Phenotyping bronchiectasis based on aetiology, exacerbation characteristics and response to erythromycin

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Thesis submitted to the University of Leicester for the degree of Doctorate of Medicine (MD)

May 2017

Abstract

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Background

Recurrent infections and daily symptoms are the main features of non-cystic fibrosis bronchiectasis. The beneficial effect of low-dose macrolides in these patients had been noted but at the time of initiation of this study no clinical trials had been undertaken. The aims were to assess the response to the drug and to determine how best to monitor the response. Informal clinic data had suggested a large improvement in the FEV_1 would be seen particularly in those with small airway disease, evident in the lung clearance index and the CT scoring data, and neutrophilic disease would have the best response to erythromycin.

Methods

Forty participants with CT proven non-cystic fibrosis bronchiectasis were recruited onto a single centre, open label, non-randomised clinical trial involving 7 visits at 12 week intervals. The first year (visits 1-5) were observational and provided the control data for the intervention of 250mg daily erythromycin which was taken for 12 weeks between visits 5 and 6. The remaining 12 weeks were used to monitor whether the response was maintained. A further 28 participants were recruited to provide further baseline data. Data was collected to assess quality of life, lung function, airway inflammation and airway micro- and mycobiology.

Results

The population predominantly had post-infectious and idiopathic bronchiectasis with normal spirometry but an abnormal lung clearance index. *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae* were the most commonly cultured bacteria and *Aspergillus fumigatus* the most commonly identified filamentous fungus. The primary end-point of a 200ml improvement in FEV_1 was not found however a response to erythromycin was seen in terms of a reduction in sputum production, improved lung clearance index and transient bacterial clearance. The lung clearance index demonstrated a significantly negative correlation with FEV_1 and a significantly positive correlation with the visual analogue scale scores but not the St George's Respiratory Questionnaire or Leicester Cough Questionnaire.

Conclusions

Erythromycin therapy was well-tolerated and had a beneficial effect on the daily symptoms of some participants so would be a useful therapy to trial in non-cystic fibrosis bronchiectasis.

Acknowledgements

I would like to thanks my supervisors Professor Andrew Wardlaw for his patience and encouragement and Professor Ian Pavord for making this research idea a reality. Drs Simon Range and Chandra Ohri provided helpful clinical input.

This research was funded by the Leicester Respiratory Biomedical Research Unit and without their financial backing the study would not have been possible.

Marcia Soares, Kate Haddon, Bev Hargadon, Michelle Bourne and the rest of the clinical team at the BRU were so very helpful in performing patient visits and investigations when time was tight. Dr Rob Free was excellent at solving problems with the RedCAP database. Sarah Terry provided invaluable support with the ethics aspects of the study. Hetan Bhatt, Will Monteiro and the rest of the BRU sputum lab tirelessly processed my samples and dealt with any queries in a cheerful manner. Cat Pashley and her dedicated team in the University of Leicester fungal laboratory kindly cultured and identified all my fungal samples and Cat spent many hours teaching me about respiratory fungi. Nicola Mills and her team of physiotherapists happily saw my exacerbation patients at short notice.

The rest of my colleagues in the BRU, and the SpRs at Glenfield Hospital, kept me cheerful during this time. Extra special thanks goes to Dr Rachael Evans who made it possible for me to write during a busy job.

The patients involved in this study dedicated many hours of their time to undergo multiple investigations with the hope of furthering the body of research in non-cystic fibrosis bronchiectasis. It was their selfless generosity that kept me focused on completion.

And finally thank you to my husband Hamish who has been amazingly supportive during the period of this research.

Declaration

I hereby declare that this thesis has been composed by myself and the work undertaken by myself except where acknowledgement has been given.

The patient information booklets and GP information letters were written, posted and filed by myself. I undertook the majority of the patient visits and investigations myself with the exception of a few visits which were performed by Kate Haddon, a research nurse. Marcia Soares, a respiratory physiologist, performed around a third of the multiple breath washouts and analysed all the data from this investigation using a programme devised by the department. Dr Sumit Gupta (radiology SpR) analysed all the CT scoring data. All the data was analysed in Excel and SPSS by myself with some initial statistical input from Dr Chris Newby, a statistician.

No part of this thesis has been submitted in any previous application as part of a higher degree.

I hereby give permission for this thesis to be made available for consultation, photocopying and use by other libraries directly or via the British Library.

Alys Jane Scadding

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Chapter One: Introduction

1.1 Background to bronchiectasis

1.11 Rationale and background for the clinical trial

At the time of designing this study in 2011 there were no large randomised controlled trials published in the field of non-cystic fibrosis bronchiectasis and low dose erythromycin. There had been some published evidence for the use of either low dose macrolides or other antibiotics in bronchiectasis and the majority of the data published was in the field of Asian Diffuse Panbronchiolitis. This is discussed in a later section of the introduction. In Glenfield Hospital there was anecdotal evidence of large improvements in both symptoms and spirometry in bronchiectasis patients following a 12 week course of erythromycin at 250mg daily, an eighth the dose for acute respiratory tract infections. The dose used was comparable to that used in the Diffuse Panbronchiolitis studies. As part of the background to this study I reviewed the clinic data in terms of spirometry pre- and post-erythromycin, reported symptoms and pathology seen on the CT. The most striking finding was that the mean improvement in FEV_1 pre- and post-treatment was 336mls (sd. 0.255l), an impressive change following a reasonably short duration of therapy. A clinical trial was designed to replicate the improvements seen in the clinical setting. Initially it was hoped a randomised, double-blind, placebo-controlled trial could be carried out but unfortunately due to financial and time constraints this was not possible. Therefore the study was designed to observe the recruited bronchiectasis patients for 1 year as a control group prior to the addition of the erythromycin at 250mg daily for 12 weeks. The study participants were then followed for a further 12 weeks to monitor whether the improvements seen with erythromycin therapy were sustained. The rationale for this was related to the observation from these clinic patients that the response had lasted after the erythromycin was completed. This data was presented at the East Midlands Thoracic Society meeting in 2012.

1.1.2 Discovery and definitions

The term bronchiectasis is derived from the Greek words *bronkhia* (meaning bronchial tubes) and *ektasis* (meaning extension or stretching) which describes some of the pathophysiological changes seen in non-cystic fibrosis bronchiectasis. The first recognisable description of bronchiectasis comes from the writings of Rene Theophile Hyacinthe Laennec (1781-1826), a French physician known for inventing the early stethoscope. Of bronchiectasis, he writes:

"*This affection of the bronchia is always produced by chronic catarrh or by some other disease attended by long, violent and often repeated bouts of coughing."* (1)

The latter part of his career involved post-mortem examinations, where he encouraged the attending physician to link the findings from auscultation with those at dissection. In bronchiectasis the dilated bronchi and bronchioles were noted to extend to the lung peripheries and were measured according to crow quills (2-3mm diameter) and goose quills (4mm diameter)(2). William Ewart described bronchiectasis following post-mortem examination which he documented in his textbook *A System of Medicine* published in 1898 (3). Further classification of the condition followed Lynne Reid's studies in the 1950s, during which she performed bronchograms on post-mortem specimens and described the dilated airways as cylindrical, varicose or saccular in appearance. She also noted the striking appearance of luminal obstruction from mucus casts and bronchial wall thickening with inflammation and fibrosis (4). These finding correlate with the pathophysiology we recognise today.

Today, the British Thoracic Society now uses the following description for bronchiectasis:

"Bronchiectasis is a persistent or progressive condition characterised by dilated thick-walled bronchi. The symptoms vary from intermittent episodes of expectoration and infection localised to the region of the lung that is affected to persistent daily

expectoration often of large volumes of purulent sputum. Bronchiectasis may be associated with other non-specific respiratory symptoms including dyspnoea, chest pain and haemoptysis, and may progress to respiratory failure and cor pulmonale."(5)

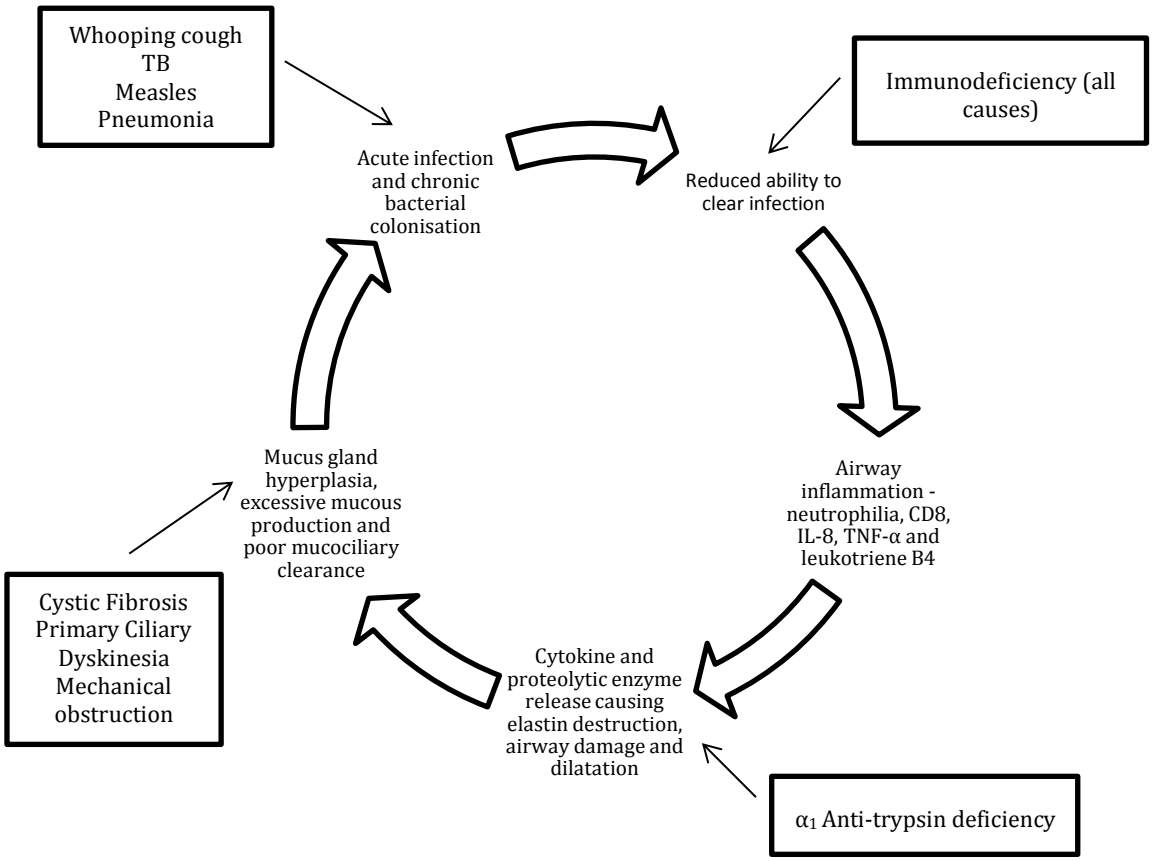
Our modern definition of bronchiectasis involves a combination of CT findings and symptoms. The changes on CT include dilated bronchi wider than the accompanying pulmonary arteriole or a lack tapering if there is no accompanying vessel (6). There are now several bronchiectasis severity scoring systems available which take into account bronchial dilatation, wall thickness, distribution, number of affected lobes and the presence of small airway involvement. These are described in more detail further on.

This section outlines the underlying pathophysiology, aetiology, underlying inflammation, microbiology and describes the severity scoring systems used.

1.1.3 Pathophysiology

Non-cystic fibrosis bronchiectasis is a chronic, usually progressive and potentially disabling respiratory condition which is characterised by chronic sputum production and recurrent chest infections. The underlying pathophysiology behind the airway dilatation process is only now being discovered resulting in exciting changes to the way the condition is being approached. The diagram below demonstrates the vicious cycle of infection, inflammation, airway damage and reduced mucus clearance we now understand to be central to this process.

The vicious cycle hypothesis



1.1.4 Bronchiectasis and airway inflammation

Mucosal inflammation has a key role to play in the vicious cycle hypothesis and is felt to underpin the development of the airway damage and progression of non-cystic fibrosis bronchiectasis. Histological analysis of lungs with bronchiectasis demonstrated mucus gland and goblet cell hyperplasia and hypertrophy with the mucus glands far outnumbering the goblet cells (7,8). This suggests that the mucus glands contribute significantly to the characteristic symptom of increased and daily sputum production.

A precipitating event such as an infection initiates a macrophage response which, along with epithelial cells, causes the release of IL-8. This leads to neutrophil migration to the airways and further release of pro-inflammatory mediators such as TNF-α, transcription factors, myeloperoxidase and proteolytic enzymes such as neutrophil elastase (9,10). Various studies have demonstrated elevated levels of IL-8 in the sputum of stable bronchiectasis patients (8,11,12). IL-8 correlates with sputum neutrophilia and neutrophil elastase (13–15). Neutrophil elastase is thought to be one of the key causes of the bronchial wall remodelling which leads to the permanent dilatation and the inflammatory infiltrate and an increase in bronchial wall thickness. In chronic inflammation there is thought to be a down-regulation of α_1 antitrypsin causing increased activity of bacterial proteinases resulting in further tissue destruction (16).

1.2 The microbiology of bronchiectasis and the host-bacterial interaction

1.2.1 The lung microbiome

The scientific community once believed healthy lungs to be sterile with bacterial infection and colonisation only occurring in the event of chronic disease, such as bronchiectasis and COPD, and acute infection, such as pneumonia. Bacteria were simply not supposed to live within the lungs and the aim of disease management was eradication to achieve a sterile environment once again. We now know there are multiple bacterial genera existing in the lungs at any one time in both diseased and healthy lungs (17–19). The majority of bacteria live harmoniously in a symbiotic relationship with our cells and immune systems.

The sterile lung theory has been driven by laboratory sputum culture results which often report sputum samples as having "no significant growth." This was always taken to mean that there was no bacterial growth but it is increasingly becoming understood that no "pathogenic" bacteria have been cultured, only "normal lung flora." But what actually constitutes normal lung flora? As new culture-independent techniques have been developed it has become increasingly clear is that we only know about the bacteria that we can culture.

The lung microbiome is formed from aerobic, anaerobic, gram negative and gram positive bacteria. *Pseudomonas* species, *Staphylococci, Moraxella catarrhalis* and *Streptococci* are aerobic and readily grow in the lung whereas *Klebsiella* species, *Escherichia* species, *Haemophilus* species and *Proteus* species are facultative anaerobes so will live well in both aerobic and anaerobic conditions. *Haemophilus* is also a capnophile, preferring an environment of 15% oxygen and 5-10% carbon dioxide. This is a similar environment to the lungs in chronic respiratory disease. In non-cystic fibrosis bronchiectasis the most commonly identified bacteria from standard laboratory cultures are in descending order, *Haemophilus influenzae,*

Pseudomonas aeruginosa, Moraxella catarrhalis, Streptococcus pneumoniae and *Staphylococcus aureus*.(20) By contrast, culture-independent analysis of the lung microbiome has revealed the most prevalent to be: *Haemophilus influenzae, Pseudomonas aeruginosa*, *Veillonella* species, *Prevotella* species, and *Streptococcus* species (21).

There is conflicting evidence regarding the origins and stability of the lung microbiome. We know that the microbiome from any organ system is individual to a person and can be dated back to the bacteria acquired at birth with modifications in the bacterial genera with events affecting the respiratory system. Evidence is emerging that the healthy population have the most diverse microbiomes while those with severe lung disease have a dramatic reduction in the number of genera identified (19). It has been suggested that lung microbiomes are host specific and similar to their oral microbiome (18) while other authors have found that the lung microbiome is different to the recognised oral microbiome (19). This has also been recognised in the gut microbiome in patients with inflammatory bowel disease (22). In the setting of COPD it has been suggested that inhaled corticosteroids and bronchodilators can affect the oral microbiome, increase periodontal disease and increase the likelihood of micro-aspiration thus changing the lung microbiome (18). In cystic fibrosis the microbiome remains relatively stable despite exacerbations. It can be hypothesised that this is the case in non-cystic fibrosis lungs (23).

1.2.2 Biofilms

Bacterial colonies exist in two different formations – freely mobile, individual cells (planktonic) or a sessile colony known as a biofilm. From the 1800s bacteria were thought to be free-floating planktonic organisms. The environmental microbiological community recognised the formation of biofilms long before the medical microbiological community. A biofilm is the polysaccharide based extra

cellular matrix produced by almost any bacteria to ensure species survival in stressful environmental conditions such as hypoxia and lack of nutrition (24). They have been detected on both organic surfaces such as mucus membranes and inorganic materials such as medical devices and have been estimated to cause a significant number of bacterial infections (25). Bacteria within a biofilm are relatively protected from antibiotic therapy which poses a challenge in the treatment of infection in chronic lung disease. Bacteria can switch from a non-mucoid colonising formation to a biofilm community provoked by environmental stressors such as antibiotic therapy or a reduced nutrition supply. The role of environmental stress is clear but additionally mutations in the mucus producing genes such as *mucA22* which is commonly seen in the *Pseudomonas* colonising cystic fibrosis patients may favour biofilm formation (26). The development of mucus plugging in the small airways has the effect of reducing alveolar oxygen concentration to 5% from the normal of around 15% and increasing CO_2 concentrations from 5% to 6%. This has been described as a sufficient stressor to induce bacterial biofilm formation (27,28).

It is thought that once a bacterial biofilm has been formed within the airways there are two possible outcomes. One is that planktonic cells germinate from the biofilm and are released to cause exacerbations. The other possibility involves an immunomodulatory reaction between the host and the alginate layer beneath the biofilm (27). Bacteria communicate within the biofilm using chemical based quorum sensing systems with differing targets, from increasing gene transcription for virulence factors such as proteases, exotoxins and antimicrobial products to co-ordinating communities and forming the biofilm. Biofilms cause slowed bacterial metabolism and division, increased mutation rates and increased antibiotic resistance (27).

1.3 Aetiology and associated conditions

1.3.1 Idiopathic

This category involves those patients with bronchiectasis which cannot be attributed to another cause. Previous studies characterising the non-CF bronchiectasis cohort have reported this group to be the most common and were most likely to be middle aged, non-smoking, Caucasian females (29–31). However, the proportion of people who fit this category is likely to diminish as our increasingly complex investigations accurately identify more underlying conditions. For a large proportion of patients there is not currently an identifiable underlying cause but some will have an unrecognised immunological deficit (32).

1.3.2 Post-infectious

Historically post-infectious bronchiectasis has been attributed to childhood pulmonary infections such as whooping cough, pneumonia and TB. Of course most people, both with and without bronchiectasis, can recall a previous lung infection but most recover quickly without any lasting or detectable damage. We may not truly know to what extent historical infection causes bronchiectasis. It is possible that the widespread use of vaccinations and antibiotics will result in a decline in infections and therefore post-infectious bronchiectasis although as vaccinations have been around for some years this will only become apparent, if true, later on.

1.3.3 Immunodeficiencies

Immunodeficiency can be divided into primary and secondary categories. The primary causes are discussed below. Secondary causes include immunosuppression therapy in the cause of inflammatory or autoimmune conditions, chemotherapy for cancer, prolonged steroid use and HIV infection.

Primary immunodeficiencies

Investigations for subtle immunodeficiencies are becoming increasingly complex. The BTS non-CF bronchiectasis guidelines recommend routine investigation of immunoglobulins A, G and M and the antibody response to a vaccination for encapsulated bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenza* type b and *Neisseria meningitidis* (6,33). However, the vast majority of patients with bronchiectasis will have normal immunoglobulin levels and no identifiable immunodeficiency.

Common variable immunodeficiency (CVID)

This is characterised by low IgG +/- low IgM and IgA levels. Sometimes there is a poor functional antibody response to vaccination. There is a predisposition to autoimmune conditions, some endocrine conditions, granuloma formation and some malignancies especially gastrointestinal and haematological.

Selective IgA deficiency

This is the most common primary immunodeficiency and has a prevalence of 1:500 in the Caucasian population (34). It is characterised by low or absent blood IgA levels and has been associated with autoimmune disease (30%), asthma and rhinitis. The clinical manifestations range from asymptomatic to recurrent infections with a similar picture to CVID (35,36).

Selective IgM deficiency

Declining IgM levels are common with advancing age but very low levels combined with recurrent or severe infections indicates an immunodeficiency, which may be part of a genetic condition (37). It has been estimated in 1:3000 of the general population and has been associated with allergy, asthma, atopy and autoimmune disease (38,39). An undetectable IgM level is very rare.

Sub-class and specific antibody deficiency

Some patients with bronchiectasis have an impaired antibody response to encapsulated bacteria. This has been termed selective anti-polysaccharide antibody deficiency and has been emerging as an area of interest in the aetiology and cause of recurrent infections in bronchiectasis since the 1980s (40). In adults, the diagnosis is made if in <70% of serotypes there is an inadequate IgG response to the vaccination given (often the 23-valent pneumococcal vaccination) or the immunological response is not maintained for more than a couple of months. The responses can be measured in terms of the total IgG response or the specific IgG subclass response to the bacterial serotypes.

There are four IgG sub-classes – IgG_1, IgG_2, IgG_3 and IgG_4. This immunodeficiency usually results in normal total IgG levels and predominantly involves the IgG_{2-4} sub-classes as these are the least abundant. IgG_1 deficiency often results in total low IgG levels and is therefore diagnosed and treated as either CVID or specific antibody deficiency (SPAD). The sub-classes have different functions: IgG_1 and IgG_3 are predominantly active against diphtheria and tetanus toxins and viruses while IgG_2 is active against the lipopolysaccharide molecules of gram negative bacteria such as *Haemophilus influenzae* and *Streptococcus pneumoniae* although in high quantities will inhibit bacterial killing (41). Patients with IgG_2 deficiency have the most severe clinical symptoms.

A combined IgA and IgG_2 subclass deficiency has been found to be an important risk factor for pulmonary infection (42). This is illustrated by a study of 22 patients with presumed idiopathic bronchiectasis, normal total immunoglobulin levels and a normal total antibody response to the 23-valent pneumococcal vaccination who were experiencing between 3 and 12 respiratory tract infections a year. Fifty percent of these had an inadequate IgA and IgG_2 subclass response to the vaccination. The patients with the inadequate humoural responses had a greater

frequency of positive sputum culture and had more extensive bronchiectasis in the lung peripheries on CT scan than the group with the adequate responses (43).

The Innate Lung Immunity and the host-bacterial interaction

The immune system has evolved to fight all kinds of pathogens successfully. However, pathogens are continually evolving as their cell turnover is so rapid. They have developed many ways to evade the immune system and antibiotic therapy in order to survive as a species.

Surfactant proteins

There are four surfactant proteins (SP) within the lung – A, B, C and D. SP-A and SP-D are two of the four surfactant proteins produced by the type II alveolar cells and clara cells (non-ciliated epithelial cells). These proteins bind to bacteria, predominantly *Streptococci, Staphylococcus aureus, Klebsiella, Haemophilus influenza* and *Pseudomonas aeruginosa,* viruses, especially *Influenza A* and fungi, in particular *Aspergillus* species in order to enhance opsonisation and phagocytosis. SP-A and SP-D also play a role in the intracellular killing of *Mycobacterial* species (44). Various studies have demonstrated a reduction in the SP-A and SP-D levels in chronic lung conditions and acute viral infections though it is unclear as to whether this is due to consumption in the infected state, lysis by bacteria or primary immunodeficiency (45,46).

Host endotoxin binding proteins and their inhibitors

The lung's surfactant layer also contains a group of innate immunity defence proteins comprising the PLUNC proteins (palate, lung, nasal, epithelial clone), the Bactericidal/Permeability Increasing protein (BPI) and Lipopolysaccharide Binding Protein (LBP) (47–49). These proteins are all coded within chromosome 20 and are collectively termed Host Endotoxin Binding Proteins. BPI and LBP bind to lipopolysaccharide A and the o-antigen of the lipopolysaccharide cell wall of

gram negative bacteria enabling and enhancing bactericidal activity (50). This binding activates both CD14 dependent, via LPS-LBP binding, and independent cytokine cascades to neutralise endotoxin.

In 1995 Zhao et al discovered an anti-neutrophilic cytoplasmic antibody against BPI proteins called BPI-ANCA. It has been detected in chronic inflammatory lung conditions, such as diffuse panbronchiolitis and bronchiectasis, ulcerative colitis and rheumatoid arthritis. In 1998 Kobayshi recognised an association between BPI-ANCA and chronic lung infections and postulated that it is the gram negative bacterium itself which drives the ANCA production thereby rendering the innate immunity sub-optimal to such infections (49).

We also know from work in cystic fibrosis and bronchiectasis patients colonised with *Pseudomonas aeruginosa* have over-production of IgG_2 against the bacterial LPS which inhibits the immune mediated *Pseudomonas* killing (41). From research in cystic fibrosis we know that patients with the highest and most rapidly rising anti-*Pseudomonas* antibodies have the worst prognosis despite a strong immunological response (51). Unfortunately the anti-*Pseudomonal* antibodies form immune complexes which drive cytokine mediated tissue inflammation and destruction (27).

