – 2.84	0.301	2
COPD [Yes]	Insufficient variance	Insufficient variance	Insufficient variance	2
colour	0.87	0.35 – 2.21	0.774	3
age bl	1.02	0.91 – 1.14	0.714	3
sex [Male]	0.81	0.12 – 5.64	0.827	3
ever smoker [Yes]	4.11	0.51 – 33.24	0.182	3
COPD [Yes]	12.35	0.72 – 213.19	0.083	3
colour	1.13	0.40 – 3.23	0.817	4
age bl	0.91	0.81 – 1.04	0.167	4
sex [Male]	2.25	0.26 – 19.38	0.453	4
ever smoker [Yes]	1.42	0.14 – 14.68	0.766	4
COPD [Yes]	Insufficient variance	Insufficient variance	Insufficient variance	4
Observations	84			
R ² / R ² adjusted	0.295 / 0.288			

8.2.2 Sputum bacterial load and mMRC

Table A1.2: No association between bacterial load and mMRC score. Covariates age, sex, smoking status and COPD truncated for simplicity.

mode mMRC				
<i>Associations</i>	<i>Odds Ratios CI</i>		<i>p</i>	<i>mMRC score</i>
Bacterial load (organisms x10 ⁻³ /ml)	1.00	1.00 – 1.00	0.375	1
Bacterial load (organisms x10 ⁻³ /ml)	1.00	1.00 – 1.00	0.617	2
Bacterial load (organisms x10 ⁻³ /ml)	1.00	1.00 – 1.00	0.531	3
Bacterial load (organisms x10 ⁻³ /ml)	1.00	1.00 – 1.00	0.660	4
Observations	277			
R ² / R ² adjusted	0.150 / 0.148			

8.3 Z polymer levels and mMRC

Table A15.2: No association between mMRC score and Z polymer levels

mode mMRC				
Associations	Odds Ratios	CI	p	mMRC score
mean z polymer ugl	0.98	0.95 – 1.02	0.277	1
age bl	1.16	1.09 – 1.23	<0.001	1
sex [Male]	1.68	0.59 – 4.79	0.333	1
copd [Yes]	1.54	0.49 – 4.81	0.453	1
ever smoker [Yes]	3.39	1.12 – 10.21	0.031	1
mean z polymer ugl	0.99	0.96 – 1.02	0.575	2
age bl	1.14	1.07 – 1.21	<0.001	2
sex [Male]	1.74	0.59 – 5.15	0.318	2
copd [Yes]	12.29	2.27 – 66.56	0.004	2
ever smoker [Yes]	6.06	1.82 – 20.24	0.004	2
mean z polymer ugl	0.97	0.93 – 1.01	0.145	3
age bl	1.13	1.06 – 1.21	<0.001	3
sex [Male]	1.73	0.54 – 5.55	0.356	3
copd [Yes]	5.04	1.11 – 22.89	0.036	3
ever smoker [Yes]	8.39	2.18 – 32.30	0.002	3
mean z polymer ugl	0.97	0.93 – 1.02	0.221	4
age bl	1.07	0.98 – 1.16	0.111	4
sex [Male]	2.44	0.57 – 10.39	0.224	4
copd [Yes]	Insufficient variance	Insufficient variance	Insufficient variance	4
ever smoker [Yes]	Insufficient variance	Insufficient variance	Insufficient variance	4
Observations	176			
R ² / R ² adjusted	0.291 / 0.288			

8.4 Patients with outlying mean biomarker values

Table A1.3: Selected characteristics those with statistically outlying biomarker levels.