The lung also produces anti-microbial proteins such as lysozyme and lactoferrin. Lysozyme predominantly has activity against gram-positive bacteria while lactoferrin chelates iron away from gram-negative bacteria resulting in weakening of the cell wall resulting in bacterial death (52).

Toll-like Receptors (TLRs)

Toll-like receptors are a group of thirteen immune-modulatory pattern recognition proteins which span the surface of macrophages and dendritic cells. They identify the pathogen-associated molecular patterns (PAMPs) on bacteria, viruses and fungal cells leading to the activation of the immune response (53). Toll-like

receptors sometimes work in pairs and bind to different pathogens. TLR1/2 pairs bind to bacterial lipopeptides, TLR2/6 pairs bind with gram positive bacteria, fungi and mycoplasma. TLR3 binds to viral dsDNA, TLR4 primarily binds to gram negative bacterial lipopolysaccharides, but can also bind with protozoa, candida and host cells. TLR5 binds to bacterial flagellae, TLR7 and 8 to viral ssRNA and TLR 9 to DNA in viruses, bacteria and protozoa (53–56). Binding to the pathogen initiates an immune cascade. Specific TLR deficiencies have been implicated as a cause of deep seated infection by *Staphylococcus aureus, Streptococcus pneumoniae and Pseudomonas aeruginosa* in young children (34).

1.3.4 Mucociliary clearance disorders

Primary ciliary dyskinesia

This is an autosomal recessive condition which results in defective structure, function or both in the central or peripheral microtubules of the cilia or in the inner or outer dynein arms of respiratory and reproductive epithelial cells. Mutations are numerous and have been identified in over 21 genes (57–59). The clinical picture is characterised by sinusitis, bronchiectasis and infertility. The hallmark symptom is respiratory distress in the early neonatal period. There is an association with varying degrees of *situs inversus* due to defective ciliary function in the nodal cilia responsible for laterality in the early embryological period (60,61).

Diagnosis first involves a nasal nitric oxide (NNO) level screening test (62–65). The NNO level in primary ciliary dyskinesia patients has been found to be lower than that seen in cystic fibrosis, non-CF bronchiectasis patients and healthy controls though there is still some debate as to the exact cut-off point (58,62,66). One study has suggested that a combination of an exhaled nitric oxide (ENO) level <2.4ppb and NNO <187ppb identifies PCD patients with 98% specificity, positive predictive

value 92% and negative predictive value 93% (67). Should the screening test be positive, the gold standard investigation involves a nasal epithelial biopsy with the assessment of the ciliary beat function and ultrastructure under the light and electron microscopes (58).

Secondary ciliary dyskinesia

Secondary ciliary dyskinesia can be caused by ciliary paralysis as a result of bacterial or viral infection or inflammation (68). Another unusual cause has been seen in Young's syndrome which comprises sinusitis, bronchiectasis and male infertility (69). The condition was mainly confined to the UK and France and rarely seen in the USA. It was initially felt to be a *forme fruste* of cystic fibrosis but is now thought to be due to a degree of mercury poisoning from various medications (70). The incidence of the condition has declined dramatically since mercury was removed from the manufacturing process (69).

1.3.5 Allergic Bronchopulmonary Aspergillosis (ABPA)

Allergic bronchopulmonary aspergillosis is classed as a hypersensitivity to colonising *Aspergillus fumigatus* and is classically is found in patients with asthma and cystic fibrosis. The diagnostic criteria involve the following characteristics: (1) History of asthma, (2) Positive fungal IgE or skin prick test, (3) Positive fungal IgG precipitins, (4) Proximal bronchiectasis, (5) Blood or sputum eosinophilia, (6) Total serum IgE >1000ng/ml, (7) Flitting pulmonary infiltrates. The condition is discussed in more detail in chapter 6.

1.3.6 Associated inflammatory conditions

There is an increasingly recognised association between bronchiectasis and inflammatory bowel disease. The bowel and bronchial tree are both of mesodermal origin with similar mucosal associated lymphoid tissue and it has long been felt that the inflammatory cytokine activity found in inflammatory bowel disease affects the bronchial mucosa by a similar mechanism (71). The lung pathology seen in these conditions ranges from bronchiectasis and bronchiolitis to small airway changes, interstitial lung disease and nodules (72). The pathology may be sub-clinical or symptomatic and occurs independently of IBD activity (73).

There is also a recognised association between rheumatoid arthritis and bronchiectasis (74). It has been estimated that 2.9% of patients with rheumatoid arthritis have bronchiectasis (75) and 5.2-12.3% of patients with bronchiectasis have rheumatoid arthritis (76,77). One study has demonstrated that patients with co-existent bronchiectasis and rheumatoid arthritis are 2.4 times more likely to die in a 5 year period than patients with bronchiectasis alone (78).

1.4 Introduction to CT scoring to determine bronchiectasis severity

1.4.1 The radiology of bronchiectasis

The diagnosis of bronchiectasis relies upon the presence of both clinical symptoms and recognised radiological changes. Lynne Reid described bronchiectasis as "cylindrical, varicose or cystic" following her work on pathology specimens (4). Traditionally, pre-CT, a chest radiograph or a bronchogram were the main methods for diagnosis. In the early 1990s the signet ring sign on a CT image was described (79). This refers to bronchial dilatation to a size greater than the accompanying artery. Some authors have expressed feelings that the sign should more accurately be referred to as the pearl ring sign (80).

Radiological interpretation was previously qualitative and subject to inter-observer variability but has now progressed towards the development of semi-quantitative scoring systems which use the traditional direct and indirect markers of bronchiectasis. The direct markers are 1) bronchial dilatation compared to the accompanying vessel, 2) lack of bronchial tapering more than 2cm distal to the carina, 3) visible bronchi within 1cm of the pleural space. The indirect markers are 1) bronchial wall thickening more than 0.5 times the diameter of the accompanying artery, 2) tree-in-bud changes or centrilobular nodules, 3) mucoid impaction, 4) mosaic perfusion, 5) atelectasis and 6) air trapping on an expiratory scan. There remains some controversy around the diagnosis of mild bronchiectasis as some of the radiological changes such as bronchial wall thickening can be caused by infection and inflammation and therefore reversible following treatment of the underlying cause. This important in studies such as this where some patients have mild disease at the outset and potentially have radiological changes of bronchiectasis that could disappear with the initiation of antibiotic therapy and could exert a bias on the data.

1.4.2 The CT scoring systems for bronchiectasis

The CT scoring systems for non-CF bronchiectasis are derived from those developed for cystic fibrosis severity scoring. The first CT scoring system was developed by Bhalla as a means of evaluating disease progression and improvement from therapy and was based upon a series of 14 scans from cystic fibrosis patients in 1991 (81). The scoring system involves the following areas: bronchiectasis, peribronchial thickening, mucus plugging, bronchiectatic sacculations/abscesses, bullae, emphysema and lobar collapse/consolidation. The severity is scored from 0 to 3 and the number of lobes involved as 1 to 9 with the maximum, and most severe, score being 25. Several more modified scoring systems have since appeared, the majority of which are for use in cystic fibrosis (82–85) though a few have been developed for non-cystic fibrosis bronchiectasis (86–88). One study compared five different non-CF bronchiectasis CT scoring systems and found them to be both comparable and reproducible (89). The Roberts score (86) was used for this study as it was favoured by the radiology department in the hospital. It has eight categories: bronchial dilatation, bronchial wall thickness, number of bronchopulmonary segments per lobe affected, mucus plugging in large and small airways, air trapping, bronchial collapse and tracheal collapse on expiration. The score for some categories ranges from 0-3 and for others 0-1 or 0-2. The total score is 17 for each lobe and 102 for both lungs.

1.4.3 The clinical application for CT scoring systems in bronchiectasis

We know that a severity score for cystic fibrosis can be beneficial in assessing treatment response clinically and in clinical trials but does this translate to non-cystic fibrosis bronchiectasis? Can it predict exacerbation frequency or prognosis? One study demonstrated a statistically significant association between the 24 hour sputum volume and the small airway changes on the CT scan along with a significant association between FEV_1 (% predicted) and bronchial wall thickness.

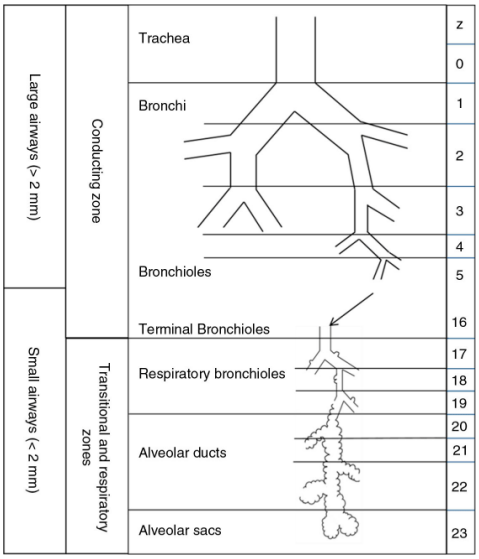
The same study also showed an inverse relationship between 24 hour sputum volume and spirometry (88). Another study by Lynch et al found that the modified Bhalla CT score was significantly higher in the group who isolated *Pseudomonas aeruginosa* when compared with the group who grew other organisms. This study also found that CT score severity was associated inversely with FEV_1 (87). The Roberts study also demonstrated excellent correlation between FEV_1, the extent and severity of bronchiectasis and bronchial wall thickening. The study also found that increasing bronchial dilatation was associated with less severe airflow obstruction (86). Again, the negative correlation between bronchial wall dilatation and airflow obstruction was seen in a Korean study of stable bronchiectasis patients (90). Linking CT severity with spirometry is clinically important but it doesn't inform us of patient symptoms and quality of life. A study from Israel compared the modified Bhalla CT score from a bronchiectasis population against the St George's Respiratory Questionnaire (SGRQ) (91). They did not find an association between the total CT score and the total SGRQ score however further analysis revealed that a cut off CT score of 15 (maximum 60) divided the group into more and less severely affected categories. The group with the more severe CT score of greater than 15 had a significantly higher, indicating a lower quality of life, SGRQ score. Several other authors (92,93) have demonstrated a correlation between symptom scores and bronchiectasis severity but the results of the studies are not as conclusive as would be imagined.

The scoring systems are not currently widely used in clinical practice. Other non-CT based scoring systems to predict mortality and need for hospital follow up have been developed (94).

1.5 The small airways and bronchiectasis

By definition the small airways are less than 2mm in diameter and do not contain cartilage (95). There are 23 branches to the bronchial tree and the last 15 are considered to be the small airways demonstrated in the figure below.

Figure 1: The divisions of the small airways



From McNulty and Usmani (96)

1.5.1 The small airways in clinical practice

Airway-related lung conditions such as asthma and COPD have traditionally been thought to only affect the larger airways, however there is increasing evidence to suggest that the peripheral airways are also affected (97–99). Spirometry has been the traditional measurement of lung function however it largely provides information about the proximal airways. The maximum mid-expiratory flow (MMEF) or the average forced expiratory flow for the middle section of the FVC (FEF_{25-75}) can provide information on the smaller airways however the results have not been more useful than standard spirometry (100).

Spirometry can be insensitive to the early clinical changes in lung disease but the inert gas washout has been shown to be more sensitive in the detection of pathology within the lung's peripheries in cystic fibrosis and primary ciliary dyskinesia (101–105). Irving et al demonstrated that lung clearance index (LCI) and $FEF_{25-75\%}$ were both more sensitive in detecting structural abnormalities on chest CT than FEV_1 in both primary ciliary dyskinesia and cystic fibrosis (106). The development of a radiation free investigation in monitoring structural lung pathology in children is very attractive. It has been proposed that a CT scan should only be performed in children with cystic fibrosis once the LCI has started to rise towards the abnormal range. The lung clearance index has been demonstrated to be a reproducible measurement in non-cystic fibrosis bronchiectasis patients. (107,108) It has been hypothesized to be a better biomarker for therapy as it is more sensitive to peripheral airway pathology, often abnormal despite a normal FEV_1 and can, therefore, allow for a smaller sample size based upon calculations (102).

1.5.2 Small airway investigations

Investigations to assess small airway function such as inert gas washout tests were first described in the 1940s but are only just emerging as clinically meaningful investigations (109). They can be categorised according to the number of breaths involved (single and multiple breath washouts) and the gas used [sulphur hexafluoride (SF_6), helium (He) and nitrogen (N_2)]. The multiple breath washout generates more clinical information than the single breath washout. It is easier to perform though more time consuming with each test taking around 8 minutes. The parameters generated which are associated with ventilation inhomogeneity are the lung clearance index (LCI) and the normalisation phase III slope gradient measurements of S_{cond} and S_{acin}. Other small airway investigations include the forced oscillation technique (FOT) and impulse oscillometry (110).

The lung clearance index (LCI)

The lung clearance index is best defined as the number of volume turnovers of the lung required to reduce the inert gas concentration to a $1/40^{th}$ of its starting value. A normal value is <7.5 and a value greater than 10 is significantly abnormal. LCI can be calculated using the following equation:

$$LCI = \frac{V_{CE}}{FRC}$$

V_{CE} is the sum of the tidal volumes and FRC is calculated as follows:

$$FRC = \frac{net\ volume\ of\ inert\ gas\ exhaled}{Cet_{start} - Cet_{end}}$$

Cet is the concentration of the inert gas at the end-tidal volume at the start (Cet_{start}) and end (Cet_{end}) of the investigation (111).

1.6 Erythromycin and non-cystic fibrosis bronchiectasis

"When patients have bacteria that are resistant to all antibiotics, prescribe erythromycin, leave them on it for a long time, and they will do much better"

Dr Harry Shwachman, Cystic Fibrosis specialist, 1950.

1.6.1 The background to erythromycin

Erythromycin was discovered within the bacterium *Saccharopolyspora erythraea* in 1949 when soil samples from the Philippines were analysed. The antibiotic was created commercially in 1981 and many derivatives have since been synthesized. Erythromycin, clarithromycin and roxithromycin are 14-membered lactone ring macrolides and azithromycin is a 15-membered ring macrolide. The clinical uses of erythromycin can be divided into short-term therapy for an acute infection and long-term therapy for chronic infection and inflammation, especially in non-cystic fibrosis bronchiectasis and COPD (112–114).

1.6.2 Diffuse panbronchiolitis and erythromycin

One of the first success stories for erythromycin was in the progressive and often fatal condition of diffuse panbronchiolitis (DPB). This condition was first described in Japan in 1969 and is still largely confined to the Far East (115). The exact causative agent is unknown although we know there is an association with the presence of HLA-B54 (116) and HLA–A11 (117). The natural progression of the condition evolves from chronic sputum production, progressive breathlessness, frequent infections and *Pseudomonas aeruginosa* colonisation to end-stage respiratory failure. The Ministry of Health and Welfare in Japan use the following criteria for diagnosis: 1) Persistent cough with increased sputum volumes, 2)

Chronic sinusitis, 3) Bilateral diffuse centrilobular nodules with a branching pattern on CT (tree in bud changes) or chest x-ray, 4) Coarse crepitations on auscultation, 5) Obstructive spirometry or PaO_2 <80mmHg, 6) Elevation by more than 64-fold of cold haemagglutinin titre. Presence of at least three of these criteria is required for the diagnosis. Outside of the Far East a biopsy is required to confirm the diagnosis.

Low-dose erythromycin was the first antibiotic to improve survival and symptoms in diffuse panbronchiolitis. A retrospective analysis of 498 patients identified from the Ministry of Health and Welfare of Japan Registry between 1970 and 1990 calculated a statistically significant survival benefit for the patients who were prescribed erythromycin compared to the patients who were not. Pre-1983 the 10-year survival rate was 33.2% which improved to over 90% in the patients treated with erythromycin although the prognosis is still poorer in those colonised with *Pseudomonas aeruginosa* (118). Other improvements have been demonstrated following erythromycin therapy, with one study publishing improvements of 730ml in the FEV_1 and 83ml reduction in sputum production with greater improvements in the group who achieved bacterial clearance (119). Bronchoalveolar lavage from patients with diffuse panbronchiolitis typically demonstrates neutrophilic inflammation (120,121). The use of erythromycin resulted in a significant reduction in the sputum neutrophil count from 71.2% to 40.6% in the patients who were not colonised with *Pseudomonas aeruginosa* (118). The characteristic response from erythromycin therapy in diffuse panbronchiolitis was summarised excellently in a paper by Kanoh and Rubin (122) From their observations they stated: 1. Response to treatment will take 3 months, 2. The response will be independent of bacterial culture, even *Pseudomonas aeruginosa*, 3. The dose required is lower than the known minimum inhibitory concentration for mechanism of action, 4. The response only occurs in 14- and 15-membered ring macrolides such as erythromycin and azithromycin.

1.6.3 Is the benefit from erythromycin antibacterial or anti-inflammatory?

Antibacterial effects

The mechanism of action of erythromycin is bacteriostatic through the binding of the 50s subunit of the bacterial RNA, preventing replication (123) Erythromycin has also been demonstrated to inhibit bacterial adhesion to epithelial surfaces, to inhibit the *Pseudomonas aeruginosa* biofilm and inhibit bacterial protein synthesis at concentrations below the minimum inhibitory concentration (MIC) for traditional antibiotic effects (124,125). For acute infections macrolides are recommended for the treatment of mild-moderate community acquired pneumonia in penicillin allergic patients given the action against gram positive bacteria such as *Haemophilus influenzae* and *Streptococcus pneumoniae* and atypical, intracellular bacteria such as *Mycoplasma* species and *Legionella pneumophila* (126,127). Erythromycin is also a guideline directed antibiotic for upper respiratory tract infections such as otitis media, laryngitis and sinusitis, especially in penicillin allergy (128,129).

Evidence for anti-inflammatory effects

The immunomodulatory effect of macrolides has been utilised in the treatment of chronic respiratory and sinus conditions (130–132) and independent of the antibiotic effect of erythromycin (122,124,133). In diffuse panbronchiolitis erythromycin therapy improvement and survival benefit was seen together with a reduction in the sputum neutrophil count and therefore neutrophil derived interleukins, inhibition of mucus production and improvement in peribronchiolar inflammatory changes on radiological imaging (118,121). The improvement is greater in the group colonised with *Pseudomonas aeruginosa* but is not due to bacterial clearance (118). None of the large clinical trials using macrolides have suggested an antibiotic effect such as sputum bacterial clearance from the drug despite improvements in exacerbation frequency (112,114,134).

1.6.4 Potential side-effects of erythromycin

Gastrointestinal disturbance

The most common side effects of macrolides are gastrointestinal disturbance such as nausea, diarrhoea and abdominal cramps. The acidic environment of the stomach results in the degradation of erythromycin to a motilin receptor agonist which has a pro-kinetic effect. As well as intestinal symptoms deranged liver function tests can occur and are usually both asymptomatic and transient. Hepatotoxicity is rare.

Cardiac toxicity

All 14 and 15 member ring macrolides have been implicated as a cause of polymorphic ventricular tachycardia (torsades de pointes), ventricular tachycardia (VT) and QTc interval prolongation (135). The risk of sudden cardiac death from these arrhythmias is small but degeneration into ventricular fibrillation or pulseless ventricular tachycardia is possible. There are a number of additional risk factors: Females, increasing age, structural heart disease, genetic predisposition, co-prescription of other drugs causing QT prolongation (class I and III anti-arrhythmics and anti-histamines are a few) and electrolyte disturbance such as hypokalaemia (136,137). Most written reports of macrolide related cardiac toxicity are either singular case reports or occur in patients with underlying cardiovascular problems and contraindicated medication usage, particularly those on high dose, intravenous and prolonged courses (138–141). Only a small number of macrolide trials have monitored ECG changes but have not reported any significant cardiac abnormalities such as QT interval prolongation (114).

Ototoxicity

This is a known side-effect of erythromycin therapy but the exact risk its occurrence is unknown. To date it has only been demonstrated in those receiving high dose (4g/24 hours) erythromycin and has been shown to be reversible (142,143). Other risk factors include poor liver function and concomitant use of cytochrome P450 medications (144). There is very little, if any, risk of ototoxicity in patients taking low-dose erythromycin.

Microbial resistance

Officially, a strain of *Streptococcus pneumoniae* is resistant if the minimum inhibitory concentration of a drug required to inhibit bacterial growth is greater than 1μg/ml, where the usual level is 0.06μg/ml (145). The Global Resistance to Antimicrobials with *Streptococcus pneumoniae* (GRASP) project demonstrated that 29.6% of clinically important isolates were not sensitive to macrolide treatment (145). Similarly, the incidence of macrolide resistant *Streptococcus pneumoniae* in a Canadian study increased from 8% in 1998 to 22% in 2008 (146). More recently multi-drug resistance has been reported (147). It has been hypothesized that increasing azithromycin use is the largest cause of macrolide resistance, partly due to its long half-life of 3 days (148). One large study saw macrolide resistance increase significantly after azithromycin therapy from 26% of pathogens tested in the placebo group to 88% of pathogens tested in the intervention group (134). Risk factors for infection with macrolide resistant strains include: living a care home, age under 5 or over 65 and a recent admission to hospital (149).

1.6.5 Overview of the recent clinical trials using low-dose erythromycin

There has only been one large, well-designed randomised, double-blind, placebo-controlled trial of low-dose erythromycin in non-cystic fibrosis bronchiectasis. This was the BLESS trial by Serisier et al. in Australia (112). One hundred and

seventeen patients with at least two exacerbations in the preceding year were stratified for the presence of *Pseudomonas aeruginosa* and randomised to take either placebo or 250mg erythromycin twice daily for 48 weeks with a 4 week washout period. The study completion rate was 91.5% suggesting good tolerance of the erythromycin. The primary outcome was the number of exacerbations over the 12 months. The secondary end-points were spirometry, quality of life questionnaires, 6 minute walk, c-reactive protein, sputum neutrophils (%) and 24 hour sputum volume. There was a significant reduction in the number of protocol defined exacerbations in the intervention group. The spirometry changes were not significant. There were no significant changes in the other secondary outcome measures apart from a significant reduction in 24 hour sputum weight. Data was also collected on pharyngeal *Streptococcal* macrolide resistance which increased by 27% in the intervention group. The long term clinical implications of this are as yet unknown.

Other non-cystic fibrosis macrolide trials have been conducted using azithromycin. The BAT study by Altenburg et al. used 250mg azithromycin or placebo daily in 83 participants. Again, the primary outcome was the number of exacerbations over the 12 month study period. The secondary outcomes were quality of life questionnaires, spirometry, c-reactive protein, microbiological changes and blood white cell count. There was a significant improvement in exacerbation frequency and a significant improvement in the rate of decline of FEV_1 over the study period. The placebo group had a decline of 0.10% every three months over the year whereas the macrolide group had an improvement of 1.03% every three months. This study also saw an increase in respiratory pathogen macrolide resistance following therapy (134).

Finally, the EMBRACE study was conducted by a New Zealand group led by Wong. This study used azithromycin 500mg or placebo three times a week for 6 months in 141 subjects. The co-primary end-points were number of exacerbations and

change in FEV_1 at the 6 month mark and the benefits were followed up for another 6 months. There was a non-significant fall in FEV_1 of 10ml in the macrolide group and 60ml in the placebo group at the end of 12 months. At 6 months the symptom domain of the St George's Respiratory Questionnaire suggested a significant improvement between the placebo and intervention group however this was not sustained by the end of the 12 month study period. There was also a reduction in the exacerbation rate over 6 months from 1.57 in the placebo group and 0.59 in the azithromycin group (150).

Chapter Two: Methods, hypotheses, aims and study design

2.1 Primary aim and secondary objectives

Hypothesis

- Participants with non-cystic fibrosis bronchiectasis demonstrate a diverse pattern of clinical characteristics and have a variable response to erythromycin.

Aims

- To assemble a cohort of participants with non-cystic fibrosis bronchiectasis to investigate the underlying aetiology and to describe the airway inflammation and the bacterial and fungal microbiome.
- To investigate whether there was an improvement in the FEV_1 of at least 200ml following a 3 month course of erythromycin at 250mg once a day.

Secondary objectives

- We hypothesise that the study participants who demonstrate the best response to erythromycin 250mg daily for 12 weeks will have neutrophilic airway inflammation and demonstrate small airways disease in the form of an increased lung clearance index on multiple breath washout testing and tree in bud changes on the CT scan.
- To evaluate whether the response to 12 weeks erythromycin will be sustained after the course is completed.
- To investigate the benefit of low-dose erythromycin and to determine biomarkers of responsiveness.
- To describe the nature of exacerbations in non-cystic fibrosis bronchiectasis.
- To describe the fungal microbiome in non-cystic fibrosis bronchiectasis.

2.2 Study design and sample size calculation

2.2.1 Study design

This study was designed as a single centre, open label, non-randomised trial. The participants were recruited from respiratory clinics in Glenfield Hospital following confirmation of a CT based diagnosis of bronchiectasis. Potential participants were sent a study information booklet and responded by post. There was always more than 24 hours between receiving the study information and signing the consent form. Participants were allowed to discontinue the study at any time. In keeping with Good Clinical Practice one copy of the consent form was filed in the notes, one given to the patient and the original stored in the site file. Each participant was assigned a Unique Study Number. Initially the 40 participants were recruited for the clinical trial. Recruitment commenced in July 2012 and the first patient was consented in December 2012. After a period of time a further 28 participants were recruited for a single visit. They were not prescribed any erythromycin and hence have more straight forward inclusion and exclusion criteria. The final patient visit was in May 2015.

Ethics approval was obtained from the Research and Ethics Committee (REC). REC reference number: 12/EM/0370. The trial is registered with EUDRACT with a trial number: 2012-002792-34.

2.2.2 Inclusion and exclusion criteria

Inclusion Criteria (clinical trial cohort)

- Bronchiectasis proven on CT scan and presence of symptoms (cough, sputum production, recurrent infections sufficient to indicate a diagnosis of bronchiectasis to the referring consultant)
- Aged 18 to 100 inclusive
- Ability to give valid consent
- Willingness to attend the hospital every 3 months for the study duration.

Exclusion Criteria (clinical trial cohort)

- Active TB
- Aged under 18 and over 100
- Unable to perform procedures such as pulmonary function tests, spirometry or unable to attend visits due to ill health
- Patients with known cystic fibrosis
- Patients with traction bronchiectasis due to fibrosis as a primary diagnosis
- Patients who are unable to consent
- Patients already on long term antibiotics
- Patients with macrolide allergy / severe intolerance / long QT interval on ECG
- Patients on medications with a proven interaction with erythromycin, with the exception of simvastatin.

Inclusion Criteria (single visit cohort)

- Bronchiectasis proven on CT scan and presence of symptoms (cough, sputum production, recurrent infections sufficient to indicate a diagnosis of bronchiectasis to the referring consultant)
- Aged 18 to 100 inclusive
- Ability to give valid consent
- Willingness to attend the hospital for one visit

Exclusion Criteria (single visit cohort)

- Active TB
- Aged under 18 and over 100
- Unable to perform procedures such as pulmonary function tests, spirometry or unable to attend visits due to ill health
- Patients with known cystic fibrosis
- Patients with traction bronchiectasis due to fibrosis as a primary diagnosis

- Patients who are unable to consent

2.2.3 Sample size calculation

The number of participants was determined by a sample size calculation. Data from a retrospective review of clinics for patients with bronchiectasis over the last 6 years who received a 3 month course of erythromycin was used to inform the calculation. This data demonstrated that the difference in the change in FEV_1 (delta FEV_1) pre- and post-treatment between the patients who had the best and worst improvement in symptoms overall was 336mls (sd. 0.255l). As discussed in the introduction chapter the data was presented at the East Midlands Thoracic Society meeting in 2012. The decision to use a value of 200ml was made using the data discussed above after discussion with a statistician. A value of 156ml was twice the known standard deviation for the variability of spirometry. A value of 200ml was felt to be a good point between 336ml and 156ml.

The following calculation uses 90% power and a significance level of 5%:

$$n = f(\propto, \beta) \cdot \frac{2s^2}{\delta^2}$$

Where $f(\propto, \beta)$ is 10.5, s is the standard deviation of the FEV_1 in the sample and δ is the desired change in FEV_1.

Using the values above the required sample size to achieve a 200ml improvement in FEV_1 was 34.1 participants. Estimating for an approximate 20% drop out rate the number recruited was 40. The sample size calculation was performed again using the data from the observation year and the actual standard deviation of the spirometry. This calculation demonstrated that 25.4 participants were required to provide meaningful data.

2.2.4 Trial progression and visit outline

Stable visits occurred at 12-weekly intervals and 6 or more weeks after an exacerbation. There were 7 visits on the study. Visit 5 marked the end of one year of observation and the start of the erythromycin intervention. This lasted for 12 weeks until visit 6. The last study visit was visit 7. Exacerbation visits were scheduled as a soon as possible after the development of symptoms if the protocol criteria were met.

Visit 1

This visit included background questions on demographics and medical history including:

- Age of symptom onset
- Age of diagnosis
- Potential causes – ear nose and throat symptoms, gastrointestinal symptoms, childhood infections, previous TB, fertility problems, joint, eye and skin symptoms that may suggest a connective tissue disease or rheumatoid arthritis.
- Family history
- Smoking history, alcohol consumption, occupation and pets.
- Past medical history.
- Medication history was taken and checked against the list of contraindicated medications (see appendix)

The participants completed the following quality of life questionnaires – Leicester Cough Questionnaire (LCQ), St George's Respiratory Questionnaire (SGRQ) and Visual Analogue Scale (VAS) assessment of cough, breathlessness and sputum production and purulence. Blood tests were taken to include:

- Full blood count, urea and electrolytes, c-reactive protein and liver function tests

- Immunology tests to include immunoglobulin levels (IgA, IgE, IgG, IgM and *Aspergillus fumigatus, Penicillium notatum, Candida albicans* specific IgE and *Aspergillus fumigatus* specific IgG), autoantibodies (ANA, ANCA, RhF, ENA), alpha 1 anti-trypsin levels and pneumococcal serotypes
- Exhaled oral nitric oxide (ENO) and nasal nitric oxide (NNO) levels were collected as a marker of airway inflammation and as a screening test for Primary Ciliary Dyskinesia (PCD). If the nasal NO was below 100ppb the test was repeated at the next stable visit. If the level was consistently low the patient was referred for formal investigations
- Spontaneous and induced sputum collection. The spontaneous samples were sent for microbiological tests (bacterial and mycobacterial) and the induced samples for fungal culture and differential cell counts
- Multiple breath washout
- Genetic cystic fibrosis testing. If the history was suggestive and the patient developed respiratory symptoms under the age of 40 a blood sample was taken to test for the 50 most common cystic fibrosis mutations as per normal clinical practice
- Full pulmonary function tests

Visits 2-4 (months 3, 6 and 9)

The following investigations were performed:

- Quality of life questionnaires (Leicester Cough Questionnaire, St George's Respiratory Questionnaire and Visual Analogue Scale for cough, dyspnoea and sputum production and purulence)
- Exhaled nitric oxide to assess airway inflammation
- Pre- and post-bronchodilator spirometry
- Blood tests for full blood count, c-reactive protein and urea and electrolytes

- Spontaneous and induced sputum collection. The spontaneous samples were sent for microbiological tests (bacterial and mycobacterial) and the induced samples for fungal culture and differential cell counts
- Research protocol CT scan in order to calculate the bronchiectasis severity according to the Robert's scoring system
- At this visit I wrote to the GP or cardiologist of the participants who were taking simvastatin asking for their agreement to substitute the drug with atorvastatin for the 12 week erythromycin intervention period

Visit 5 (month 12)

Participants received 250mg erythromycin daily for 12 weeks. The study drug was discontinued if there was an allergic reaction, a life-threatening event which was not related to the underlying condition or intolerable side-effects.

The following investigations were performed:

- Quality of life questionnaires (Leicester Cough Questionnaire, St George's Respiratory Questionnaire and Visual Analogue Scale for cough, dyspnoea and sputum production and purulence)
- Exhaled nitric oxide to assess airway inflammation
- Pre- and post-bronchodilator spirometry
- Blood tests for full blood count, c-reactive protein and urea and electrolytes
- Spontaneous and induced sputum collection. The spontaneous samples were sent for microbiological tests (bacterial and mycobacterial) and the induced samples for fungal culture and differential cell counts
- ECG to exclude a pre-existing prolonged QT interval
- Multiple breath washout

Visits 6 and 7 (months 15 and 18)

The following investigations were performed:

- Quality of life questionnaires (Leicester Cough Questionnaire, St George's Respiratory Questionnaire and Visual Analogue Scale for cough, dyspnoea and sputum production and purulence)
- Exhaled nitric oxide to assess airway inflammation
- Pre- and post-bronchodilator spirometry
- Blood tests for full blood count, c-reactive protein and urea and electrolytes
- Spontaneous and induced sputum collection. The spontaneous samples were sent for microbiological tests (bacterial and mycobacterial) and the induced samples for fungal culture and differential cell counts
- Multiple breath washout
- ECG to exclude a drug-related prolonged QT interval (visit 6 only)
- Blood samples for drug levels if applicable (visit 6 only)
- Adverse symptom profile (visit 6 only)

Compliance was checked at visit 6. The participant was asked to keep the erythromycin packaging and return all unused tablets.

2.2.5 Physiotherapy

Physiotherapy was optimised for each patient both in the stable state and during exacerbations, particularly if it was felt there was insufficient familiarity with the sputum clearance and active cycle breathing techniques. This was in-keeping with the current BTS guidelines for non-cystic fibrosis bronchiectasis (6).

2.2.6 Data

Data was entered onto a paper case report form (CRF) and inputted into a password protected REDCap™ online database, which I designed. The data was only identifiable by the study number. After all the data was collected it was exported into MS Excel and SPSS for further analysis. The data was subject to monitoring

visits from the Research and Ethics Committee where the data entry was checked against the source data documents and inaccuracies amended.

2.3 Clinical Methods

2.3.1 Quality of life questionnaires

This study used the St. George's Respiratory Questionnaire (SGRQ), the Visual Analogue Scale for cough, breathlessness and sputum production and purulence (VAS) and the Leicester Cough Questionnaire (LCQ) which have all been validated for use in non-cystic fibrosis bronchiectasis (92,151,152). The SGRQ was used with permission for the St George's Respiratory Unit in London. All the questionnaires referred to the symptoms experienced in the two weeks prior to the appointment except for section one of the SGRQ which referenced the previous three months. During exacerbations, the questionnaire answers were based around the time since the onset of the exacerbation symptoms.

St George's Respiratory Questionnaire

The SGRQ comprises of 76 weighted questions divided into 3 domains – symptoms, activities and impacts. The total score ranges from 0-100 with a high score indicating more symptoms. Participants ticked the most appropriate answer to the questions which was then inputted to a computer-based marking system. The score was given as a total score and broken down into the symptom domains.

Leicester Cough Questionnaire

The LCQ has 19 questions with 3 domains – physical, social and psychological impacts. The total score ranges from 3-21 with a lower score indicating more symptoms.

Visual Analogue Scale

The VAS is a linear scale ranging from 0-100mm with a higher score indicating more severe symptoms. This has been validated in COPD (153). There were domains covering cough and breathlessness severity, sputum production and sputum purulence. Each participant was asked to place a vertical mark along the scoring line to rate their current symptoms. The distance from 0mm to the mark was recorded (in mm).

2.3.2 Blood Sampling

Blood samples were collected in the appropriate blood sample bottles using a standard aseptic technique. They were labelled and sent to the laboratory on foot.

2.3.3 Exhaled Nitric Oxide

This investigation was performed using a hand-held NIOX MINO® device (Aerocrine AB, PO Box 1024, SE-171 21, Solna, Sweden). The participant was required to inhale through the mouthpiece and exhale at a steady rate for 14 seconds according to the instructions on the screen of the device. The test was performed twice and the mean value was recorded in parts per billion.

2.3.4 Nasal Nitric Oxide

This result was collected using a hand-held NIOX MINO® device (Aerocrine AB, PO Box 1024, SE-171 21, Solna, Sweden) using a nasal adaptor. The participant was asked to first blow their nose then place the nasal adaptor just inside one nostril. They were instructed to breathe gently through their mouth ensuring the hard palate was closed. Air was extracted from the nostril at 5ml/s. The test was then repeated on the opposite side and the average value calculated and recorded. If the

value was below 100ppb the test was repeated at the next stable visit. A further mean reading below 100ppb was taken to indicate a positive screening test for primary ciliary dyskinesia. The participant was then referred to the Primary Ciliary Dyskinesia clinic for further investigations including a nasal epithelium biopsy.

2.3.5 Spirometry

Pre-bronchodilator spirometry was carried out according to the departmental standard operating procedure using a Vitalograph® spirometer (Vitalograph, Maids Moreton, Buckingham, MK18 1SW). The participant was then given 400mcg salbutamol via a spacer device and spirometry was then repeated after 20 minutes. The best of three values was recorded. If this was the third test a fourth and possibly fifth test was carried out.

2.3.6 Pulmonary Function Tests

Full pulmonary functions tests were carried out using a standard protocol in the Respiratory Physiology Department at Glenfield Hospital, Leicester at the baseline visit.

2.3.7 Multiple Breath Washout

Participants wore a nose clip and inhaled a known concentration (0.2%) of an inert and non-absorbed gas, sulphur hexafluoride (SF6), via a mouthpiece connected to an Innocor®photoacoustic gas analyser (Innovision AS, Odense, Denmark). An inspired volume of 1000ml at around 12 breaths per minute was achieved using a visual display on the monitor as a guide. This wash-in phase continued until the SF6 level in the exhaled air was greater than 0.2% for three consecutive breaths, or until there was less than 0.004% difference between inspiratory and expiratory

SF6 concentration. The gas reservoir bag was then removed and the wash-out phase began with participants following the same breathing pattern. This continued until the end-tidal concentration of expired SF6 fell below 1/40th of the original concentration (<0.005%) for three consecutive breaths. The test was repeated three times to ensure there were two calculated FRC values within 10% of each other. The lung clearance index and the concentration normalised phase III slope indices (S_{cond} and S_{acin}) were calculated. The lung clearance index was calculated as the number of lung volume turnovers needed to lower the end-tidal SF6 concentration to less than $1/40^{th}$ of the starting concentration. A lung volume turnover is the cumulative expired volume divided by the functional residual capacity. The multiple breath washout was carried out at visits 2, 5, 6 and 7 and at the single visit for the additional participants.

2.3.8 CT scanning

An inspiratory and expiratory CT scan was carried out using the standard research protocol. The scans were reported clinically for safety and graded for severity using the Roberts score (86) by two independent radiologists. The correlation co-efficient was calculated between the two radiologists. The scoring system is outlined in the table below. There are eight categories: bronchial dilatation, bronchial wall thickness, number of bronchopulmonary segments per lobe affected, mucus plugging in large and small airways, air trapping, bronchial collapse and tracheal collapse on expiration. The score for some categories ranges from 0-3 and for others 0-1 or 0-2. The total score for each lobe is 17. The maximum score for both lungs is 85. This scoring system can be seen on the following page.

2.3.9 Sputum Sampling

Spontaneous sputum samples were collected during the visit and sent for bacterial and mycobacterial culture. An induced sample was collected for the differential cell count, bacterial colony forming unit counts and fungal culture using the standard operating procedure employed by the research department. Pre-procedure spirometry was performed and sputum induction carried out only if the FEV_1 was greater than 1.0 litre or greater than 50% of the predicted value. The subject was given 4ml 3% saline through an EASYneb II® ultrasonic nebuliser (FlaemNuova, Via del Passero, 3 - 20147 Milano, Italy) for 5 minutes followed by 4% and 5% nebulisation if a sputum sample was not collected. Post-nebulisation spirometry was carried out after each saline dose and the test terminated if the FEV_1 fell below 80% of the post-bronchodilator value. The samples were immediately taken to the microbiology and sputum laboratories where they were stored on ice prior to processing. See the laboratory methods section for further details. Samples were stored at -80 °C until study closure.

The Robertson CT scoring method

Category	Score				
	0	**1**	**2**	**3**	**Maximum (all lobes affected)**
Inspiratory Scan					
Severity of bronchial dilatation	None	100-200% of accompanying arterial diameter	200-300% of arterial diameter	>300% arterial diameter	18
Bronchial wall thickness	None	<50% of accompanying arterial diameter	50-100% of accompanying arterial diameter	>100% of accompanying arterial diameter	18
Extent – number of bronchopulmonary segments / lobe	None	Localised – one or part of one BP segment	Extensive – in more than one BP segment	Generalised Cystic	18
Mucous plugging in large airways	None	Present			6
Mucous plugging in centrilobular airways	None	Present			6
Expiratory Scan					
Regional decrease in attenuation	None	<50% lobar volume	>50% lobar volume		12
Bronchial collapse	None	<30% decrease in diameter	30-80% decrease in diameter	>80% decrease in diameter	18
Tracheal collapse	None	>30% decrease in diameter			6
Total					**102**

2.4 Laboratory Methods

2.4.1 Sputum Differential Cell Counts

The sputum was processed under a class II hood to prevent any outside factors interfering with analysis. The sputum weight was multiplied by four and this volume in millilitres of Dithiothreitol (DTT) and Phosphate Buffered Saline (PBS) was added. The sample was rotated on ice in a Spiromix for 15 minutes then centrifuged for 10 minutes at 700G. The solution was then filtered into a second tube through a 48 micron nylon gauze. The sample was stained with Tryphan Blue to stain dead cells and the number of viable cells counted. Seventy five microliters of the solution were mounted on a slide and stained with red Eosin Y and Methylene Blue. Four hundred cells were counted under the microscope to give the absolute differential cell count and cell type percentages.

2.4.2 Sputum Colony Forming Units

Following the previous processing method five sterile micro-tubes were filled with 900µl of sterile PBS. One hundred microliters of the filtrate were pipetted into the first tube and the sample inverted 5 times. From this tube 100µl were pipetted into the second micro-tube filled with 900µl PBS. The solution was inverted 5 times and the process repeated until all 5 tubes had been sampled. Quadrants were drawn onto the lid of a Columbia blood agar plate (Oxoid Ltd, Wade Road, Basingstoke, Hampshire, RG24 8PW) and 3x20µl samples of the solution starting with the second micro-tube (the 10^{-2} dilution) were pipetted onto the surface in the designated area. The process was repeated for the remaining concentrations and the sample stored in an incubator at 37°C for 24 hours. After 24 hours the colonies were counted using an inverted microscope and a tally counter. The colony forming unit/ml (CFU/ml) was calculated from the average of three colonies using the following formula:

Average of the 3 colonies counted x dilution factor x 20

eg: (25+24+26)/3 = 25 (average) X 10^3 (1000) X 20 (to make 20 µl drop to 1000µl) =5x10^5

2.4.3 Fungal cultures

The centrifuged sputum sample was plated on an agar medium and sent to the University of Leicester laboratories for fungal culture and type analysis.

A sputum plug of around 150mg was selected, serially diluted from 10^{-1} to 10^{-7} and distributed in quadrants across an agar plate. The plate was cultures at 37°C before the colonies were read and the colony forming units (CFU/ml) calculated. These methods are discussed in more detail in chapter 6.

2.4.4 Statistical methods

A statistician was consulted for the initial statistics plan but all subsequent statistics calculations were performed by myself.

Bray-Curtis Index of similarity and dissimilarity

This index was used to determine the similarity and dissimilarity in overall microbial culture between visits. The formula for this calculation is as follows. Essentially the differences in absolute values between the samples are added and divided by the total sum of microbial isolates.

$$bii' = \frac{\sum_{J=1}^{J} |nij - ni'j'|}{n_{i+} + n_{i'+}}$$

For example: The following table contains the number of isolates for the total study population of participants 1-40 for each sputum bacterial culture result at visits 1 and 2. To calculate the Bray-Curtis Index the absolute number for the

difference between each number of isolate for each bacteria between visits 1 and 2 are added. The number is the divided by the sum of the isolates for these visits.

	H. influenzae	M. catarrhalis	S. pneumoniae	P. aeruginosa	Coliform sp.	Proteus sp.	S. aureus	Other	No sig growth	Total
V1	9	2	3	4	1	1	2	2	11	35
V2	6	2	2	1	2	1	2	2	9	27

This is calculated as follows:

$$\frac{3+0+1+3+1+0+0+0+2}{35+27} = 0.161$$

A value of 0 means absolute similarity between the two samples and a value of 1 means absolute dissimilarity. For the dissimilarity index one subtracts the similarity index from 1.

Chapter Three: Demographic data and baseline characteristics

3.1 Introduction

This chapter introduces the bronchiectasis study cohort. In total there were 68 participants who took part in at least one visit. In this chapter the baseline and demographic data collected at visit 1 for the entire study cohort is analysed and discussed. The aim for the first part of the chapter is outlined below. The second part discusses the role of potential biomarkers to phenotype the cohort.

Secondary objective

Describe the cohort in terms of baseline characteristics, demographic data and results of radiological and inflammatory investigations.

3.2 Results

3.2.1 Demographics

The age, sex, body mass index, age of onset of symptoms and post-bronchodilator spirometry are summarised in table 1. The mean age of the 68 participant cohort was 69 years. There were 54.4% females with a body mass index of 26.9 kg/m^2 (sd 4.8). The age of onset had a biphasic pattern. The median age of onset was 27 years with a broad interquartile range of 57 years. The youngest self-reported onset was several months of age and the oldest 80 years. The majority of the group had never smoked (61.8%) and only 1 person was a current smoker 51 pack year history.

Table 1: Summary of the demographic data for all 68 participants

Demographic information		Value
Age (years), mean (sd)		69 (7.6)
Sex, n (%)	**Female**	37 (54.4)
Body Mass Index (kg/m^2), mean (sd)		26.9 (4.8)
Age of onset of bronchiectasis symptoms (median IQR)		27 (57)
Smoking history, n (%), [pack years# median (IQR)]	**Never smoked**	42 (61.8)
	Ex-smoker	25 (36.8), [15 (21)#]
	Current smoker	1 (1.5), [51 (0)#]

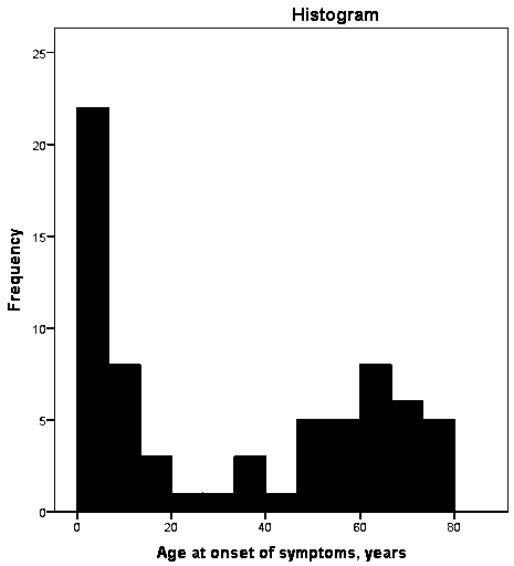


Figure 2: The distribution of the age at symptom onset

3.2.2 Aetiology and associated conditions

The most commonly self-reported associated conditions in this bronchiectasis population were gastro-oesophageal reflux disease, followed by recurrent sinusitis (41.2%) and the presence of auto-immune diseases (25%), the majority of whom had been diagnosed with rheumatoid arthritis, inflammatory bowel disease both with and without an inflammatory arthritis and auto-immune hypothyroidism. Thirteen (19.1%) of the participants had a pre-study physician diagnosis of asthma. The most common recognised aetiology were a self-reported history of whooping cough (50%) and childhood pneumonia (32.4%) followed by a pre-study physician diagnosis of allergic broncho-pulmonary aspergillosis (ABPA). A self-reported history of TB was a potential underlying cause in 2.9% of the study cohort.

Table 2: A summary of aetiology and associated conditions

Aetiology	Number (%)
Whooping cough*	34 (50.0)
Childhood pneumonia*	22 (32.4)
Allergic broncho-pulmonary aspergillosis#	4 (5.9)
Previous tuberculosis*	2 (2.9)
Associated conditions	
Gastro-oesophageal reflux disease*	34 (50.0)
Recurrent sinusitis*	28 (41.2)
Recurrent otitis media*	22 (32.4)
Confirmed autoimmune condition#	17 (25)
Asthma#	13 (19.1)
Confirmed other inflammatory arthritis#	8 (11.8)
Confirmed rheumatoid arthritis#	4 (5.9)
Inflammatory bowel disease#	3 (4.4)

**Self-reported condition, #Pre-study physician diagnosis*

Rheumatoid factor and other autoimmune conditions

The rheumatoid factor was measured regardless of whether the participant was known to have rheumatoid arthritis. Four of the 68 participants had been diagnosed with rheumatoid arthritis and of these 3 had a raised rheumatoid factor. Sixteen of the group had a raised rheumatoid factor (median level 40.5, maximum value 399). A quarter of this group had other diagnosed autoimmune conditions such as inflammatory bowel disease, Sjogren's syndrome and autoimmune hypothyroidism. Of the 52 participants who had a negative rheumatoid factor, one had a confirmed diagnosis of rheumatoid arthritis. Nine of this group (17.3%) had confirmed additional autoimmune conditions, including inflammatory bowel disease, other inflammatory arthritis, pernicious anaemia, vitiligo, coeliac disease and cold urticaria.

Table 3: Summary of rheumatoid factor status and concurrent auto-immune conditions

	Number (%)	Rheumatoid factor level, median (IQR)	Known Rheumatoid arthritis, n (%)	Other auto-immune conditions, n (%)	Positive serum auto-antibodies
Rheumatoid factor positive	16/68 (23.5)	40.5 (82.75) Highest 399	3 (18.8)	4 (25) Hypothyroidism IBD Palindromic arthritis Sjogren's	6 (37.5)
Rheumatoid factor negative	52/68 (76.5)	NA	1 (1.9)	9 (17.3) IBDx2 Hypothyroidism Coeliac disease Non-RA inflammatory arthritis x3 Lichen planus Pernicious anaemia Vitiligo Cold urticaria	19 (36.5)

Alpha-1 anti-trypsin

The alpha-1 anti-trypsin level was measured in 55 of the 68 participants. The mean level was within the normal range (mean 1.47, sd 0.24) with a minimum level of 0.78 and a maximum level of 1.96. The level was below the normal range in 4 (7.3%) of the participants (mean 0.92, sd 0.10, range 0.78-1.00) with all four subjects carrying a PiMZ phenotype. In addition another three participants had an abnormal alpha-1 anti-trypsin phenotype of PiMS with normal or only mildly reduced serum levels (mean 1.29, sd 0.08). In total seven (12.8%) had an abnormal phenotype overall.