Mean biomarker reading	Baseline age	Sex	Genotype	Ever smoker	COPD	Bronchiectasis	Other relevant detail
Z polymers (µg/l)							
105.9	57	F	ZZ	Yes	Yes	No	
86.3	48	M	ZZ	Yes	Yes	No	
85.1	64	M	ZZ	Yes	No	No	
80.4	46	M	ZZ	Yes	Yes	No	
Eosinophils (x10⁹/L)							
1.01	61	F	ZZ	No	Yes	Yes	
0.80	48	M	ZZ	Yes	Yes	No	
0.77	26	F	ZZ	Yes	No	Yes	Asthma
0.73	66	F	ZZ	No	Yes	No	
0.70	32	F	ZZ	No	No	No	
0.68	52	F	SZ	Yes	No	No	
0.66	20	F	ZZ	No	No	No	
0.60	65	F	ZZ	No	Yes	No	
0.57	27	F	SZ	No	No	No	
0.56	52	M	ZZ	Yes	Yes	Yes	
0.55	73	M	ZZ	Yes	No	No	
Platelet (x10⁹/L)							
498	43	M	ZZ	Yes	Yes	Yes	
489	65	F	ZZ	Yes	Yes	Yes	
467	33	M	ZZ	Yes	Yes	No	
462	54	M	SZ	Yes	Yes	No	
459	41	M	Z-Null	Yes	Yes	No	
459	49	F	SZ	Yes	Yes	No	
450	54	F	ZZ	No	Yes	No	
444	58	M	SZ	Yes	Yes	Yes	
440	43	F	ZZ	Yes	Yes	No	