Table 4: Summary of the alpha-1 anti-trypsin serum levels and phenotypes

A1AT genotype	Number (%)	Serum A1AT level mean (sd)	Range
All phenotypes	55 (100.0)	1.47 (0.24)	0.78-1.96
PiMM	37 (67.3)	1.58 (0.16)	1.35-1.96
PiM	11 (20.0)	1.27 (0.06)	1.20-1.37
PiMS	3 (5.5)	1.29 (0.08)	1.20-1.34
PiMZ	4 (7.3)	0.92 (0.10)	0.78-1.00

Immunodeficiency

None of the 68 participants had a severe immunodeficiency such as panhypogammaglobulinaemia. Data was collected on the number with drug related immuno-insufficiency states and more subtle primary and secondary immunodeficiencies. The data is represented in the table below but to summarise, 4 participants had a monoclonal gammopathy of uncertain significance (MGUS), 4 had type II diabetes and one had been diagnosed with a specific antibody deficiency. In terms of the medication related secondary immunodeficiencies, of 9 participants 3 were taking methotrexate, 3 were taking hydroxychloroquine, 4 were taking long term prednisolone, 1 was taking azathioprine and mesalazine and 1 participant was taking mercaptopurine. The immunoglobulin levels were measured routinely. One participant had a borderline low level of IgA and eleven participants had low IgM levels. The values ranged from 0.12g/L to 0.48g/L, with the lower limit of normal considered to be 0.5g/L.

Table 5: Primary and secondary immunodeficiency in the study population

Type of immunodeficiency		Number (n=68)
Primary	Specific antibody deficiency (diagnosed)	1
	Low IgA level (borderline)	1
	Low IgG level	0
	Low IgM level	11
Secondary	Diabetes	4
	Monoclonal gammopathy of uncertain significance	3
	Medication (n=9): Methotrexate	3
	Medication (n=9): Prednisolone	4
	Medication (n=9): Hydroxychloroquine	3
	Medication (n=9): Azathiaprine	1
	Medication (n=9): Mesalazine	1
	Medication (n=9): Mercaptopurine	1

3.2.3 Self-reported symptoms

Stable and exacerbation symptoms

The self-reported symptoms are summarised below. The majority of patients (87%) experienced a cough on a daily basis when stable, producing around 10ml of sputum a day increasing to 30ml during exacerbations. Only two (3%) of the participants experienced recurrent haemoptysis when well, but this figure rose to 21 (31%) for periods of infection. Most (80%) of the study group were MRC group 1 or2 when well, falling to 20.5% during an exacerbation. Ten percent of the group felt too breathless to get out of bed during an exacerbation (MRC 5).

Table 6: Self-reported symptoms when stable and during exacerbations

Self-reported characteristics			Value
Self-reported stable symptoms	**Self-reported cough, n (%)**		59 (87)
	Self-estimated sputum volume (ml), median (IQR)		10 (20)
	Self-reported haemoptysis, n (%)		2 (3)
	Medical Research Council dyspnoea scale, n (%)	**1**	14 (21)
		2	40 (59)
		3	10 (15)
		4	4 (6)
Self-reported symptoms during exacerbations	**Self-estimated sputum volume (ml), median (IQR)**		30 (35)
	Self-reported haemoptysis, n (%)		21 (31)
	Medical Research Council dyspnoea scale, n (%)	**1**	1 (1.5)
		2	13 (19)
		3	28 (41)
		4	19 (28)
		5	7 (10)

3.2.4 Medications

Two thirds of the patients in the group were taking an inhaled bronchodilator, with therapy ranging from lone short or long acting beta-adrenoceptor antagonist or anti-muscarinic antagonist to three inhaled bronchodilators, most commonly SABA, LABA and LAMA (12, 17.6%). The LABA was most frequently combined with steroid as Symbicort or Seretide, although one patient was taking Salmeterol alone. The LAMA was most commonly Tiotropium although one patient took Glycopyrronium.

Almost half of the study group were prescribed inhaled steroids with a median BDP equivalent of 800mcg (IQR 0-800mcg). In contrast only 6% of the group were taking long term prednisolone, with a median dose of 0mg (IQR 0mg).

Table 7: The use of inhaled bronchodilators across the group

Treatment group	Specific medication	
Bronchodilator	**None, n (%)**	23 (33.8)
	LABA only, n (%)	4 (5.9)
	SABA only, n (%)	13 (19.1)
	SAMA only, n (%)	1 (1.5)
	LAMA only, n (%)	1 (1.5)
	LABA and LAMA, n (%)	1 (1.5)
	SABA and LABA, n (%)	9 (13.2)
	SABA and LAMA, n (%)	3 (4.4)
	SABA, LABA and LAMA, n (%)	12 (17.6)
	SABA, LABA and SAMA, n (%)	1 (1.5)
Inhaled steroid	**Number taking inhaled steroid, n (%)**	32 (47.1)
	Beclomethasone Diproprionate equivalent dose, mcg (mean, sd)(median, IQR)	543 (700), 0 (0-800)
Oral steroid	**Number taking oral prednisolone, n (%)**	4 (5.9)
	Dose 2.5mg	1 (25.0)
	Dose 5mg	3 (75.0)
	Prednisolone dose, mg (mean, sd) (median, IQR)	0.3 (1.1) 0 (0)
Mucolytic	**Carbocysteine, n (%)**	6 (8.8)
Expectorant	**Hypertonic (7%) nebulised saline, n (%)**	5 (7.4)

SABA – short-acting beta-adrenergic antagonist; LABA – long acting beta-adrenergic antagonist; SAMA – short-acting muscarinic antagonist; LAMA – long-acting muscarinic antagonist.

3.2.5 Quality of life questionnaires

The scores for the St George's Respiratory Questionnaire, the Leicester Cough Questionnaire and the visual analogue scales are summarised below in the table. The mean SGRQ total score was 36.18 (sd. 15.3) and the mean LCQ total score was 15.60 (sd. 3.62). The median (IQR) visual analogue scale (VAS) scores for each domain were as follows: Cough 32mm (15-54mm), dyspnoea 25mm (10-46mm), sputum production 37mm (18-58mm) and sputum purulence 38mm (9-53mm).

Table 8: The scores for the respiratory symptom questionnaires for 68 patients at the baseline visit

Questionnaire		Value
St George's Respiratory Questionnaire (SGRQ) mean (sd)	**Total score**	36.18 (15.3)
	Symptoms domain	55.84 (20.33)
	Activity domain	43.83 (22.96)
	Impact domain	25.81 (13.88)
Leicester Cough Questionnaire (LCQ) mean (sd)	**Total score**	15.60 (3.62)
	Physical domain	5.03 (1.09)
	Psychological domain	5.28 (1.36)
	Social domain	5.29 (1.40)
Visual Analogue Scale (VAS) median (IQR)	**Cough domain**	32 (15-54)
	Dyspnoea domain	25 (10-46)
	Sputum production domain	37 (18-58)
	Sputum purulence domain	38 (9-53)

Correlation between the quality of life scores with spirometry

The correlation between quality of life questionnaires and spirometry was analysed as the assumption would be that quality of life would diminish with reducing lung function. From this study population the SGRQ total score and the

Leicester cough questionnaire both demonstrated a statistically significant correlation with FEV_1 (% predicted) with the *p*-values of 0.05 and 0.002 respectively. There was no correlation between the visual analogue scale scores and the FEV_1 (% predicted).

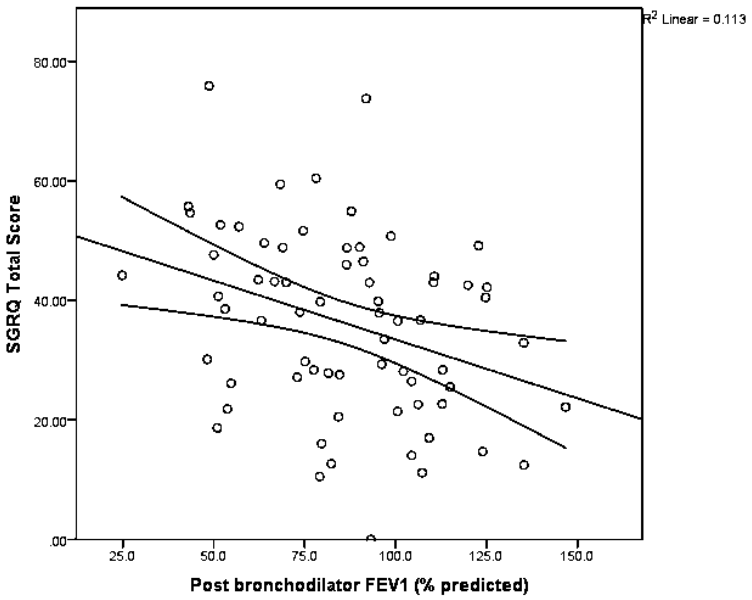


Figure 3: Scatter graph demonstrating the significant correlation between post-bronchodilator FEV1 and St George's respiratory questionnaire total score

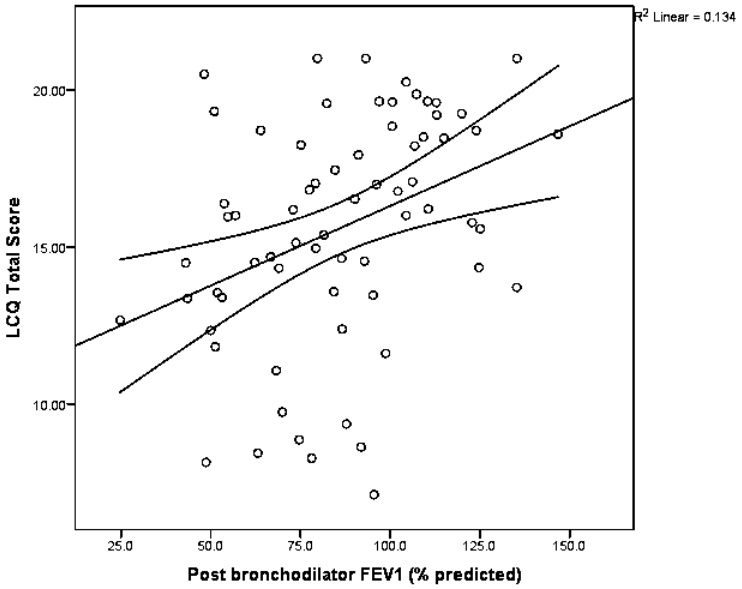


Figure 4: Scatter graph demonstrating the significant correlation between post-bronchodilator FEV1 and Leicester cough questionnaire total score

3.2.6 Airway function investigations

The results for the spirometry and multiple breath washouts are described below.

Spirometry

The mean post-bronchodilator FEV_1 was 2.04l (86.1% predicted) and FVC was 2.90l (0.84). These results are demonstrated in the table below.

Table 9: Summary of spirometry data from 68 participants

Investigation	Mean (sd)
Post bronchodilator FEV_1 (l)	2.04 (0.73)
Post bronchodilator FEV_1 (% predicted)	86.1 (26.1)
Post bronchodilator FVC (l)	2.90 (0.84)
Post bronchodilator FVC (% predicted)	97.0 (22.6)
Post bronchodilator FEV_1/FVC ratio (%)	70.2 (11.9)

The relationship between FEV_1 and quality of life questionnaires was reported in the previous section. The baseline spirometry was analysed with respect to the sputum bacterial culture results, whether *Pseudomonas aeruginosa* positive or negative. Of the 68 participants 46 were able to produce a sufficient sample to send for bacterial culture. There were 26 participants in the non-*Pseudomonas* culture group and 4 who cultured *Pseudomonas aeruginosa*. The mean FEV_1 was 1.54 (75.8%) in the Pseudomonas group and 1.97 (82.1%) in the non-Pseudomonas group. The 16 participants who did not have any significant pathogenic bacteria cultured have an FEV_1 between the two groups at 1.85 (71.4%). The group with the highest FEV_1 were those who were unable to produce a sample.

The data was also analysed after it was stratified by a positive or negative sputum bacterial culture and although the mean FEV_1 (% predicted) was higher in the group who had a positive baseline culture (80.34% vs. 73.50%) the difference was not significant (p=0.343). The box-plot is presented below. There was no correlation between FEV_1 and the sputum colony forming units (p=0.186).

Table 10: Spirometry at the baseline visit grouped according to sputum culture result

Sputum bacterial culture	n	FEV_1 (l) mean (sd)	FEV_1(% predicted) mean (sd)	FVC (l) mean (sd)	FVC(% predicted) mean (sd)	FEV_1/FVC ratio (%) mean (sd)
Pseudomonas aeruginosa	4	1.54 (0.55)	75.8 (13.1)	2.24 (0.95)	90.1 (24.7)	71.3 (7.6)
Non-*Pseudomonas aeruginosa*	26	1.97 (0.50)	82.1 (22.4)	2.84 (0.64)	93.6 (22.0)	69.3 (9.1)
No significant growth	16	1.85 (0.66)	71.4 (26.2)	2.96 (0.76)	91.0 (22.7)	61.5 (13.5)
Insufficient sample	21	2.38 (0.94)	103.5 (23.8)	3.05 (1.07)	106.0 (21.6)	77.6 (10.2)

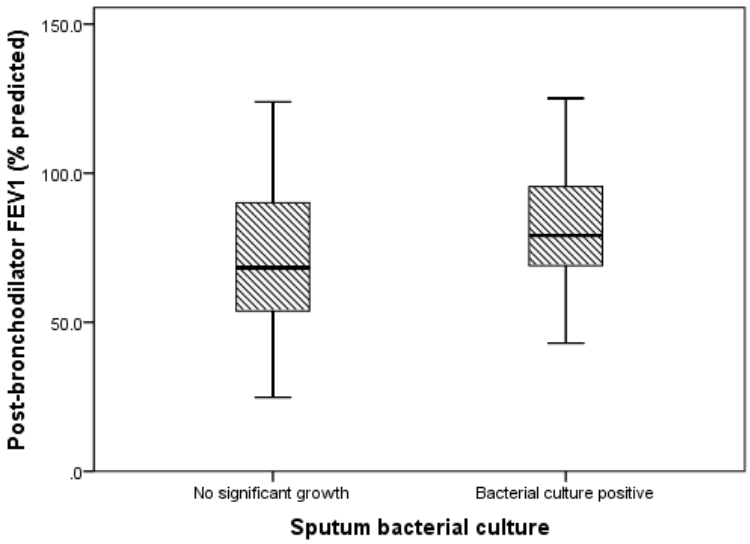


Figure 5: Post-bronchodilator FEV1 (% predicted) categorised by positive and negative sputum bacterial culture (p=0.343). Bacterial culture positive n=30; no significant growth n=16.

The analysis regarding whether the FEV_1 was affected by the degree of airway inflammation was performed. The scatter plots below both demonstrate a negative, significant correlation between the post-bronchodilator FEV_1 (% predicted) and total sputum cell count (Pearson correlation -0.431, p=0.00) and post-bronchodilator FEV_1 the absolute sputum neutrophil count (Pearson correlation -0.425, p=0.001). There was no correlation between the post-bronchodilator FEV_1 and absolute sputum eosinophil count (p=0.299).

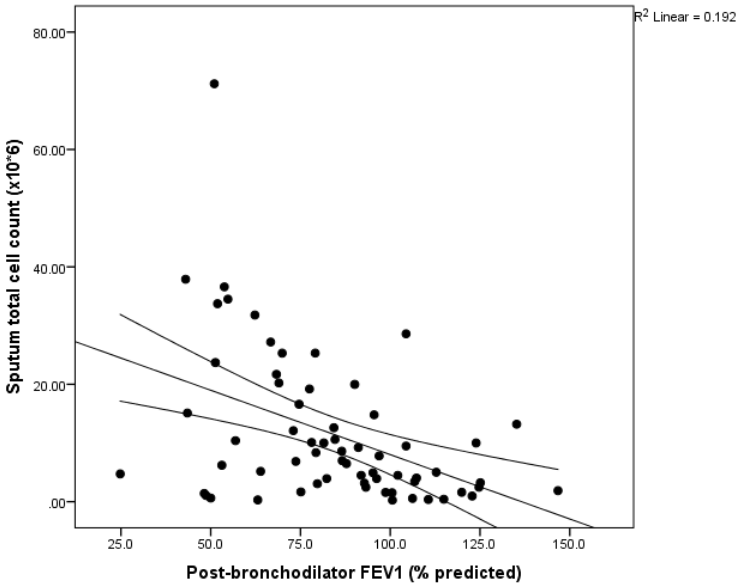


Figure 6: Negative significant correlation between the FEV_1 (% predicted) and the total sputum cell count (x10^6) (*p*=0.000)

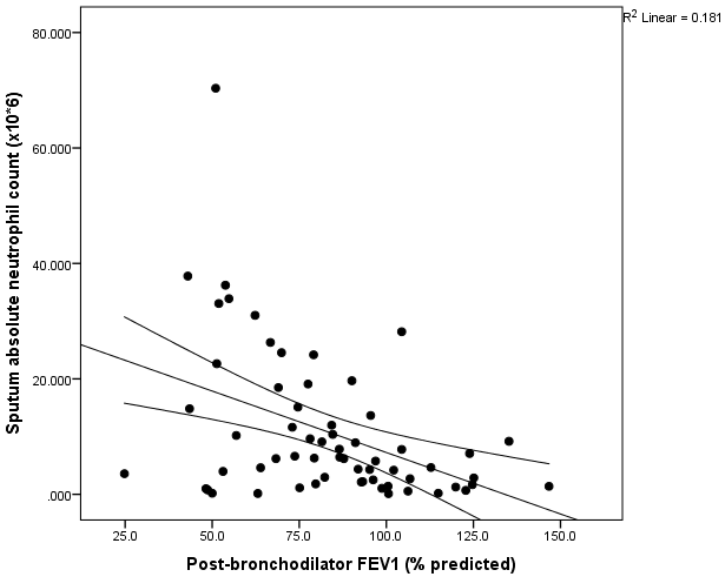


Figure 7: Scatter plot demonstrating a negative correlation between the sputum neutrophil count (x10^6) and the post-bronchodilator FEV_1 (% predicted) (*p*=0.001)

Small airway testing – multiple breath washouts results

The multiple breath washout was completed a minimum of three times to achieve at least two FRC results within 10% of each other. The test was carried out on 52 participants due to the machine being sent back to Denmark to be serviced. The

table below demonstrates the mean values for the multiple breath washout testing completed at the baseline visit. The most familiar measurement is the lung clearance index which has a value of 9.919 (sd 1.889). The cut-off for a normal LCI is <7.5.

Table 11: The multiple breath washout results at the baseline visit

	Value
Number of participants	52
Lung clearance index, mean (sd)	9.919 (1.889)
S_{cond} mean (sd)	0.069 (0.045)
S_{acin} mean (sd)	0.410 (0.200)

Lung clearance index and quality of life questionnaires

The lung clearance index is not routinely used in clinical practice so the correlation between this and other clinical investigations were analysed. For this population there was no correlation between the LCI and the either the SGRQ total score (Pearson correlation 0.029, p=0.838) or the LCQ total score (Pearson correlation -0.021, p= 0.880). There were mildly positive but significant correlations demonstrated between the lung clearance index and the dyspnoea (p=0.033), sputum production (p=0.033) and sputum purulence (p=0.010) domains of the visual analogue scales. The scatter plots are presented below.

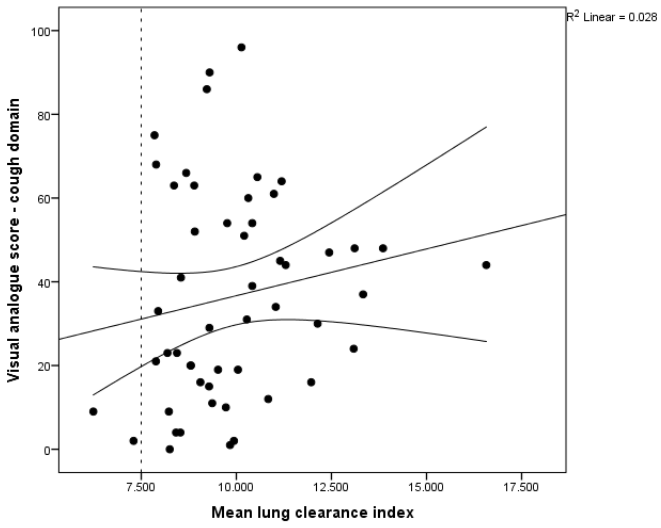


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Figure 8: Scatter plot demonstrating the non-significant correlation between the lung clearance index and the cough domain of the visual analogue scale (*p*=0.232)

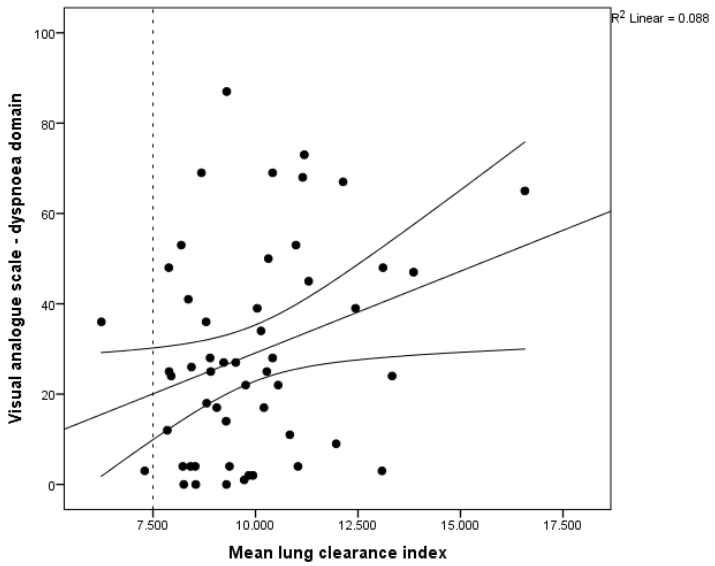


Figure 9: Scatter plot demonstrating the significant correlation between the lung clearance index and the dyspnoea domain of the visual analogue scale (*p*=0.033)

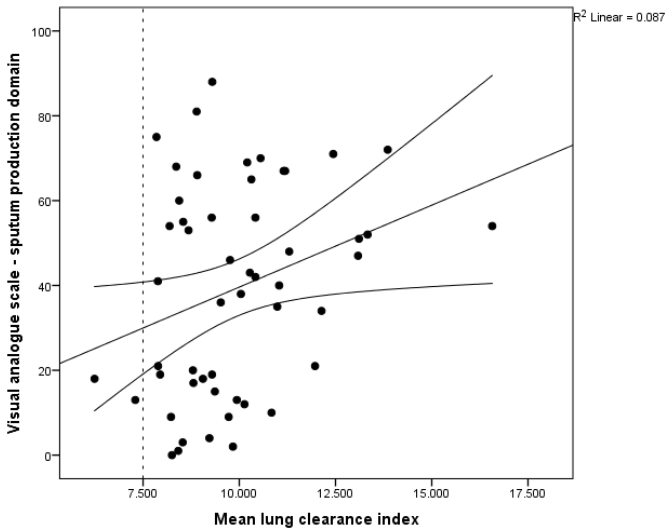


Figure 10: Scatter plot demonstrating the significant correlation between the lung clearance index and the sputum production domain of the visual analogue scale (*p*=0.033)

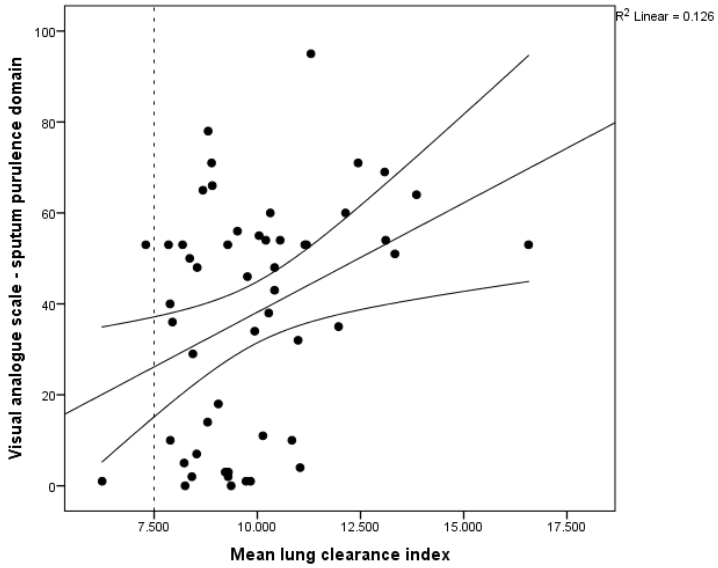


Figure 11: Scatter plot demonstrating the significant correlation between the lung clearance index and the sputum purulence domain of the visual analogue scale (*p*=0.010)

Lung clearance index and spirometry

There was a significant negative correlation between post-bronchodilator FEV_1 and the lung clearance index (Pearson correlation -0.526, *p*=0.000), and the post-

bronchodilator FEV_1 and the S_{acin} result (Pearson correlation -0.534, p=0.000). There was not any correlation between FEV_1 and S_{cond}.

The link between FEV_1 and LCI was investigated further as the LCI is known to decline earlier than the FEV_1. Many of the study population had a normal FEV_1. The data demonstrates that there were two participants (3.8%) with normal FEV_1 and LCI, 28/52 (53.8%) had a normal FEV_1 and abnormal LCI and 22 (42.3%) had an abnormal FEV_1 and abnormal LCI.

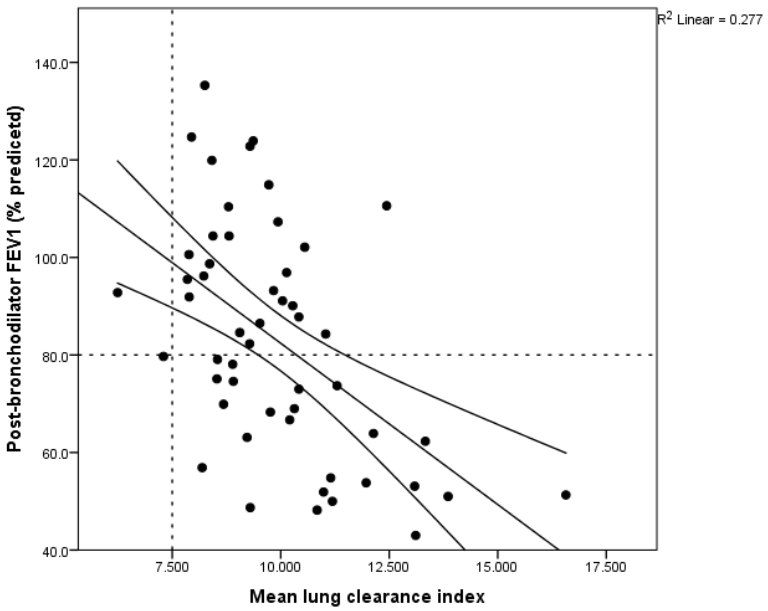


Figure 12: Scatter plot demonstrating the correlation between the lung clearance index and post-bronchodilator FEV_1 (% predicted). Dotted lines indicate limits of normality – FEV_1> 80% and LCI <7.5.

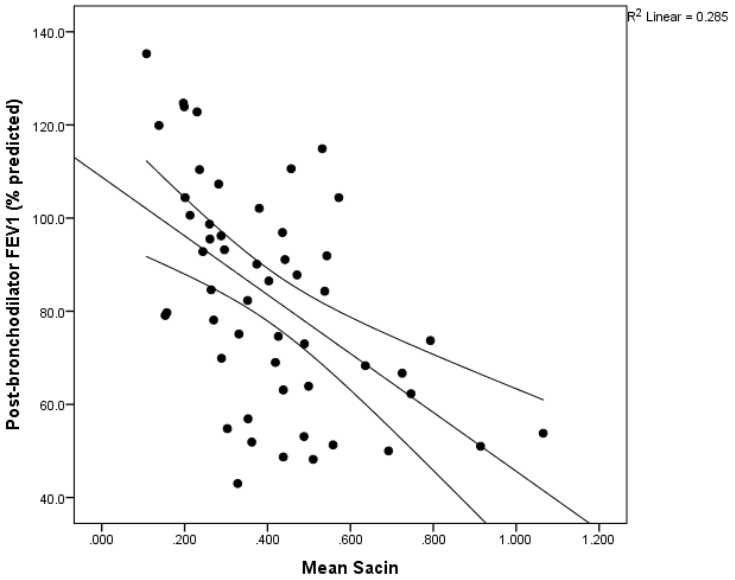


Figure 13: Scatter plot demonstrating the correlation between S_{acin} and post-bronchodilator FEV_1 (% predicted)

Lung clearance index and airway inflammation

The scatter plot below shows that there is a significantly positive correlation with LCI and sputum total cell count (Pearson correlation 0.475, p=0.000). There is an outlier with a very high sputum total cell count but the association still remains when the outlier is removed (graph not shown). As with the post-bronchodilator spirometry there is no correlation between S_{cond} and sputum total cell count (p=0.814) but a significantly positive correlation between Sacin and the total sputum cell count (p=0.001). There was no significant correlation between the LCI and the sputum neutrophils (%) (p=0.060). (Graph not shown.)

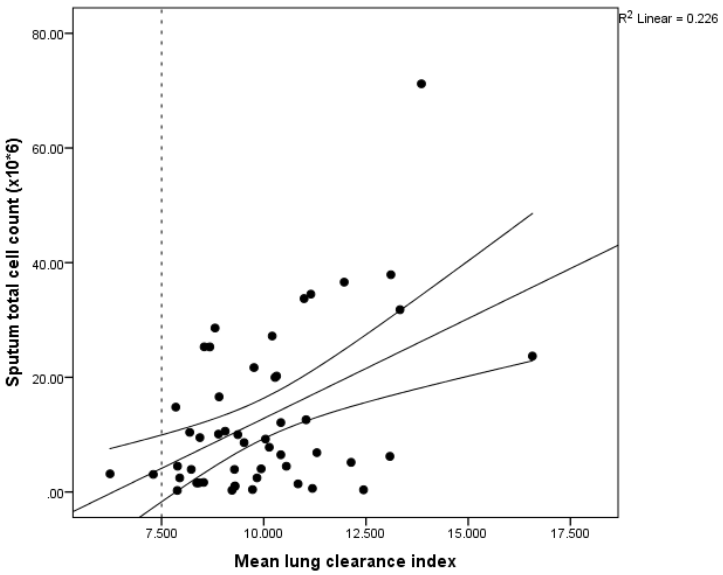


Figure 14: Scatter plot demonstrating the significant correlation between the lung clearance index and the sputum total cell count (x10^6). Dotted line indicates limit of normality – LCI <7.5. (*p*=0.000)

Lung clearance index and microbiology

The lung clearance index was analysed according sputum culture (positive / no significant growth) but there was no significant difference between the groups (*p*=0.136). (Data not shown)

3.2.7 Sputum and airway inflammation

The results from the exhaled and nasal nitric oxide investigations and the induced sputum cell counts are described in the following section. The table below lists the mean values (sd) for the nasal and exhaled nitric oxide tests.

Table 12: The nasal nitric oxide and exhaled nitric oxide mean values (ppb)

Investigation	Result
Number	64
Mean exhaled nitric oxide level (ppb), mean (sd)	25 (14)
Mean nasal nitric oxide level (ppb), mean (sd)	292 (196)

Exhaled nitric oxide levels

The mean exhaled nitric oxide level was 25ppb (sd 14 ppb) from 64 participants. Levels were not recorded in 4 participants due to equipment failure. Elevated exhaled nitric oxide levels indicate towards eosinophilic airway inflammation therefore the study population was divided into those with a high sputum eosinophil count (>3%) and a low sputum eosinophil count (<3%). Six participants were unable to produce an induced sputum sample for analysis. There were 16 participants with a raised sputum eosinophil count and 46 with a normal sputum eosinophil count. The difference in the mean level between the two groups was not significant (p=0.189).

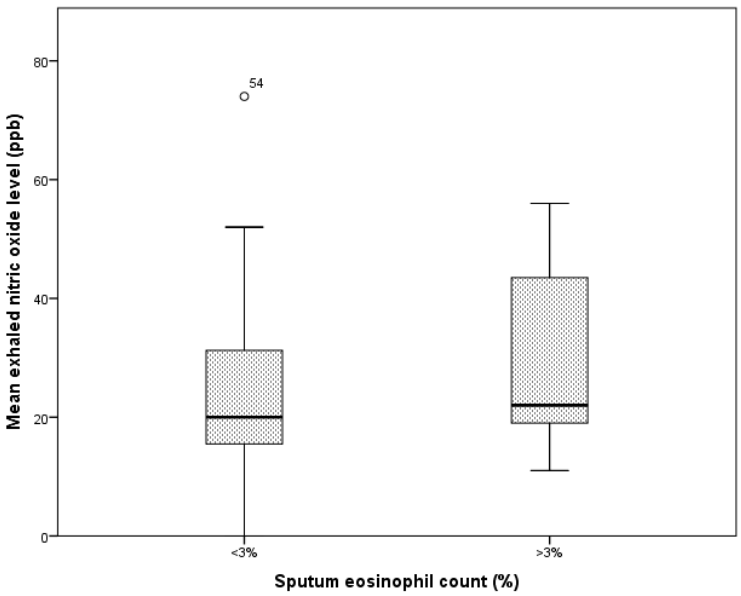


Figure 15: A comparison of the mean exhaled nitric oxide level (ppb) according to the sputum eosinophil counts (n=62, >3%=16, <3%=46)

Nasal nitric oxide levels

The mean nasal nitric oxide (NNO) level was 292ppb (sd +/- 196 ppb). The nasal NNO level was not recorded in 4 participants due to equipment problems. There were 7 participants with an average level lower than 100ppb, the cut off used as a screening tool for primary ciliary dyskinesia, but this was not persistent on repeat testing after 3 months.

Sputum differential cell counts

The median total cell count was 6.93x10^6/ml (IQR 2.76-15.85 x10^6/ml). Sixty-two of the sixty-eight participants produced an induced sputum sample for differential cell analysis. The sputum samples were predominantly neutrophilic (median 91%, IQR 70.75-96.80%). The median sputum eosinophil count was 1.25% (IQR 0.5-3.0%). The data was categorised according to whether the subject was taking inhaled or oral corticosteroids. The proportion of participants with a raised sputum eosinophil count was higher in the groups taking some form of steroid medication.

Table 13: Summary of the sputum cell counts with relation to whether the participant was taking oral or inhaled steroid medication

	Inhaled steroid medication		Long-term oral steroid medication	
	No (n=31)	**Yes (n=31)**	**No (n=58)**	**Yes (n=4)**
Sputum total cell count, x10^6/ml, median (IQR)	6.93 (2.76-15.85)			
Sputum total cell count, x10^6/ml, median (IQR)	8.60 (3.43-15.10)	6.21 (1.67-16.60)	7.40 (2.76-15.85)	5.36 (2.96-19.01)
Sputum differential cell count (%), median (IQR)				
Neutrophils	91.80 (70.75-98.00)	89.25 (69.80-96.80)	91.00 (71.50-96.75)	83.30 (66.90-97.15)
Leucocytes	0 (0-0.40)	0 (0-0.5)	0 (0-0.40)	0.13 (0-0.75)
Eosinophils	0.80 (0.50-2.25)	1.25 (0.5-5.00)	1.25 (0.50-2.25)	2.63 (0.38-9.88)
Eosinophils (n) **<3%**	26	20	44	2
Eosinophils (n) **>3%**	5	11	14	2
Macrophages	4.00 (1.00-21.00)	6.00 (2.00-17.00)	5.13 (1.75-16.80)	11.00 (2.00-20.63)
Epithelial cells	0.50 (0.25-2.75)	0.75 (0.25-3.00)	0.63 (0.25-2.75)	0.88 (0.38-2.00)
Sputum absolute cell count (x10^6/ml), median (IQR)				
Neutrophils	7.782 (2.50-18.501)	4.609 (1.264-13.660)	6.350 (2.125-14.836)	4.167 (2.479-17.685)
Leucocytes	0 (0-0.014)	0 (0-0.017)	0 (0-0.014)	0.005 (0-0.014)
Eosinophils	0.055 (0.001-0.189)	0.067 (0.028-0.259)	0.066 (0.011-0.227)	0.044 (0.015-0.500)
Macrophages	0.260 (0.135-0.680)	0.324 (0.120-0.743)	0.279 (0.135-0.680)	0.470 (0.195-0.941)
Epithelial cells	0.050 (0.006-0.100)	0.046 (0.012-0.090)	0.050 (0.008-0.100)	0.036 (0.015-0.052)

Sputum inflammation and bacterial infection

The sputum neutrophil count was categorised by whether the bacterial culture was positive for *Pseudomonas aeruginosa* or not. The sputum neutrophil count was high and similar across all groups including the group with no significant bacterial growth. The sputum bacterial colony forming unit count was lowest in the group whose sputum did not demonstrate any significant growth and highest in those who cultured *Pseudomonas aeruginosa*.

Table 14: Sputum inflammation and bacterial colony forming units according to sputum bacterial culture

Sputum sample culture result	Sputum neutrophils (%) median (IQR)	Sputum bacterial colony forming units median (IQR)
No significant growth n=16	91.80 (71.50-98.25)	0.380 (0.261-1.080)
Non-*Pseudomonas* bacteria n=26	95.00 (87.80-96.75)	3.300 (0.492-27.750)
***Pseudomonas aeruginosa* n=4**	94.50 (83.00-98.75)	3.720 (1.460-16.620)

Association with asthma and ABPA

The study participants with a sputum eosinophil count over 3% were analysed to determine the extent to which this is due to asthma or ABPA. The diagnosis of either condition had been made by a senior doctor prior to the onset of the study. From this group 7/13 (53.8%) had a pre-study physician diagnosis of asthma with a higher median eosinophil count compared to 6 (46.2%) who did not. Of those without asthma the majority (81.6%) had a normal sputum eosinophil count. All the participants with a pre-study physician diagnosis of ABPA had a raised sputum eosinophil count. There was a significant difference between all the categories.

Table 15: Comparison of sputum eosinophils by diagnosis of ABPA and asthma

		Sputum eosinophils <3%	Sputum eosinophils >3%	*p*-value
		n (%)	n (%)	
Asthma	No	40 (81.6%)	9 (18.4%)	0.009
	Yes	6 (46.2%)	7 (53.8%)	
ABPA	No	46 (79.3%)	12 (20.7%)	0.000
	Yes	0	4 (100%)	

3.2.8 Microbiology

Sputum cultures were sent for culture for all patients who were able to produce a sample. Forty-seven (69.1%) samples were produced from 68 participants. Of these samples, 53 results were generated which included either no significant growth or a positive culture. This is greater than the number of samples produced as five samples cultured 2 organisms and one sample cultured 3 organisms. The multiple cultures were:

- *Haemophilus influenzae*/*Streptococcus pneumoniae* (3)
- *Haemophilus influenzae*/*Moraxella catarrhalis* (1)
- *Haemophilus influenzae*/*Pseudomonas aeruginosa* (1)
- *Haemophilus influenza*/*Streptococcus pneumoniae*/*Moraxella catarrhalis* (1)

The most commonly cultured organism was *Haemophilus influenzae*, being cultured from 16/53 sample results (30.2%). After that the most commonly cultured were *Pseudomonas aeruginosa* (6), *Streptococcus pneumoniae* (5), and *Moraxella catarrhalis* (4). The organisms included in the "other" category were *beta-Haemolytic streptococcus* and *Candida* species.

3.3 Discussion

Sixty-eight patients with bronchiectasis confirmed on high resolution CT scanning were invited for a single visit, with 40 of these forming the cohort for the 7 visit erythromycin trial. The baseline characteristics were analysed including, age, sex, lung function, small airways function, symptom questionnaires and sputum microbiology and fungal culture.

3.3.1 Patient selection and potential bias

The patients were selected mainly from the outpatient clinics at Glenfield Hospital. It should be noted that as the main clinical trial involved a 12 week intervention with low dose erythromycin one of the inclusion criteria for the study was that the patients should not already be taking any low dose, long term antibiotic therapy. This might have resulted in a milder, less symptomatic population being recruited for the study. During the course of the study the Bronchiectasis Severity Index (154) was published and it would have been informative to have scored each patient against the system. However, despite the patients not already taking long term antibiotics it should also be pointed out that some were indeed very symptomatic, had multiple (up to 8) exacerbations in the 18 month period of the study and several patients restarted the erythromycin post study completion. Therefore the patients recruited had a wide spread of symptoms and radiological bronchiectasis severity but by the nature of the recruitment process and the need to attend multiple appointments there may have been a bias towards the milder end of the spectrum.

3.3.2 Demographics

In this study there were a greater number of females than males, in keeping with many studies to date, for reasons that are as yet unknown (29,155). The group were reasonably healthy and active with a mean body mass index of 26.9 kg/m^2, just outside the normal range, and a Medical Research Council breathlessness

score of 1-2 in 85% of the group. Unlike many respiratory conditions the majority of the participants had never smoked and only one was a current smoker. The median age of onset was 27 years but the distribution was biphasic and very broad ranging from several months old to 80 years of age, with peaks in the 0-5 years of age and 60-65 years of age brackets. Pasteur et al. also identified a wide range in the onset of symptoms with a skewed distribution, greatest under the age of 20 and reducing as the age progressed to 90 years.

3.3.3 Aetiology and associated conditions

Traditionally the causes of bronchiectasis are idiopathic, post-infective (usually TB, pneumonia or whooping cough), genetic (cystic fibrosis and primary ciliary dyskinesia), local airway obstruction or related to all forms of immunosuppression whether severe or subtle in nature. Recently the association with rheumatoid arthritis and inflammatory bowel disease, most commonly ulcerative colitis, and other autoimmune conditions is being explored. In our study cohort many of the participants had several possible contributing aetiologies and associated conditions for their bronchiectasis.

Childhood infections

In the study cohort 50% gave a history of childhood whooping cough and 32.4% gave a history of childhood pneumonia. Many studies attribute childhood infections as the most common cause of bronchiectasis (29,134) however the exact relationship between the two can never be fully understood without a prospective study. In theory, with advancing healthcare and antibiotic therapy in the western world the incidence of “post-infectious bronchiectasis” should decline as time goes by.

Tuberculosis

Post-TB bronchiectasis is often localised, well recalled in history taking and often involves traction bronchiectasis. Other post-infectious bronchiectasis is often widespread, difficult to relate to a specific condition and doesn't involve traction bronchiectasis. In my study cohort 2 patients (2.9%) had a history of TB infection. A Korean study of patients undergoing a CT chest for health screening demonstrated that of the 129 patients with bronchiectasis 15 (11.6%) had a history of previous TB compared to 3% of the group without CT evidence of bronchiectasis (31). By contrast a London study of bronchiectasis aetiology demonstrated that only one of the 136 bronchiectasis patients had post-infectious TB (155). The differences are likely to be related to the TB risk of that particular country.

Recurrent sinusitis and otitis media

It was apparent in the data that there was a large proportion of patients with a history of recurrent sinusitis (41.2%) or recurrent otitis media (32.4%) which had settled in later adulthood. Primary ciliary dyskinesia was not felt to be an underlying cause. There has long been an association between chronic rhinosinusitis and bronchiectasis, excluding primary ciliary dyskinesia and Kartagener's syndrome, but the exact relationship to each other is unclear (156) although the concept of a "united airways" has been proposed (157). There are several hypotheses as to the underlying factors for the development of a sino-pulmonary syndrome: 1) The two similar mucosal surfaces are equally affected by inhaled pathogens. 2) There is an element of post-nasal drip and transfer of infection. 3) An underlying immunodeficiency, however subtle, is likely to affect these two mucosal surfaces equally thereby predisposing to concurrent infections. 4) There are underlying subtle deficiencies with mucus clearance and possibly underlying ciliary function without clear evidence of primary ciliary dyskinesia.

The link between bronchiectasis and recurrent otitis media is less well described in the literature. Acute otitis media is common bacterial infection affecting 71% of children by the age of 3 years (158) with the most frequently identified pathogen being *Streptococcus pneumoniae.* In recent years there has been increasing evidence that a deficient IgG2 antibody response to Streptococcus pneumoniae has played a role in the infection (159).

Gastro-oesophageal reflux disease (GORD)

The study cohort contained 34 patients (50%) who gave a history of recurrent gastro-oesophageal reflux symptoms, although this was not clinically proven. The Papworth group found that only 4% of their cohort had bronchiectasis attributable to aspiration and GORD (29). Micro-aspiration is increasingly implicated in the development of bronchiectasis. There are novel methods of investigating the role of reflux using invasive methods such as pH manometry (160,161) and non-invasive methods such as sputum and exhaled pepsin levels (160,162). One study identified micro-aspiration as an independent risk factor for an increased frequency of exacerbations (163). The difficult area remains how best to resolve the aspiration. One retrospective observational case series reported an improvement in respiratory symptoms and exacerbation frequency in bronchiectasis patients who underwent Stretta radiofrequency treatment or laparoscopic fundoplication surgery to cure their reflux (164).

Genetic conditions

Primary ciliary dyskinesia was excluded in the study cohort on the basis of either history or a normal nasal nitric oxide reading. None of the participants were referred for nasal brushings and none were suspected as having underlying primary ciliary dyskinesia.

Alpha-1 anti-trypsin deficiency

Alpha-1 antitrypsin deficiency, in particular the PiZZ phenotype, has been associated with bronchiectasis. Our study did not demonstrate any patients with this phenotype although 7/55 (12.7%) were proven to have the partial deficiency PiMS and PiMZ phenotypes. Similar results were demonstrated by Pasteur and colleagues (29). In a study of 3000 Irish people, the prevalence of the MS allele was calculated to be 10% and the MZ allele 4%, in keeping with my study findings (165). Another study of patients with confirmed bronchiectasis also demonstrated that 15.3% of their study population carried this phenotype, with the suggestion that those more deficient in alpha-1 antitrypsin had an increased incidence of emphysema, with bronchiectasis developing secondary to this (166).

Autoimmune and inflammatory conditions

In the study there were 16 participants (23.5%) who had a form of autoimmune disease, including rheumatoid arthritis, inflammatory bowel disease, autoimmune hypothyroidism, vitiligo, cold urticaria, coeliac disease, lichen planus and pernicious anaemia. Some had more than one condition. Altenburg's azithromycin study of 83 bronchiectasis patients demonstrated a 12% incidence of autoimmune disease (134). The Papworth group found that 4% of their group had an autoimmune related condition (29).

Rheumatoid arthritis

The most commonly associated autoimmune disease in my study was rheumatoid arthritis. Research suggests that 2.9% of patients with rheumatoid arthritis have bronchiectasis (75) and 5.2-12.3% of patients with bronchiectasis have rheumatoid arthritis (76,77). These patients are 2.4 times more likely to die in a five year period than patients with bronchiectasis alone (78). Increasing availability to laboratory tests and a growing desire to undercover any possible underlying cause for the bronchiectasis has meant that a growing number of bronchiectasis patients have an elevated rheumatoid factor without overt features of a destructive serositis. In the study cohort 16 participants (23.5%) had an

elevated rheumatoid factor, with only three of these having diagnosed RA. The predictive value of these autoantibodies remains under-researched, however several studies have demonstrated that a raised rheumatoid factor level could indicate pre-rheumatoid arthritis, predisposing overt symptoms by 3-10 years (167,168). It has been proven that rheumatoid factor, an autoantibody against the constant domain of IgG, is raised in other autoimmune conditions and that anti-citrullinated protein antibodies are far more specific for rheumatoid arthritis, but the role for this in bronchiectasis is unknown (169,170). Research has suggested that rheumatoid arthritis autoimmunity has its origins in mucosal sites with lung inflammation playing a significant role regardless of smoking history (168). Therefore the bronchiectasis may be a primary association with a positive rheumatoid factor but for symptomatic rheumatoid patients early immunosuppression medication is the cornerstone of the treatment pathway. This can lead to recurrent infections and initiate the vicious cycle of bronchiectasis development.

Inflammatory bowel disease

The concept of gut-lung crosstalk has been developing for some 40 years (171). The bowel and bronchial tree are both of mesodermal origin with similar mucosal associated lymphoid tissue and it has long been felt that the inflammatory cytokine activity found in inflammatory bowel disease affects the bronchial mucosa by a similar mechanism (71). The mucosal surface of both the bowel and the lung is often the first line in defence to the outside world and the inflammatory cascade is similar in both areas. The lung pathology seen in these conditions ranges from bronchiectasis and bronchiolitis to small airway changes, interstitial lung disease and nodules (72). The pathology may be sub-clinical or symptomatic and occurs independently of IBD activity (73). It has been suggested that lung disease is more commonly associated with ulcerative colitis than Crohn's disease, in contrast to other systemic manifestations (172). In my study cohort there were three participants who had inflammatory bowel disease, with one participant having rheumatoid arthritis in addition. In keeping with Camus's findings described above

two of these three had ulcerative colitis and one had Crohn's disease. None had any of the other classical extra-intestinal manifestations.

Other autoimmune conditions

My study contained two participants with autoimmune hypothyroidism and several more with non-autoimmune hypothyroidism. There is no specific link in the literature between hypothyroidism and bronchiectasis. Autoimmune hypothyroidism is reasonably common with one study suggesting the incidence is in the region of 350/100 000/year in women and 80/100 000/year in men across all ethnicities and ages (173).

The other autoimmune conditions that were present in my study were cold urticaria, Sjogren's syndrome, lichen planus, coeliac disease, autoimmune hypogonadism, polymyalgia rheumatica and pernicious anaemia. It is not recommended that autoantibodies routinely be sent in bronchiectasis patients who do not show clinical symptoms of systemic disease. One study suggested that 38% of patients with primary Sjogren's syndrome had bronchiectasis on HRCT imaging (174). Other conditions with a close association to bronchiectasis are systemic lupus erythematosus (SLE) and all vasculitides.