439	53	F	ZZ	Yes	Yes	No	
436	65	F	SZ	No	No	No	
434	44	F	ZZ	Yes	Yes	No	
CRP (mg/dl)							
76.6	56	M	ZZ	Yes	Yes	No	
60.7	49	F	ZZ	Yes	Yes	No	
53.0	43	F	ZZ	Yes	Yes	No	Single reading
45.0	46	M	ZZ	No	Yes	No	Single reading
40.2	43	M	ZZ	Yes	Yes	No	
31.8	53	F	ZZ	Yes	Yes	No	
TNFα (pg/ml)							
8.22	39	M	ZZ	Yes	No	No	
7.58	42	F	ZZ	Yes	No	No	
7.41	52	F	SZ	Yes	No	No	
IL-6 (pg/ml)							
10.45	51	M	ZZ	Yes	Yes	Yes	
6.57	40	F	ZZ	Yes	Yes	Yes	
IL-8 (pg/ml)							
25.4	20	F	ZZ	No	No	No	
25.0	57	M	ZZ	Yes	No	Yes	
24.0	64	F	ZZ	No	No	No	
20.8	79	M	SZ	Yes	Yes	Yes	

8.5 Heatmaps

Figure A1.4a: Heatmap showing correlation between continuous data in biomarkers and clinical outcomes, whole AATD cohort

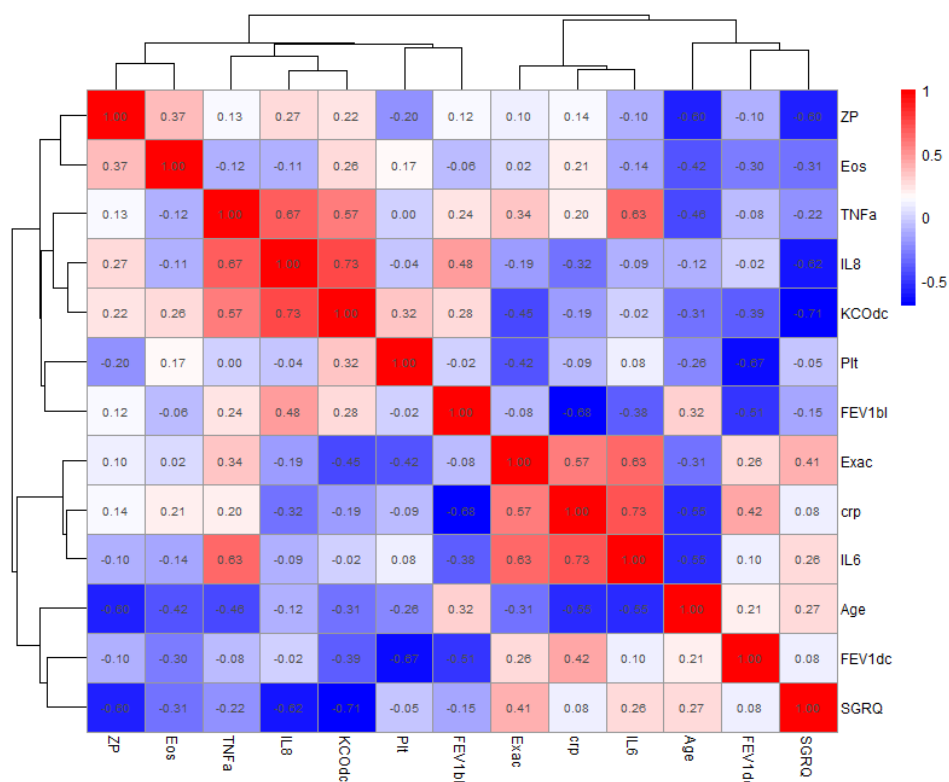
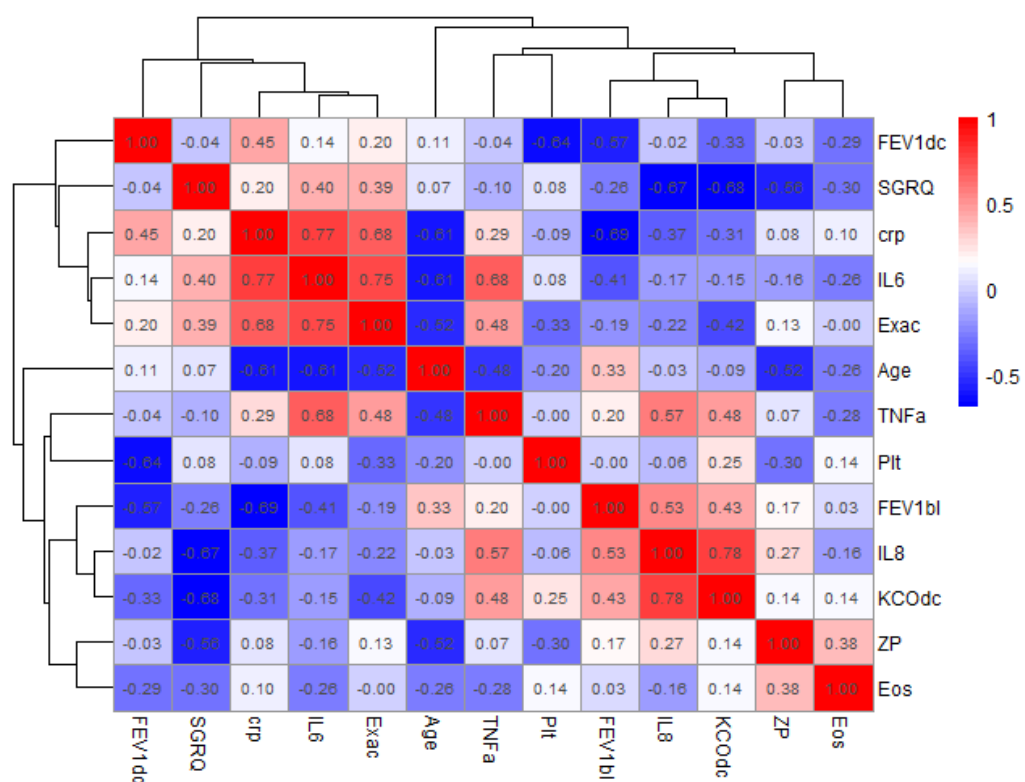