Immunodeficiency

Immunodeficiency is often divided into primary and secondary. The secondary causes are far more common, being related to age, immunosuppressive medications, acute and chronic infections and disorders affecting the bone marrow. In terms of the primary conditions, one participant had previously been diagnosed with a specific antibody disorder. The secondary causes were far more prevalent. Four participants had a diagnosis of monoclonal gammopathy of uncertain significance (MGUS) and 4 had type II diabetes. Medication was a common cause of the related secondary immunodeficiencies – from 9 participants, some prescribed several agents, there were 3 records of methotrexate, 3 of hydroxychloroquine, 4 of long term prednisolone, 1 of azathioprine and

mesalazine and 1 of mercaptopurine. The immunoglobulin levels were measured routinely. One participant had a borderline low level of IgA of doubtful significance. More surprisingly eleven participants had low IgM levels. The values ranged from 0.12g/L to 0.48g/L, with the lower limit of normal considered to be 0.5g/L. It is not uncommon for a low IgM level to be seen with advancing age and the low levels don't often result in a clinical pre-disposition to infection. An abnormally low level is considered to be less than two standard deviations below the normal cut-off level. One study demonstrated the incidence of common variable immune deficiency to be 2/83 patients (2.4%) (134). Clinically low IgM levels have been associated with allergy, asthma, atopy and autoimmune disease (38,39).

Allergic broncho-pulmonary aspergillosis

Four participants (5.9%) had pre-study diagnosis of allergic broncho-pulmonary aspergillosis (ABPA). This subject will be discussed at length in chapter 6.

3.3.4 Quality of life questionnaires

St George's Respiratory Questionnaire (SGRQ)

The St George's Respiratory Questionnaire contains three domains – symptoms, activity and impact – made from 76 sub-questions. The symptom domain covers sputum production, cough, breathlessness and wheezing. The activity domain is based around which activities and limited by breathlessness and chest symptoms. The impact domain covers the area of medication and side effects, effect of symptoms on daily life and anxiety symptoms. The scores are given for each of the three domains as well as the total score, which is given out of 100. The questionnaire has been validated for bronchiectasis (92), COPD and asthma. A higher score indicates more symptoms.

The mean total SGRQ score for the study cohort at the baseline visit was 36.18 (sd. 15.3). These results are similar to the scores recorded for the placebo group in the BLESS trial (mean 38.1, sd. 15.4) and the placebo group in the BAT azithromycin trial (mean score 40.2, sd. 20.9) (126,130).

The correlation between the SGRQ scores and FEV_1 were reviewed as the logical assumption suggests better quality of life is linked with better lung function. There was a weakly negative correlation with FEV_1 and SGRQ total score (p=0.05), meaning that a higher score indicating worse symptoms was more likely to be associated with a lower FEV_1. The same findings have been seen in COPD studies where the change in FEV_1 might be expected to have more of an impact on the SGRQ total score and overall quality of life (176).

Leicester Cough Questionnaire (LCQ)

The Leicester Cough Questionnaire is 19 item questionnaire which covers three domains – the physical, psychological and social impacts of coughing and its associated respiratory conditions (177). Unlike the SGRQ a lower LCQ score indicates more symptoms. It has been validated in bronchiectasis and found to be a good discriminator between mild and severe disease as well as reliable and repeatable (151).

The mean total LCQ score for the 68 baseline visit participants was 15.6 (sd. 3.62). The total score is comparable to that reported for the placebo group in the BLESS study (mean 14.7, sd. 3.4) (175). The correlation between the Leicester cough questionnaire total score and FEV_1 was reviewed. As with the SGRQ the LCQ score demonstrated a weakly positive but significant correlation with FEV_1 suggesting better spirometry is associated with a better quality of life (p=0.002).

Visual Analogue Scale of cough, breathlessness and sputum production (VAS)

This study used the visual analogue scales in the areas of cough, breathlessness, sputum production and sputum purulence which have been validated for repeatability and reliability. The subjective nature of these scales is such that intra-subject rather than inter-subject comparisons can be made.

The median scores for all the domains were on the lower third of the scale with a reasonably large range. The scores were compared against those obtained in COPD research (unpublished). The bronchiectasis cohort report lower symptoms in the breathlessness and cough domain but unsurprisingly higher symptoms in the sputum production and purulence domains (178).

Correlation with spirometry

Interestingly there was no correlation between the visual analogue scale domains and the FEV_1 value.

3.3.5 Airway investigations

The airway investigations include nasal and exhaled nitric oxide levels, spirometry and induced sputum for differential cell counts.

Spirometry

On average, the spirometry for the 68 participants in the study was within the normal range. This is a common finding in bronchiectasis studies. The BLESS study reported normal post-bronchodilator spirometry in both the placebo and intervention groups (175). This was also the case for the BAT bronchiectasis trial (134) and the descriptive paper by Pasteur et al. of the bronchiectasis patients in East Anglia (29). The definition of bronchiectasis does not involve a description of airflow obstruction and so it should be anticipated that a proportion of the group have normal spirometry. Some participants had other airway conditions which would have affected their lung function results and many were taking either

inhaled or oral corticosteroids which may have had an impact on improving the FEV_1. It is also possible that excessive mucosal thickening and mucus secretion in the airway causes turbulent airflow and a reduced FEV_1 despite the airways being wider, by the definition of the condition.

Stratification by sputum bacterial culture

The spirometric data at the baseline visit was categorised according to sputum culture. It is perhaps unsurprising that the group with the overall highest FEV_1 could not produce a sputum sample, suggesting fewer symptoms when stable. The lowest mean FEV_1 was found in the group who cultured *Pseudomonas aeruginosa* while the groups who cultured bacteria other than Pseudomonas or did not have any significant pathogenic bacteria cultured from their sample had FEV_1 values in between. There was also no correlation between the sputum bacterial colony forming units and the FEV_1. These findings contrast with a study by Guan et al. who identified a significant reduction in FEV_1 (% predicted) in participants who had a positive sputum bacterial culture compared to those who did not (179). There have been some prospective studies that have suggested that bronchiectasis patients who become colonised with *Pseudomonas aeruginosa* have lower lung functions than those who don't and that their rate of FEV_1 decline is worse once than colonised than previously (146,172,173).

Correlation with sputum inflammation and differential cell counts

Data analysis revealed that the FEV_1 (% predicted) had a negative, significant correlation with the sputum total cell count and the absolute neutrophil count which is similar to previous findings (181). The assumptions for this finding are that airway inflammation contributes to more turbulent airflow and therefore a reduced FEV_1. It is also possible that patients with reduced spirometry are more prone to bacterial infection which in turn might increase the sputum total and neutrophil count. However, my data did not demonstrate any association between FEV_1 and bacterial colony forming units. There was no significant difference between the FEV_1 and the sputum eosinophil count despite the association with asthma which is more likely to lead to airflow obstruction.

Small Airway Investigations – Multiple Breath Washout

Multiple breath inert gas washout (MBW) is a useful tool for assessing ventilation heterogeneity, an early feature of obstructive airway disease. The tool has been increasingly used in cystic fibrosis (101–103,108) and has been evaluated in non-cystic fibrosis bronchiectasis (107,182). Lung clearance index has been shown to be a better predictor of early CT changes than spirometry, and has been demonstrated to be both repeatable and reproducible (107).

The inert gas multiple breath washout test was performed on 52/68 subjects at the baseline visit. The equipment had to be sent back to Denmark to be serviced part way through the study hence the test not being carried out on 16 participants. Some participants found the technique straight forward but others required 5 tests to obtain three FRC results within 10%. The test itself is quite challenging for very breathless patients and was not possible to carry out during exacerbations. Only on one occasion were there technical issues with the equipment where a leak was detected and that tests was discarded and the entire investigation started from the beginning after the problem was fixed.

The mean values determined from a healthy population in one study are as follows: Lung clearance index 6.7 (0.6), S_{cond} 0.010 (0.015) and S_{acin} 0.112 (0.055) (183). The mean lung clearance index from our study population was 9.919 (sd 1.889) with S_{cond} 0.069 (sd 0.045) and S_{acin} 0.410 (sd 0.200). These values are all higher than found within the healthy population. It has been demonstrated that S_{acin} only rises with more advanced lung disease, whereas S_{cond} is abnormal early in the disease course (183).

Lung clearance index and quality of life questionnaires

There was no correlation identified between the total score of the SGRQ and LCQ, although one study did demonstrate a significantly positive relationship between the symptom domain of the SGRQ and the LCI (107). Three of the visual analogue scale domains (dyspnoea, sputum production and purulence) demonstrated a significant positive correlation perhaps as the small airways are the drivers of the

sputum production and purulence. It could be postulated that an increase in small airway mucus will have a significant impact on the lung clearance index and ventilation heterogeneity.

Correlation with spirometry

There were significant correlations between the LCI and FEV_1 (p=0.000) as seen in other studies (107). The data shows that only two participants had a normal FEV_1 and a normal lung clearance index. The rest of the population were divided into two groups: (1) Normal FEV_1 with abnormal lung clearance index, (2) Abnormal FEV_1 and abnormal lung clearance index. In cystic fibrosis the phenomenon of normal spirometry with abnormal CT imaging and lung clearance index has been termed the "silent years" – a time when lung function is deteriorating but has not been identified by other investigations (104,184).

Correlation with airway inflammation

There was also a positive significant correlation between the lung clearance index and the total sputum cell count but no correlation with the sputum neutrophil count (%).

Lung clearance index and microbiology

My study did not demonstrate any significant difference in the LCI when analysed according to specific bacterial growth, positive or negative sputum culture or sputum bacterial colony forming units. One recent study demonstrated an inverse, significant correlation between LCI and sputum colony forming units, suggesting that a worse lung clearance index is associated with a reduced bacterial burden (185). The lack of association in my study may be related to the culture methods employed. PCR techniques might produce results more representative of the lung environment.

3.3.6 Sputum inflammation

Exhaled nitric oxide

Exhaled nitric oxide is well recognised as a useful tool for diagnosing eosinophilic airway inflammation (186). However the role of the test in bronchiectasis is not as well understood. Previous work has hypothesised that the chronic cytokine-mediated inflammatory nature of the lungs in bronchiectasis and cystic fibrosis will naturally result in increased nitric oxide levels.

My study group demonstrated a mean level of 25ppb with a standard deviation of 14ppb. The normal range is below 50ppb with a grey area from 25-50ppb. The exhaled nitric oxide level was not significantly different between the group with a raised sputum eosinophil count >3%, and the group with the sputum eosinophil count below 3%. The normal exhaled nitric oxide levels found in this study are comparable to other research in the area (67,187,188). Fractional exhaled nitric oxide is very useful in detecting eosinophilic inflammation and has been used to phenotype asthma and determine expected steroid responsiveness with good success (186). Some of the participants in my study had asthma and so the FeNO levels in my data demonstrated some variation in keeping with this.

Nasal nitric oxide (nNO)

The mean nasal nitric oxide level was 292ppb (sd 196ppb). The cut off for screening recommended for the equipment used in this study is 100ppb. In the study cohort 7/64 participants had a level below 100ppb. Four of these were on the erythromycin trial and so had the test repeated at a subsequent visit. The repeat values were greater than 100ppb, and no participants were referred for a nasal biopsy. The clinicians overlooking the care of the remaining three participants with low nasal nitric oxide levels were alerted to the results and advised to consider referral for further testing if clinically relevant. Although nNO has been found to be a useful screening tool in excluding primary ciliary dyskinesia

it has been clearly demonstrated that non-PCD bronchiectasis patients can also have nNO levels below the screening cut-off (67).

Induced sputum cell counts

Induced sputum samples were obtained from 62/68 participants attending the baseline visit. So far there is not a standard reference range for sputum differential cell counts but a couple of papers have been published with data from healthy subjects. The method has previously been validated (189). Spanevello et al. tested 96 non-atopic, non-smoking healthy volunteers and found the mean percentages to be 69.2% (sd 13%) for macrophages, 27.3% (sd 13%) for neutrophils and 0.6% (sd 0.8%) for eosinophils (190). Another study carried out by Belda et al. found neutrophil values of 34-40% in their atopic and non-atopic healthy participants (191). This is similar to the findings published by Thomas et al. who additionally found that the sputum neutrophil count increased with age along with a corresponding reduction in the macrophage count. The proportional values in 66 healthy volunteers were as follows: 26.9% (sd 19.8%) sputum neutrophils in the under 30s and 68.5% (sd 20.6%) in the over 60s.(192) In contrast a Brazilian study carried out by Veras et al. did not demonstrate an increase in the mean neutrophil percentage with age. The mean value was 20.2% (sd 14.5%) in the 18-29 year old group and 23.1% (sd 14.8%) in those greater than 50 years old (193).

At the baseline visit the median (IQR) values were 91% (70.75-96.80%) for neutrophils, 1.25% (0.5-3.0%) for the eosinophils and negligible counts for the leucocytes and epithelial cells. The remainder was made up of the macrophages in keeping with the normal value data. The sputum counts in this population were predominantly neutrophilic which is in keeping with other research on non-CF bronchiectasis (150,175). My data also found that there were a higher proportion of participants with a raised sputum eosinophil count who were taking some form of long-term steroid, whether inhaled or oral. Steroid treatment is indicated for eosinophil airway conditions and it appears as if the participants taking steroids were more likely to have eosinophilic airway inflammation. However, steroid

treatment would usually suppress the eosinophil count, which had not entirely been the case in these participants.

Some groups have studied neutrophil derived mediators such as elastase, leukotriene B4, interleukin 8 and myeloperoxidase. The elevated level of these products seen in bronchiectasis indirectly suggests a neutrophilic inflammatory process (11,13,194). It is hypothesised that the cycle of insults to the bronchial airways leads to increased neutrophil recruitment through the circulation and bronchial tissues using a complex mechanism of cytokines and their adhesion molecules. The pro-inflammatory cytokine release from this process causes airway goblet cell hypertrophy and increased mucus secretion which both traps bacteria and inflammatory products for efficient clearance and traps the same products to remain stationary in the airway to exert further insult.

Sputum inflammation and bacterial infection

In this study the sputum neutrophil count was mostly over 90% and was not affected by the sputum bacterial culture. However, the sputum neutrophil percentage has previously been shown to increase with bacterial infection and colonisation. Angrill et al. found that the value rose from 1% (range 0-4%) in healthy controls to 8% (range 0-93%) in non-colonised participants and 57% (range 0-98%) in colonised participants (195).

Association with asthma and ABPA

The sputum samples were predominantly neutrophilic, although 14 participants largely those with asthma or allergic bronchopulmonary aspergillosis demonstrated a raised sputum eosinophil count >3%. This is the result that would be expected given the underlying lung conditions.

3.3.7 Sputum microbiology

There were 47 sputum samples produced at the baseline visit. These yielded 53 outcomes such as ‘no significant growth’ or a bacterial growth. Thirty samples had

a positive culture result of which 6 grew multiple organisms. The most commonly cultured organism was *Haemophilus influenzae* which was identified on 16 occasions (30.2%) and in every sample which yielded a multiple bacterial culture. *Haemophilus influenzae* is the most prevalent organism cultured in non-cystic fibrosis bronchiectasis. In descending order the most commonly cultured organisms at the baseline visit were: *Haemophilus influenzae, Pseudomonas aeruginosa, Streptococcus pneumoniae, Moraxella catarrhalis, Staphylococcus aureus, Proteus species* and *Coliform species.* This is comparable to other studies using both culture methods and culture-independent methods.(17,134,150,196) One study found that the most common bacterial phylum in diseased lungs were those belonging to the *Proteobacteria* group which contains common pathogens such as the *Haemophilus*, *Escherichia, Moraxella* and *Pseudomonas* genera.(197) However, another suggested that the *Firmicutes* were more commonly found in the lungs of COPD subjects (19).

The sputum samples taken during this study were all analysed in the NHS laboratory of Glenfield Hospital. We know that the interpretation of the bacterial cultures can be subjective and dependent upon whether the underlying lung condition is known. The lab were aware that all the patients had bronchiectasis but not that the patient was on a clinical trial. The sputum culture samples that had been reported as not demonstrating any significant bacteria will have cultured bacteria, only the results have been interpreted by the lab technician as "normal respiratory flora". The concept of normal respiratory flora is increasingly questioned and the role of all bacteria in underling lung disease is being considered.

As described in the introduction chapter, research is conflicted around whether the lung microbiome is similar (derived from) or different to the oral microbiome, and whether the microbiome diversity remains or decreases in damaged lungs. The lung microbiome seems to be formed from aerobic, anaerobic, gram negative and gram positive bacteria. For example *Pseudomonas*, *Staphylococci, Moraxella* and *Streptococci* are aerobic, so will grow in the lung well where as *Klebsiella*,

Escherichia, *Haemophilus* and *Proteus* are facultative anaerobes so will live well in both aerobic and anaerobic conditions. *Haemophilus* is also a capnophile, meaning it prefers an environment of 15% oxygen and 5-10% carbon dioxide. This is similar environment to the lungs in chronic respiratory disease.

The results of this study were achieved through standard laboratory culture techniques and many detailed studies in this field involve culture-free methods such as 16s PCR techniques and 454 pyrosequencing. There are pros and cons for each investigation – the laboratory culture method will only allow for detection of a limited number of bacteria but the 16s PCR method with detect both live and dead bacterial DNA but be unable to differentiate between the two thereby overstating the number of bacteria detected. Research has shown that sputum samples will be representative of a small pocket of the lung which might explain the variation in repeated sputum results (19).

3.4 Conclusion

The chapter above has discussed all aspects of the baseline data in terms of demographic details, aetiology, lung function – both spirometry and small airway function – airway inflammation in terms of exhaled and nasal nitric oxide and sputum cellularity and characterisation of the lung microbiology.

The aim of the analysis of the data was to characterise the cohort in terms of the investigations listed above. The 68 participants were in middle age and the most common causes of the bronchiectasis were post-infectious and idiopathic. A higher proportion than previous seen in studies have an autoimmune condition.

The cohort on average had normal spirometry although there is a wide spread of results as expected. Interestingly all but two participants had an abnormal lung clearance index. The lung clearance index has been shown to correlate with FEV_1 (% predicted), the visual analogue scale scores and the sputum total cell count.

The vast majority of the cohort has a sputum neutrophilia but a proportion also have a raised sputum eosinophil count in addition. The most commonly cultured sputum bacterium was *Haemophilus influenzae* which was detected within a sample growing multiple bacteria more commonly than other organism. The participants who cultured Pseudomonas aeruginosa had a higher sputum colony forming unit count than those who cultured other pathogenic bacteria and those who cultured what was identified as "insignificant" bacteria. Unexpectedly there was no correlation between the colony forming unit count or the sputum neutrophil count. A third of the sputum samples did not reveal any significant growth on standard laboratory culture.

There were some limitations of the study and study population that should be mentioned. Firstly, as previously stated, the clinical cohort was chosen as they were not taking long-term antibiotics already. This might have led to a milder and less symptomatic population and subsequently this may have impacted the spirometry, multiple breath washout and microbiological results. However, the spectrum of radiological severity and clinical symptoms were wide and certainly some of the patients went on to have multiple exacerbations during the course of

the study. Around half of the chosen study population would have met the inclusion criteria for one of the macrolide trials, such as the BLESS study. From a microbiological point of view, we were unable to finance the use of quantitative PCR techniques to characterise the lung microbiome of the cohort. It should also be noted that the sputum cultures were carried out within a standard NHS hospital laboratory. Ideally, for qualitative bacterial culture as part of a study, two independent microbiologists would interpret the results and the statistical correlation between the two reporters would be calculated.

Chapter Four: The clinical trial of low-dose erythromycin in bronchiectasis patients

4.1 Introduction

This chapter describes the clinical trial of erythromycin in 40 non-cystic fibrosis bronchiectasis patients. It involved 7 stable visits at 12-weekly intervals. The first year (visits 1 to 4) was observational in nature and the information collected formed a control data set against which the erythromycin intervention data was compared. The interventional part involved participants taking erythromycin 250mg daily for 12 weeks between visits 5 and 6. Following this the participants were observed for a further 12 weeks and the trial ended at visit 7. Along with the stable visits the participants were also seen at the time of an exacerbation for clinical assessment, antibiotic therapy and further data collection. The exacerbation data is presented in the next chapter.

The following hypotheses and objectives have formed the structure of this chapter. The data from the clinical trial is presented briefly first followed by the discussion of the first hypothesis. The subsequent hypothesis and objectives then follow.

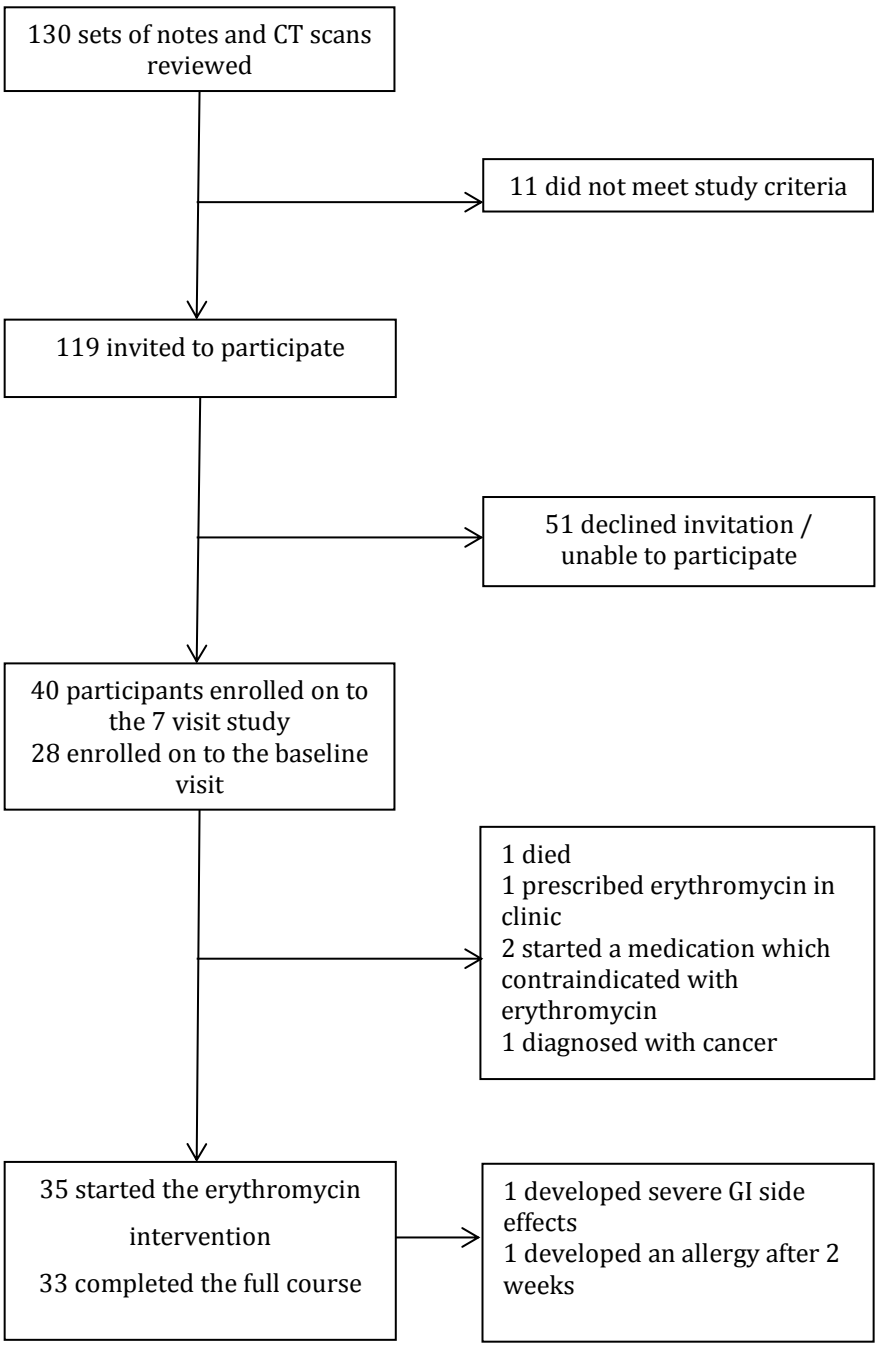
Primary aim

- *We anticipate an improvement in the FEV_1 of at least 200ml following a 3 month course of erythromycin at 250mg once a day.*

Secondary objectives

- *We hypothesize that the study participants who demonstrate the best response to erythromycin 250mg daily for 12 weeks will have neutrophilic airway inflammation and demonstrate small airways disease in the form of an increased lung clearance index on multiple breath washout testing and tree in bud changes on the CT scan.*
- *To evaluate whether the response to 12 weeks erythromycin will be sustained after the course is completed.*

4.2 Study journey



4.3 Results from the whole trial

4.3.1 Demographics

The demographic data presented here is from the 40 trial participants rather than the 68 participants recruited for baseline data. As expected the results are similar to those discussed in the demographics chapter and so have not been reiterated in detail.

The trial participants had a female predominance (55%) with a mean age of 67 years (sd. 7y). The mean body mass index of the group was in the overweight category at 27.5 kg/m2. The majority of the patients had never smoked (57.5%) and those who had smoked had a median pack year history of 22.4. Only one patient was a current smoker. Seven participants (17.5%) had a historical exposure to asbestos and 15 (37.5%) of the cohort were current pet owners.

Figure 16: A summary of the demographic data for the 40 patient erythromycin cohort

Demographic information		Value
Age, mean (sd)		67 (7)
BMI, mean (sd)		27.5 (4.9)
Age of onset of bronchiectasis symptoms (median, IQR)		27 (5-61)
Sex, n (%)	**Female**	22 (55)
	Male	18 (45)
Smoking history, n (%), mean pack years#	**Never smoked**	23 (57.5) 0#
	Ex-smoker	16 (40.0) 22.4#
	Current smoker	1 (5.0) 51.0#
Asbestos exposure, n (%)		7 (17.5)
Current pet ownership, n (%)		15 (37.5)

4.3.2 Aetiology and associated conditions

Half of the participants had a self-reported history of recurrent gastro-oesophageal reflux disease requiring either prescribed or over the counter medications. The most common aetiologies were childhood whooping cough and pneumonia accounting for 80% of the study participants. However previous TB was uncommon affecting only 2 participants. The most common associated conditions were recurrent otitis media (32.5%) and recurrent sinusitis (35.0%). After this, 27.5% had a history of a pre-study physician diagnosis of asthma 10% a pre-study diagnosis of ABPA. Twenty percent had a diagnosis of an autoimmune disease. This group included the 10% who had a diagnosis of inflammatory arthritis and 5% with a diagnosis of inflammatory bowel disease.

Figure 17: Summarising possible aetiology and associated conditions

	Condition	n (%)
Aetiology	**Whooping cough***	19 (47.5)
	Childhood pneumonia*	13 (32.5)
	Allergic bronchopulmonary aspergillosis#	4 (10.0)
	Previous TB*	2 (5.0)
Associated conditions	**Gastro-oesophageal reflux disease***	20 (50.0)
	Recurrent sinusitis*	14 (35.0)
	Recurrent otitis media*	13 (32.5)
	Asthma#	11 (27.5)
	Autoimmune disease#	8 (20.0)
	Inflammatory arthritis#	4 (10.0)
	Inflammatory bowel disease#	0 (5.0)

*Self-reported condition, #Pre-study physician diagnosis

4.3.3 Inhaled and oral steroid use

Over half (55%) of the 40 study participants were prescribed inhaled steroids with a median beclomethasone diproprionate (BDP) equivalent dose of 1200mcg. Four (10%) of the group were taking long term oral prednisolone with a median dose of 5mg (IQR 3.8-5.0mg).

Figure 18: The inhaled and oral steroids use of the 40 patient cohort

Steroid use	Result
Inhaled steroid use, n (%)	22 (55.0)
Beclomethasone Diproprionate equivalent dose (mcg), median (IQR)	1200 (800-1600)
Oral steroid use, n (%)	4 (10.0)
Oral steroid dose (mg), median (IQR)	5 (3.8-5.0)

4.3.4 Pre- and post-erythromycin data

The table below demonstrates the pre- and post-erythromycin data from visits 5 and 6. There were significant improvements in the Leicester cough questionnaire score, the visual analogue scale scores for dyspnoea and sputum production, the post-bronchodilator FEV_1 (% predicted, not absolute value) and lung clearance index.

The data is presented in more detail throughout the chapter in order to address the study hypotheses.

Table 16: The study investigations pre- and post-erythromycin

		Pre-erythromycin	Post-erythromycin	p-value
St Georges Respiratory Questionnaire total score, mean (sd)		33.38 (16.59)	30.38 (16.10)	0.106
Leicester Cough Questionnaire total score, mean (sd)		16.15 (3.10)	17.25 (3.33)	**0.010***
Visual Analogue Scale cough domain, median (IQR)		33 (18-57)	29 (11-52)	0.071
Visual Analogue Scale dyspnoea domain, median (IQR)		26 (13-57)	28 (9-46)	**0.044***
Visual Analogue Scale sputum production domain, median (IQR)		54 (19-66)	32 (11-58)	**0.016***
Visual Analogue Scale sputum purulence domain, median (IQR)		28 (10-61)	27 (7-54)	0.245
Exhaled nitric oxide (ppb), mean (sd)		27.3 (15.1)	26.4 (10.5)	0.517
Post-bronchodilator FEV_1 (l), mean (sd)		2.03 (0.65)	2.08 (0.66)	0.055
Post-bronchodilator FEV_1 (%), mean (sd)		85.5 (24.6)	87.7 (24.8)	**0.044***
Post-bronchodilator FVC (l), mean (sd)		2.86 (0.66)	2.89 (0.65)	0.385
Post-bronchodilator FVC (%), mean (sd)		97.7 (21.1)	98.7 (20.8)	0.427
Lung clearance index, mean (sd)		9.956 (2.226)	9.049 (1.844)	**0.038***
Sputum total cell count (10^6/ml), median (IQR)		6.82 (2.21-20.67)	8.24 (0.76-26.70)	0.775
Sputum neutrophils (%), median (IQR)		85.00 (74.75-96.25)	89.75 (72.50-97.0)	0.629
Sputum eosinophils (%), median (IQR)		1.25 (0.25-4.25)	1.25 (0.25-5.5)	0.180
Sputum absolute neutrophil count (10^6/ml), median (IQR)		5.881 (1.549-20.412)	6.739 (0.318-24.533)	0.885
Sputum absolute eosinophil count (10^6/ml), median (IQR)		0.106 (0.005-0.315)	0.040 (0.005-0.235)	0.808
Sputum bacterial culture (n)	**No significant growth**	7	13	
	Pseudomonas aeruginosa	3	2	
	Non-*Pseudomonas aeruginosa* bacteria	14	8	
	Insufficient sample	11	12	
	Not tested	0	0	
	Positive culture samples	17/24 (70.8%)	10/23 (43.5%)	
Sputum colony forming units (10^6/ml), median (IQR)		0.800 (0.234-12.666)	1.595 (0.300-13.400)	0.546
C-reactive protein, median (IQR)		5 (5-10)	5 (5-11)	0.574
QT_c interval, mean (sd)		409 (12)	408 (12)	0.821

4.4 Primary Aim

We anticipate an improvement in the FEV_1 of at least 200ml following a 3 month course of erythromycin at 250mg once a day.

The data has been analysed to answer this question.

4.4.1 Results

The change (delta) in the post-bronchodilator spirometry between visit 1 (baseline) and visit 5 (pre-erythromycin), visit 5 and visit 6 (post-erythromycin) then visit 6 and visit 7 (end of study) has been presented in the table below. This demonstrates little change in both the raw values and the percentage of predicted values. The FEV_1 fell by 30ml on average between visits 1 and 5, improved by 50ml during the erythromycin phase and fell again by 30ml in the 3 months between completing the erythromycin and completing the study. When looking at the change in terms of percentage of the predicted value the FEV_1 improved by 0.8% over the first year, improved by 2.2% during the 3 month erythromycin trial and fell again by 1.2% after completing of the erythromycin. The post-bronchodilator FVC values also changed by a similar amount.

Table 17: The absolute difference (delta) in spirometry values during the stable phase, erythromycin phase and post-erythromycin period

	Visit 1-Visit 5	Visit 5-Visit 6	Visit 6-Visit 7
Post-bronchodilator FEV_1 (l)	-0.03 (0.220)	0.05	-0.03
Post-bronchodilator FEV_1 (% predicted)	0.8	2.2	-1.2
Post-bronchodilator FVC (l)	-0.05	0.03	-0.02
Post-bronchodilator FVC (%)	0.9	1.0	0.7
Post-bronchodilator FEV_1/FVC ratio (%)	-0.1	1.2	-0.7

There were 12 participants who had an improvement in FEV_1 between V5 and V6. The range in improvement was 20ml to 300ml. There was only one person with an improvement in FEV_1 >200ml following erythromycin. Three people did not have any change in their FEV_1 following erythromycin treatment and there were 20 who sustained a fall in FEV_1 during this 12 week period, ranging from 40ml to 350ml. The graph below presents the spread of delta FEV_1 between the pre- and post-erythromycin visit. The mean of the delta FEV_1 was 0ml (sd. 0.22l).

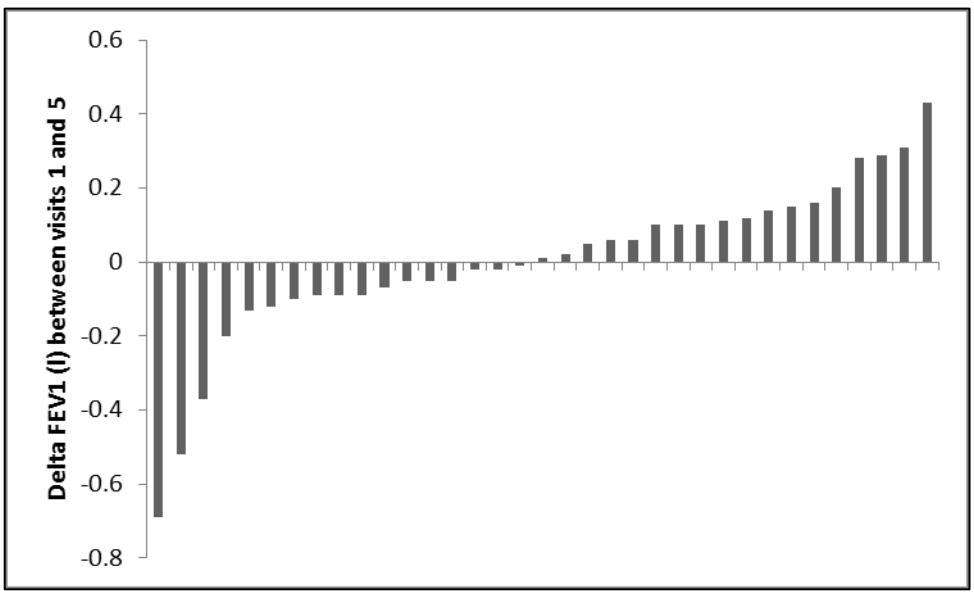


Figure 19: Delta FEV_1 (l) between the pre- and post-erythromycin visits

The original sample size calculation required 34 participants (40 recruited to account for drop outs) to demonstrate an improvement of 200ml in the post-bronchodilator FEV_1 using a 5% significance level and a power of 10%. The sample size required was then recalculated at the end of the observational year using the observed standard deviation. The sample size required to achieve a statistically meaningful change in the FEV_1 of 200ml is 25.4 participants. This means that there were sufficient participants in the sample for the results, although negative, to be statistically significant.

For comparison purposes below is a graph for the delta FEV_1 between the first visit and the pre-erythromycin visit. This clearly demonstrates variation in FEV_1 over time. It could be suggested that the inherent variation in FEV_1 between individuals even without intervention make it an unsuitable measure for the end-point of a non-cystic fibrosis bronchiectasis clinical trial.

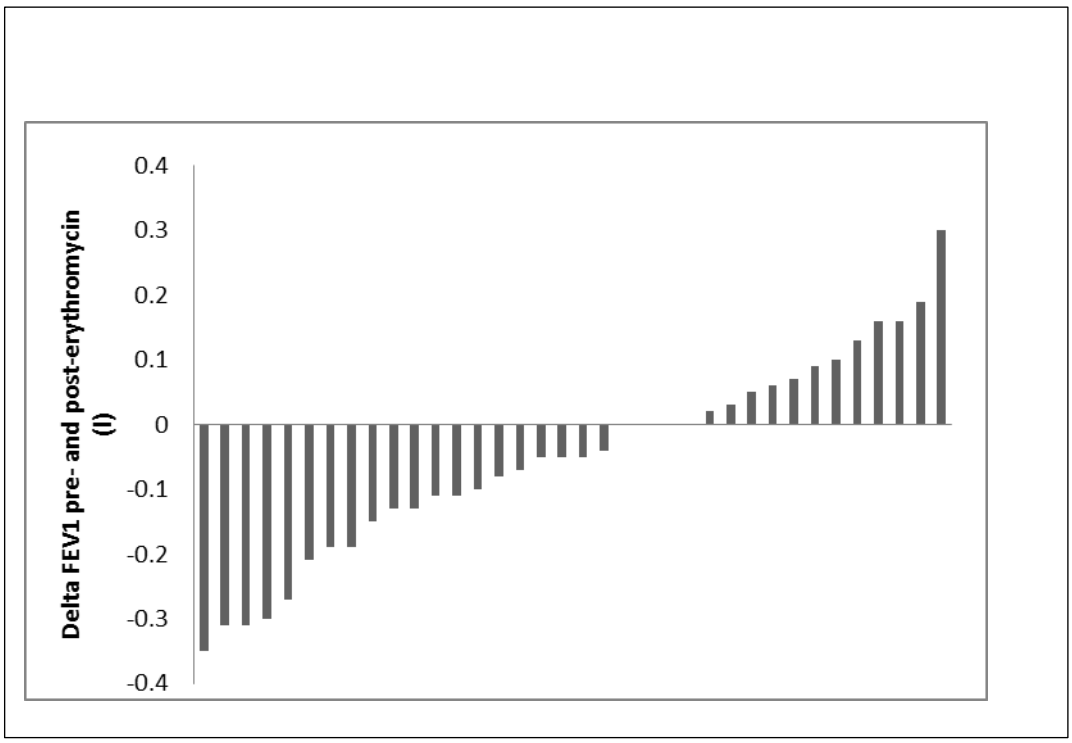
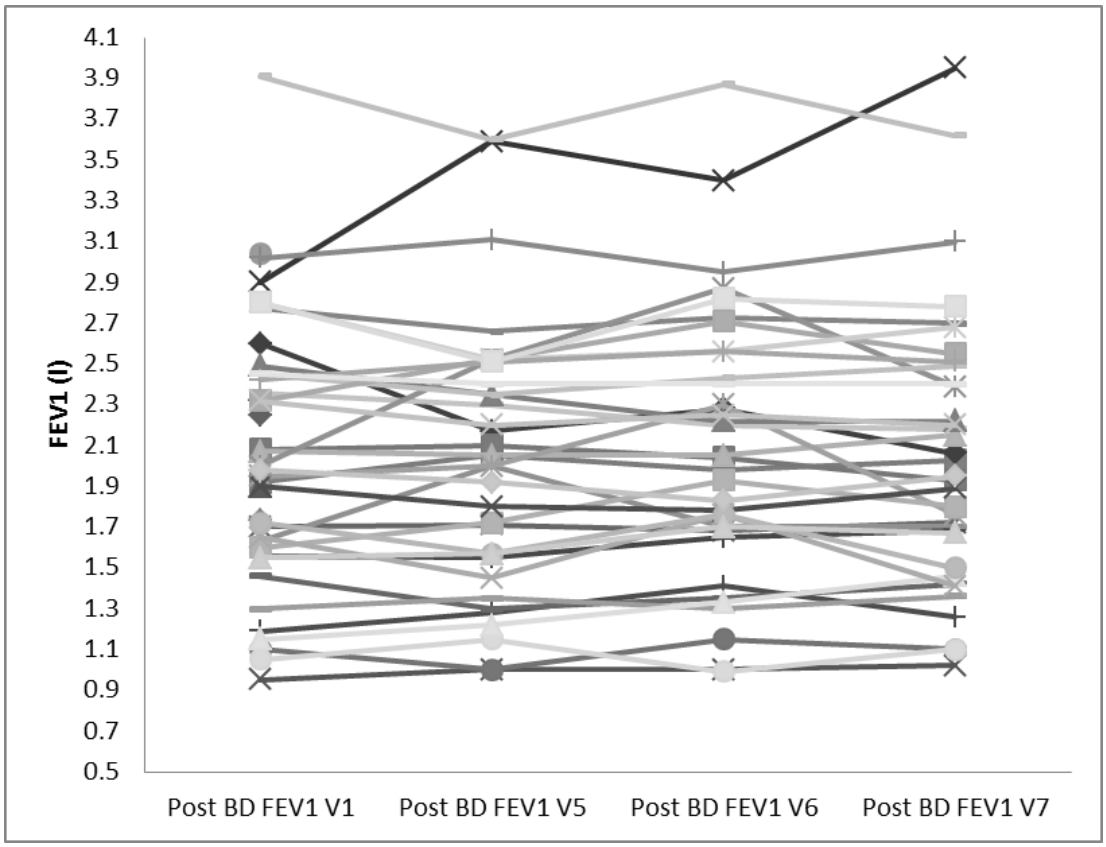


Figure 20: Delta FEV_1 (l) between visits 1 and 5

The graph below demonstrates the change in FEV_1 over the 7 study visits. Again, it can be clearly seen that there is a large variation in the FEV_1 at each point in the study. There is no consistent trend in the spread of the data.

Figure 21: The variation in FEV_1 over the 7 visit study period



Baseline characteristics of delta FEV_1 >200ml and delta FEV_1 <200ml

There was only one participant who had more than a 200ml improvement in FEV_1 between visits 5 and 6, meaning that statistical analysis between the two groups is not possible. The table is in the "Appendix – results tables" section.

4.5 Discussion

The pre- and post-erythromycin data demonstrated a statistically significant improvement in the FEV_1 (% predicted), Leicester cough questionnaire scores, visual analogue scale scores for the dyspnoea and sputum production domains and the lung clearance index. There was not a significant improvement in the absolute FEV_1 value. This was a non-blinded interventional trial with an observational year where each participant became their own control group. This design was chosen partly to collect data on the variation in bronchiectasis symptoms and investigations such as lung function and microbiology as this has never been published. There were also cost implications of using a placebo tablet which could not be overcome. Shortly after starting the recruitment process the large, well designed BLESS trial was published which involved either low-dose erythromycin or placebo in non-cystic fibrosis bronchiectasis patients.

The primary aim stated that a 12 week trial of 250mg daily erythromycin would result in a 200ml improvement in the FEV_1 was rejected. The mean improvement in the FEV_1 during this period was 50ml. The primary aim of the study was devised from anecdotal observational data from patients seen in clinical practice over many years who had been given a trial of lose dose erythromycin for a variety of clinical reasons. The patients (see Study Design section) came from a clinic that had a predominance of asthma patients. Whilst the low-dose erythromycin was given for a variety of reasons the large mean improvement of over 350ml seen at this time could have been in part due to the reduction in the inflammation in asthmatic patients. Low-dose macrolides have previously been found to be beneficial in neutrophilic asthma (132). It has already been discussed that due to the fact these particular study patients were not already taking low-dose antibiotics that they were situated towards the milder end of the bronchiectasis spectrum. In actual fact the severity of the patients recruited demonstrated a wide variation similar to a more typical clinic population however, during the course of the study the Bronchiectasis Severity Index (154) was published and it would have been informative to have scored each patient against the system. There were a

significant proportion who had demonstrated a large improvement in terms of both symptom resolution and change in FEV_1. Unfortunately such a positive response could not be replicated in this clinical trial.

The delta FEV_1 in this study was a 30ml fall between visits 1 and 5, a 50ml improvement between visits 5 and 6 and a 30ml fall between visits 6 and 7. The delta FEV_1 ranges from -700ml to +400ml between visits 1 and 5 and the standard deviation was 220ml. The large variations essentially result in a minimal net change meaning that when the group as a whole is analysed there is no significant difference in the FEV_1 over that period. The reasons behind why FEV_1 changes so much for some and not others without any intervention is not clear but this makes it unsuitable as an end point for a study. There are no longitudinal trials observing FEV_1 in non-CF bronchiectasis which take into individual rather than mean changes in FEV_1 over relatively close time periods. Most trials report that start and end data without mention of any fluctuation in between these time points. One longitudinal study of bronchiectasis patients found a mean decline in FEV_1 of 48.8ml/year, which was greater in men than women (198). Another found there to be a mean decline of 52.7ml/year.(199) Bacterial colonisation (200), especially with *Pseudomonas aeruginosa* (199), inflammation (199,201) and increased sputum production (198) have been shown to be associated with more rapid decline in FEV_1 in bronchiectasis patients. However, many studies present baseline data which shows that patients with *Pseudomonas aeruginosa* are statistically more likely to have reduced lung function compared to non-colonised participants (20) but not an accelerated decline in lung function (202).

For the few bronchiectasis drug trials that use FEV_1 as the primary end point the mean delta FEV_1 ranges from 10ml over 12 months to 160ml over 8 weeks (113,150). It is more usual for change in FEV_1 to be a secondary end point. These trials have reported a reduction in FEV_1 of 1.67% compared to 4.0% in the placebo group over 12 months (175) and an improvement in FEV1 of 1.03% over 3 months in the intervention group compared to a decline of 0.10% in the placebo group (134).

There was a single participant who had >200ml improvement in the FEV_1 following the course of erythromycin. The immediate pre-erythromycin investigations were reviewed. The validity of the differences seen in the results could not be statistically proven due to there being a single person in the comparison group. However there was a striking difference in the sputum bacterial colony forming unit numbers ($125x10^6$/ml bacteria) in the participant with the apparent improvement in FEV_1 post-erythromycin and a median value of $0.700x10^6$/ml for the rest of the group with a wide interquartile range. Interestingly this participant could not produce another induced sputum sample to send for bacterial culture on this visit so it is impossible to know whether they would have cultured any particular bacteria to account for the high bacterial load. The total sputum cell count was high in this particular participant but there were several participants at that visit who had similarly elevated total sputum cell counts and did not have such a change in FEV_1 following erythromycin therapy.

On closer review of the data this participant had seen a 370ml fall in their FEV_1 between visits 4 and 5 despite being well at the time. Unfortunately it should be concluded that the big improvement in the FEV_1 following erythromycin therapy was simply a regression back towards their usual lung function and not a result of the trial intervention.

4.6 Conclusion

The hypothesis stated that the 12 week course of erythromycin would bring an improvement to the FEV_1 of 200ml. This was not supported as there was very minimal mean improvement in the FEV_1. There was a high degree of variation in FEV_1 similarly in the observation year and the intervention period with roughly half of the participants demonstrating an improvement and half demonstrating a decline during either of these time periods. For this reason I believe that we cannot draw any conclusions from the spirometry results between two time points in bronchiectasis patients. The factors behind the apparent improvement or decline in some participants both with and without any intervention need to be researched in further detail.

Only one participant was found to have an improvement in FEV_1 greater than 200ml between the pre- and post-erythromycin visits. On closer analysis this participant had a fall in FEV_1 between visits 4 and 5 despite them being clinically well and so the apparent improvement was likely to be the lung function returning to the usual values. Therefore the apparent improvement seen post-erythromycin was unlikely to have been due to a response to the drug.

4.7 The clinical trial

The following section outlines the changes in the investigations both during the observation year, the erythromycin intervention period and the final observation period. The remaining objectives are discussed in detail.

4.7.1 Questionnaires

The data table for the questionnaire scores over the study period is below.

St George's Respiratory Questionnaire

The table below demonstrates the mean total score for the SGRQ started at 37.40 and fell consistently during the study to 33.38 (sd. 16.59) indicating fewer symptoms. There was no significant difference between the scores from the pre- and post-erythromycin visits. The data between visit 1 and visit 5 demonstrates similarity as assessed using Cronbach's alpha statistics. Between visits 6 and 7 the SGRQ total score remained static (30.38 and 30.03) and were found to have good statistical similarity.

Leicester Cough Questionnaire

The mean total score for the LCQ improved during the study and reached a peak at visit 6, the post-erythromycin visit. The score fell slightly by the final visit but was still higher, suggesting fewer symptoms, at the final visit. There was good statistical similarity between the visits. There was a statistically significant improvement (p=0.010) in the score following the erythromycin therapy when it climbed from 16.15 (sd. 3.10) to 17.25 (sd. 3.33). There was a non-significant drop in the scores after the completion of the erythromycin.

Visual Analogue Scale

The mean score for the cough domain of the visual analogue scale was 30mm (IQR 16-50mm) at the baseline visit, rising to 33mm (IQR 18-57mm) at the pre-erythromycin visit and then falling to 29mm and 27mm at the post-erythromycin and final visits respectively. Again, the Cronbach's alpha score was greater than 0.7 suggesting good similarity between stable visits. The erythromycin intervention period saw a non-significant fall in the score from 33mm to 29mm (IQR 11-52mm).

The dyspnoea domain of the VAS remained stable during all the visits with good statistical reliability between stable visits. Interestingly, there was a statistically significant increase in the score from 26mm (IQR 13-57mm) to 28mm (IQR 9-46mm) during the erythromycin intervention, suggesting a worsening in the reported breathlessness.

The sputum production domain median score started at 47mm (IQR 19-64mm) at the baseline visit and rose to 54mm (IQR 19-66mm) at the pre-erythromycin visit. During the erythromycin intervention the score improved significantly from 54mm (IQR 19-66mm) to 32mm (IQR 11-58mm) (p=0.016). The score fell again to 30mm at the final visit. Again, the stable visits demonstrated statistical reliability.