Figure A1.4b: Heatmap showing correlation between continuous data in biomarkers and clinical outcomes, limited to AATD-bronchiectasis only



8.6 Lobar involvement and mMRC score

Figure A1.5a: Collinearity between age and COPD

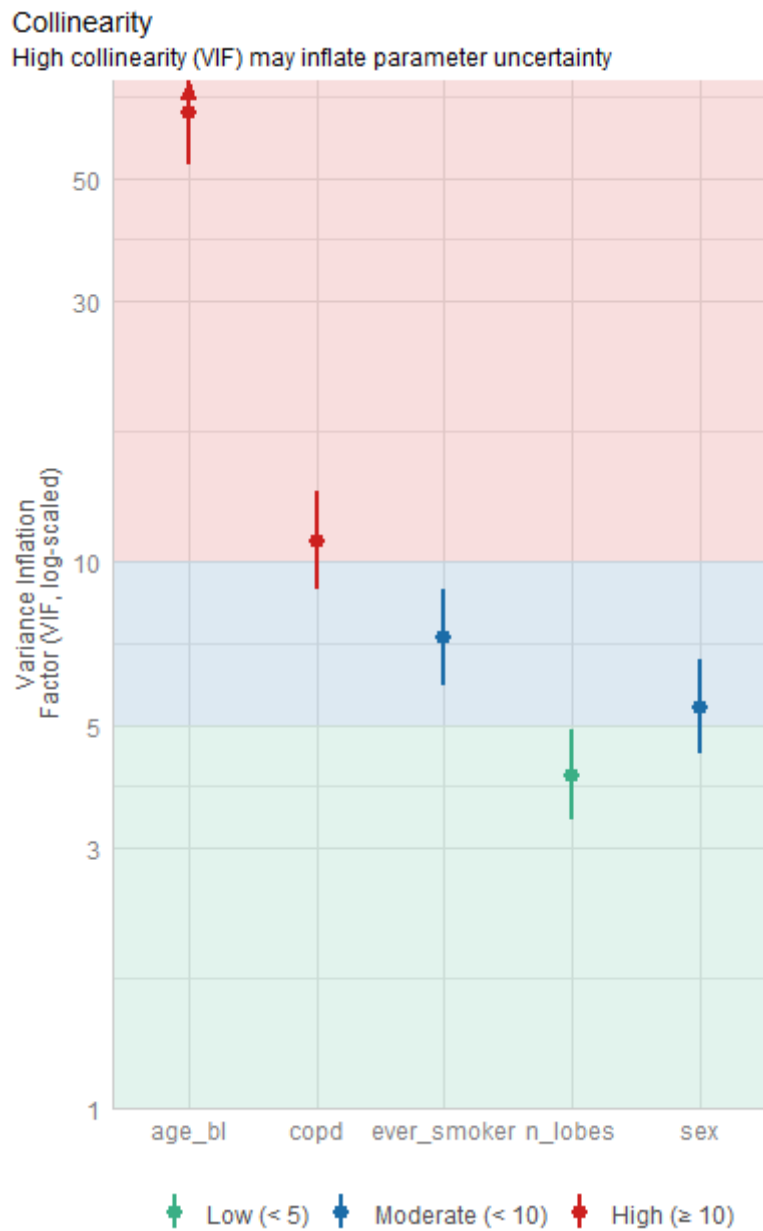


Figure A1.5b: Associations of lobar involvement with mMRC score

mode mMRC				
<i>Associations</i>	<i>Odds Ratios</i>	<i>CI</i>	<i>p</i>	<i>Response</i>
n lobes	0.88	0.71 – 1.10	0.260	1
age bl	1.07	1.02 – 1.12	0.005	1
sex [Male]	1.90	0.79 – 4.60	0.153	1
ever smoker [Yes]	3.06	1.18 – 7.95	0.021	1
copd [Yes]	0.85	0.32 – 2.28	0.747	1
n lobes	0.87	0.69 – 1.11	0.270	2
age bl	1.05	1.00 – 1.10	0.038	2
sex [Male]	1.14	0.46 – 2.85	0.771	2
ever smoker [Yes]	3.09	1.12 – 8.55	0.030	2
copd [Yes]	12.97	2.58 – 65.11	0.002	2
n lobes	0.89	0.70 – 1.13	0.342	3
age bl	1.03	0.99 – 1.08	0.166	3
sex [Male]	1.05	0.43 – 2.59	0.914	3
ever smoker [Yes]	8.12	2.78 – 23.68	<0.001	3
copd [Yes]	8.44	2.04 – 34.92	0.003	3
n lobes	1.02	0.76 – 1.38	0.884	4
age bl	1.02	0.96 – 1.08	0.503	4

sex [Male]	0.96	0.32 – 2.86	0.944	4
ever smoker [Yes]	10.71	2.28 – 50.24	0.003	4
copd [Yes]	5860861.58	968167.20 – 35479097.38	<0.001	4
Observations	259			
R ² / R ² adjusted	0.116 / 0.113			

8.7 Publications and conference abstracts arising from the thesis

8.7.1 Publications

- De Soyza J, Ellis P, Newnham M, Rickard L, Turner AM. Bronchiectasis Occurs Independently of Chronic Obstructive Pulmonary Disease in Alpha-1 Antitrypsin Deficiency. *Chronic Obstr Pulm Dis.* 2024;11(5):507-514. doi:10.15326/jcopdf.2024.0526
- De Soyza J, Pye A, Turner AM. Are clinical trials into emerging drugs for the treatment of alpha-1 antitrypsin deficiency providing promising results? *Expert Opin Emerg Drugs.* 2023;28(4):227-231. doi:10.1080/14728214.2023.2296088

8.7.2 Conference presentations

- De Soyza J, Ellis P, Rickard L, Newnham M, Turner AM. S40 Radiological bronchiectasis vs outcomes in alpha-1 antitrypsin deficiency *Thorax* 2024;79:A34.
- De Soyza J, Newnham M, Turner AM. Bronchiectasis and Disease Progression in Alpha-1 Antitrypsin Deficiency. *Am J Respir Crit Care Med* 2024;209:A3792.
- De Soyza J, Pye A, Spittle DA, Turner AM. What are the radiological, microbiological and biochemical characteristics of bronchiectasis in alpha-1 antitrypsin deficiency? A systematic review. *European Respiratory Journal* Sep 2023, 62 (suppl 67) PA394; DOI: 10.1183/13993003.congress-2023.PA394