Finally, the sputum purulence domain demonstrated a steady improvement between visits 1 and 5 with a 20 point reduction in the median score. However, despite the large drop in the score these values were statistically similar (alpha >0.7). The erythromycin intervention did not cause a significant change in the sputum purulence scores, which improved marginally from 28mm (IQR 10-61mm) to 27mm (IQR 7-54mm). The final score was 19mm and was found to be statistically significant to the previous visit.

Table 18: Questionnaire data throughout the study period

		Visit 1	Visit 5	Visit 6	Visit 7
St Georges Respiratory Questionnaire	**Total score~**	37.40 (16.89)	33.38 (16.59)	30.38 (16.10)	30.03 (16.23)
	Δ		-4.02	-3.00	-0.35
	***p*-value**		0.106		
	Cronbach's α	0.940		0.910	
Leicester Cough Questionnaire	**Total score~**	15.20 (4.03)	16.15 (3.10)	17.25 (3.33)	16.86 (3.21)
	Δ		0.95	1.10	-0.39
	***p*-value**		**0.010***		
	Cronbach's α	0.860		0.876	
Visual Analogue Scale	**Cough#**	30 (16-50)	33 (18-57)	29 (11-52)	27 (11-50)
	Δ		3	-4	-2
	***p*-value**		0.071		
	Cronbach's α	0.658		0.809	
Visual Analogue Scale	**Dyspnoea#**	25 (4-40)	26 (13-57)	28 (9-46)	26 (10-44)
	Δ		1	2	-2
	***p*-value**		**0.044***		
	Cronbach's α	0.769		0.738	
Visual Analogue Scale	**Sputum production#**	47 (19-64)	54 (19-66)	32 (11-58)	30 (4-50)
	Δ		7	-22	-2
	***p*-value**		**0.016***		
	Cronbach's α	0.836		0.885	
Visual Analogue Scale	**Sputum purulence#**	48 (10-54)	28 (10-61)	27 (7-54)	19 (5-46)
	Δ		-20	-1	-8
	***p*-value**		0.245		
	Cronbach's α	0.783		0.906	

~Mean (sd), #Median (IQR), *statistical significance

4.7.2 Spirometry and multiple breath washout investigations

Overall the spirometry for the 40 patients was within the normal range. The mean FEV_1 was 2.06l/min (sd. 0.64l/min) or 84.7% predicted (sd. 24.1%). The mean FEV_1/FVC ratio was 70.2% (sd. 11.8%). There was very little variation in the mean FEV_1 over the study period and the spirometry data is described in more detail as part of the discussion for the first hypothesis. The observational year saw a reduction of 30ml in the mean FEV_1, there was a modest improvement in the mean FEV_1 between visits 5 and 6 of 50ml. There was a significant change in the mean FEV1 % predicted value between visits 5 and 6. However, none of the other spirometric values showed any significant change. Following the completion of the

erythromycin course there was a fall in the mean post-bronchodilator FEV_1 value of 30mls, which was proven to have statistically similarity.

Small airway investigations

The mean lung clearance index was 9.794 (sd. 1.830) at the baseline visit rising to 10.493 (sd. 1.987) at the pre-erythromycin visit and then falling to its lowest value of 9.206 (sd. 1.895) at the post-erythromycin visit (p=0.038). The final visit had a mean value which was slightly higher at 9.838 (sd. 2.180). There was statistical similarity between the baseline and pre-erythromycin visits and the post-erythromycin and final visits and a significant improvement in the lung clearance index following 12 weeks of erythromycin.

Table 19: Spirometry and small airway tests throughout the study period

		Visit 1	Visit 5	Visit 6	Visit 7
Post-bronchodilator FEV_1 (l), mean (sd)		2.06 (0.64)	2.03 (0.65)	2.08 (0.66)	2.05 (0.68)
	Δ		-0.03	0.05	-0.03
	***p*-value**		0.055		
	Cronbach's α	0.972		0.978	
Post-bronchodilator FEV_1 (%), mean (sd)		84.7 (24.1)	85.5 (24.6)	87.7 (24.8)	86.5 (25.4)
	Δ		0.8	2.2	-1.2
	***p*-value**		**0.044***		
	Cronbach's α	0.969		0.976	
Post-bronchodilator FVC (l), mean (sd)		2.91 (0.64)	2.86 (0.66)	2.89 (0.65)	2.87 (0.68)
	Δ		-0.05	0.03	-0.02
	***p*-value**		0.385		
	Cronbach's α	0.965		0.972	
Post-bronchodilator FVC (%), mean (sd)		96.8 (20.7)	97.7 (21.1)	98.7 (20.8)	99.4 (21.1)
	Δ		0.9	1.0	0.7
	***p*-value**		0.427		
	Cronbach's α	0.963		0.938	
Lung clearance index, mean (sd)		9.794 (1.830)	10.493 (1.987)	9.206 (1.895)	9.838 (2.180)
	Δ		0.699	-1.287	0.632
	***p*-value**		**0.038***		
	Cronbach's α	0.968		0.907	

4.7.3 Airway inflammation investigations

The table below outlines the exhaled nitric oxide values and sputum cell differential counts for the 40 trial participants.

Exhaled nitric oxide levels

The mean exhaled nitric oxide level was 24.6ppb (sd. 12.3ppb) and values remained stable over the 7 visits (alpha >0.7). The mean pre-erythromycin value at

visit 5 was 27.3ppb (sd. 15.1ppb) and the post-erythromycin value was 26.4ppb (sd. 10.5), a non-significant reduction (p=0.517). At the final visit the mean value was 24.9ppb (sd. 10.9ppb) which was similar to the result from visit 6.

Induced sputum differential cell counts

The median total cell count had a mean value of $6.93x10^6$ (IQR $3.16\text{-}14.8x10^6$) with a median neutrophil proportion of 91%. Throughout the study the samples were predominantly neutrophilic with values ranging from 85.0% to 93.75%. The sputum samples at visit 5 (pre-erythromycin) were the least neutrophilic. The median total sputum cell count reached its highest value at the post-erythromycin visit ($8.24x10^6$/ml). There was reasonable statistical similarity between the stable visits but no significant difference between the pre- and post-erythromycin visits. This can be seen across all the sputum cell domains. The median percentage eosinophil value was within the normal range at all the visits although a proportion of participants did demonstrate an eosinophil count >3%.

Table 20: Airway and sputum inflammation data throughout the study period

		Visit 1	Visit 5	Visit 6	Visit 7
Exhaled nitric oxide (ppb), mean (sd)	**Mean (sd)**	24.6 (12.3)	27.3 (15.1)	26.4 (10.5)	24.9 (10.9)
	Δ		2.7	-0.9	-1.5
	***p*-value**		0.517		
	Cronbach's α	0.750		0.716	
Total cell count (10^6/ml), median (IQR)	**Median (IQR)**	6.93 (3.16-14.8)	6.82 (2.21-20.67)	8.24 (0.76-26.70)	6.54 (1.030-22.600)
	Δ		-0.11	1.42	-1.7
	***p*-value**		0.775		
	Cronbach's α	0.874		0.727	
Neutrophils (%), median (IQR)	**Median (IQR)**	91.0 (70.28-96.53)	85.00 (74.75-96.25)	89.75 (72.50-97.0)	93.75 (79.25-96.75)
	Δ		-6.0	4.75	4.0
	***p*-value**		0.629		
	Cronbach's α	0.616		0.768	
Eosinophils (%), median (IQR)	**Median (IQR)**	1.33 (0.5-3.38)	1.25 (0.25-4.25)	1.25 (0.25-5.5)	1.25 (0.50-6.00)
	Δ		-0.08	0	0
	***p*-value**		0.180		
	Cronbach's α	0.403		0.637	
Neutrophil count (10^6/ml), median (IQR)	**Median (IQR)**	6.515 (2.318-12.815)	5.881 (1.549-20.412)	6.739 (0.318-24.533)	5.886 (0.776-21.812)
	Δ		-0.634	0.858	-0.853
	***p*-value**		0.885		
	Cronbach's α	0.893		0.667	
Eosinophil count (10^6/ml), median (IQR)	**Median (IQR)**	0.068 (0.024-0.246)	0.106 (0.005-0.315)	0.040 (0.005-0.235)	0.094 (0.014-0.339)
	Δ		0.038	-0.066	0.054
	***p*-value**		0.808		
	Cronbach's α	-0.036		0.852	

4.7.4 Microbiology

During the study samples were sent for bacterial culture, colony forming unit analysis and fungal culture. The fungal data is described in a later chapter. The proportion of culture-positive sputum samples ranged from 63.3% to 70.8% during all the study visits. Interestingly the lowest percentage of 43.5% was seen at the post-erythromycin visit, compared to a pre-erythromycin proportion of 70.8%. There was no major change with the number of individual species.

The table below demonstrates the number of sputum samples obtained, the number of sputum "outcomes" such as a bacterial growth or no significant growth, from those samples and the total number of culture-positive samples at that visit.

The most commonly cultured bacteria were *Haemophilus influenzae,* followed by *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*. Between the pre- and post-erythromycin visits there was an increase in the number of samples without any significant growth from 7 (29.2%) to 13 (56.5%), with a subsequent reduction in the number of samples culturing either *Staphylococcus aureus* or *Coliform* species. The number of culture-negative samples had returned to the pre-erythromycin level by the final visit.

The median colony forming unit count steadily climbed during the study reaching the highest value of 2.170 $x10^6$/ml (IQR 0.320-5.340) at visit 7. The counts were neither statistically similar nor different between the visits.

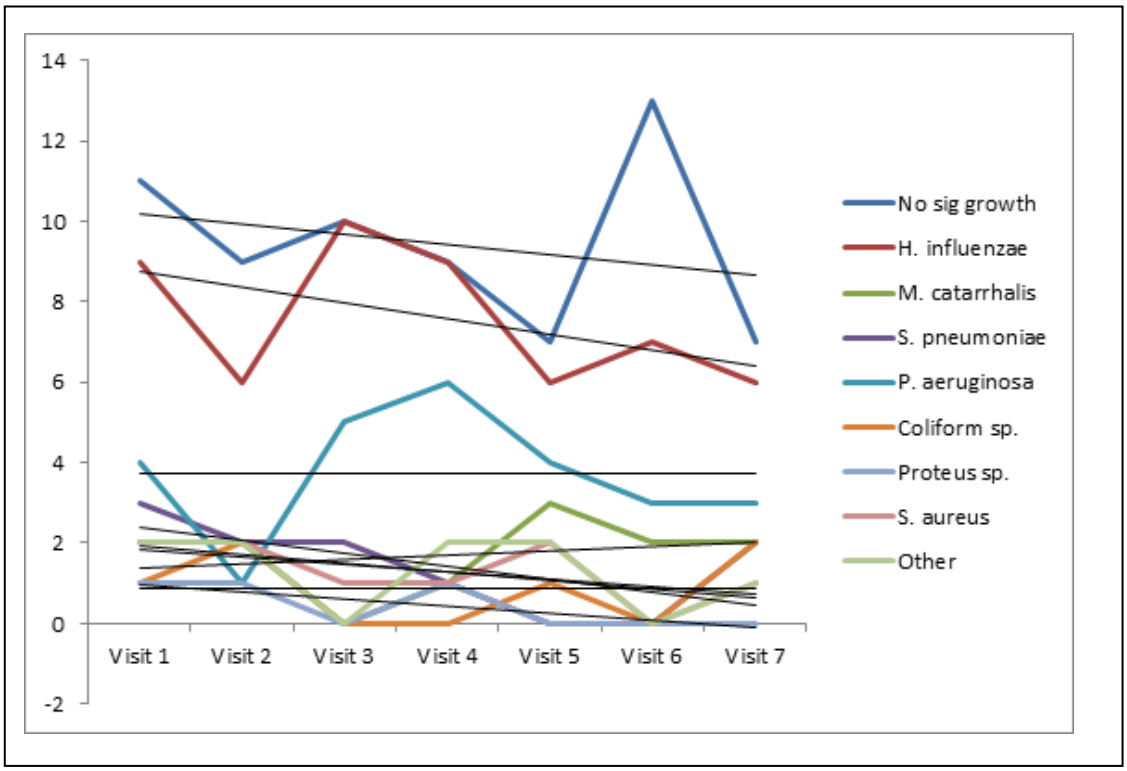
The Bray-Curtis Index for species similarity was calculated and the values were all close to zero indicating that there was little change in the bacterial diversity, despite a 12-week intervention of an antibiotic and a reduction in the number of culture-positive samples.

Table 21: Microbiology data throughout the study period

		Visit 1	Visit 5	Visit 6	Visit 7
No. samples		30	24	23	22
No. positive samples		19 (63.3)	17 (70.8)	10 (43.5)	15 (68.2)
No. positive cultures		23	18	12	17
No. participants		40	35	35	35
Sputum bacterial culture, n (%)	**No significant growth**	11 (36.6)	7 (29.2)	13 (56.5)	7 (31.8)
	Haemophilus influenzae	9 (30.0)	6 (25.0)	6 (26.1)	6 (27.3)
	Moraxella catarrhalis	2 (6.7)	3 (12.5)	2 (8.7)	2 (9.1)
	Streptococcus pneumoniae	3 (10.0)	0	0	2 (9.1)
	Pseudomonas aeruginosa	3 (10.0)	4 (16.7)	3 (13.0)	3 (13.6)
	***Coliform* species**	1 (3.3)	1 (4.2)	0	2 (9.1)
	***Proteus* species**	1 (3.3)	0	0	0
	Staphylococcus aureus	2 (6.7)	2 (8.3)	1 (4.3)	1 (4.5)
	Other	2 (6.7)	2 (8.3)	0	1 (4.5)
	Insufficient sample	10 (25.0)	11 (31.4)	12 (34.3)	13 (37.1)
Bray-Curtis Index of similarity		0.153	0.280		0.265
Sputum colony forming units (10^6/ml), median (IQR)		0.770 (0.3-2.6)	0.800 (0.234-12.666)	1.595 (0.300-13.400)	2.170 (0.320-5.340)
α		0.101		-0.143	
p-value		0.546			

The following graph demonstrates the changes in the bacterial species cultured over the course of the study. It clearly demonstrates the spike in the number of culture-negative samples at visit 6 following the completion of the erythromycin course.

Figure 22: The changes in sputum bacterial culture over the trial period



4.7.5 CT scoring

Each participant underwent a research protocol CT scan at visit 4. The CT images were scored according to the Robert's scoring system and the results listed in the table below. The highest possible score was 112. The mean total score was 35.76/112 (sd. 15.49) with the greatest degree of bronchiectasis in the lower lobes, where the median score was 8/18.

The association with the bronchiectasis severity according to the CT score and various investigations including microbiology cultures have been described below.

Table 22: The CT scoring by categories and lobes

CT score domain	Mean score (sd)
Total CT score	35.76 (15.49)
Bronchial wall dilatation	7.37 (4.68)
Bronchial wall thickening	4.89 (3.63)
Extent of bronchiectasis	8.95 (3.56)
Mucus plugging (large airways)	2.08 (1.85)
Mucus plugging (small airways)	2.87 (2.34)
Attenuation	6.11 (4.60)
Bronchial collapse on expiration	3.39 (4.27)
Tracheal collapse on expiration	0.08 (0.27)
Right upper lobe	4.71 (3.07)
Right middle lobe	6.09 (3.01)
Right lower lobe	7.87 (3.26)
Left upper lobe	3.39 (2.93)
Lingula	5.89 (3.44)
Left lower lobe	8.21 (3.09)

Associations and correlation

The CT scores were plotted against various investigations to look for correlations and associations. Only the statistically significant correlations have been described. There was a significant negative correlation between the total CT score and the FEV_1 (% predicted), suggesting an association between increasing bronchiectasis severity and large airway function. There was a significant positive correlation between the lung clearance index and the total CT score suggesting an association between increasing bronchiectasis severity and worsening small airway function. The visual analogue scale scores all demonstrated a significant, positive correlation with the CT scores suggesting that worsening symptoms of cough, breathlessness, sputum production and purulence are reflected by increasing bronchiectasis severity on CT imaging. Finally, there was a significant, positive correlation between the sputum neutrophil count (% predicted) and the CT scores without any correlation with the total cell count or bacterial colony forming units.

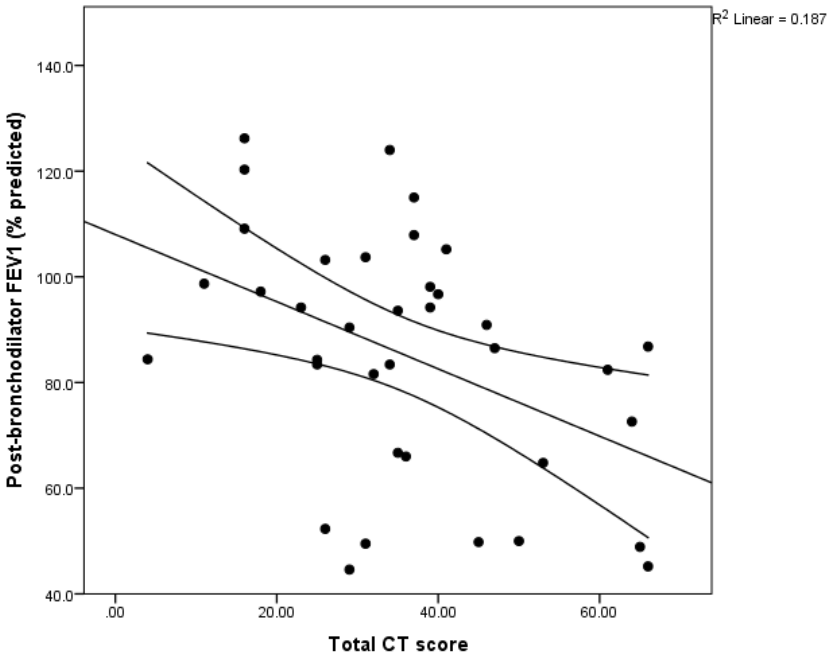


Figure 23: Scatter plot demonstrating the correlation between the total CT score and the FEV_1 (% predicted), p=0.007

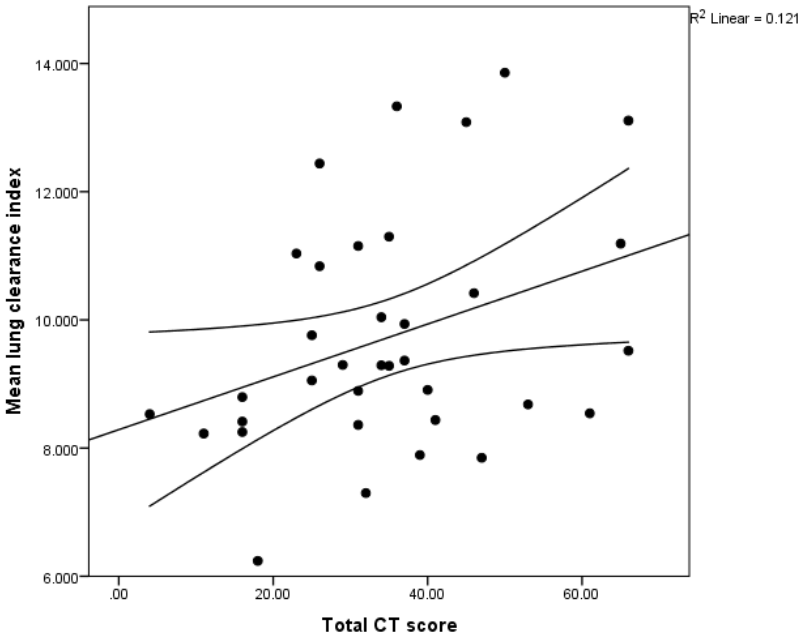


Figure 24: Scatter plot demonstrating the correlation between the total CT score and the lung clearance index, p=0.041

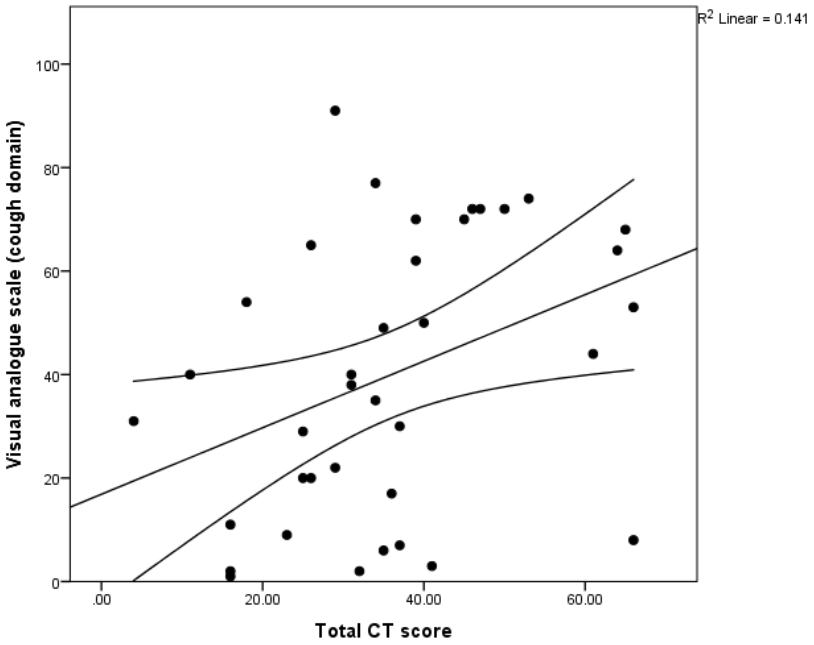


Figure 25: The scatter plot demonstrating the correlation between the total CT score and the VAS (cough domain) questionnaire score, p=0.022

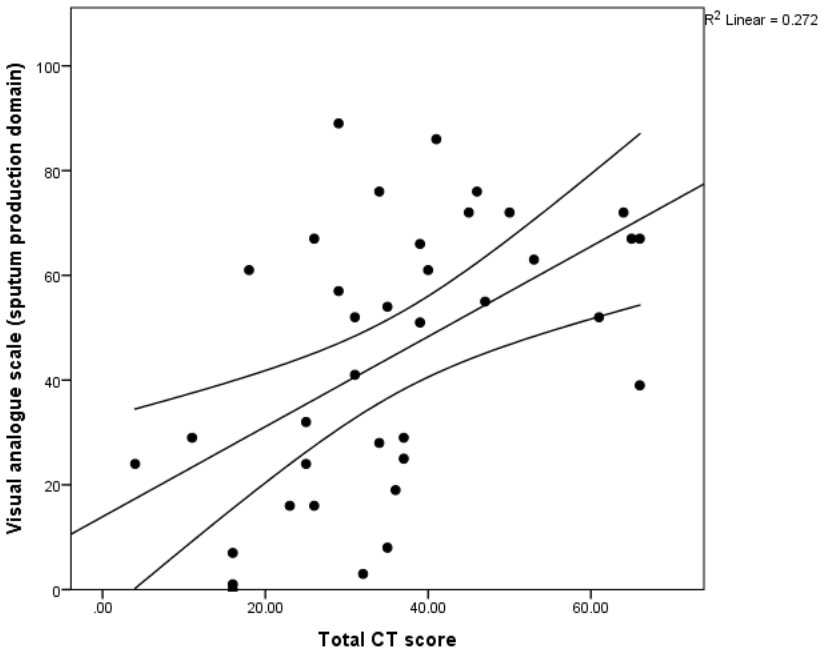


Figure 26: The scatter plot demonstrating the correlation between the total CT score and the VAS (sputum production domain) questionnaire score, p=0.001

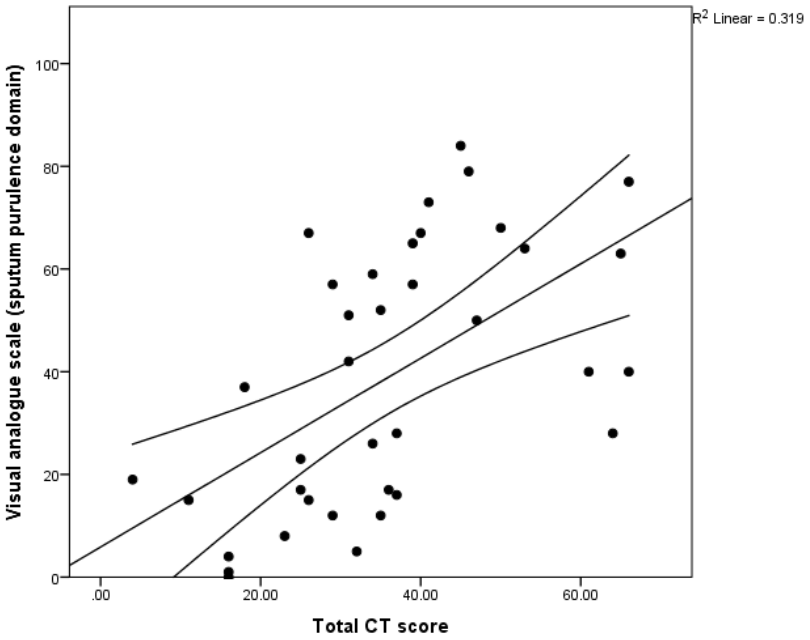


Figure 27: The scatter plot demonstrating the correlation between the total CT score and the VAS (sputum purulence domain) questionnaire score, p=0.000

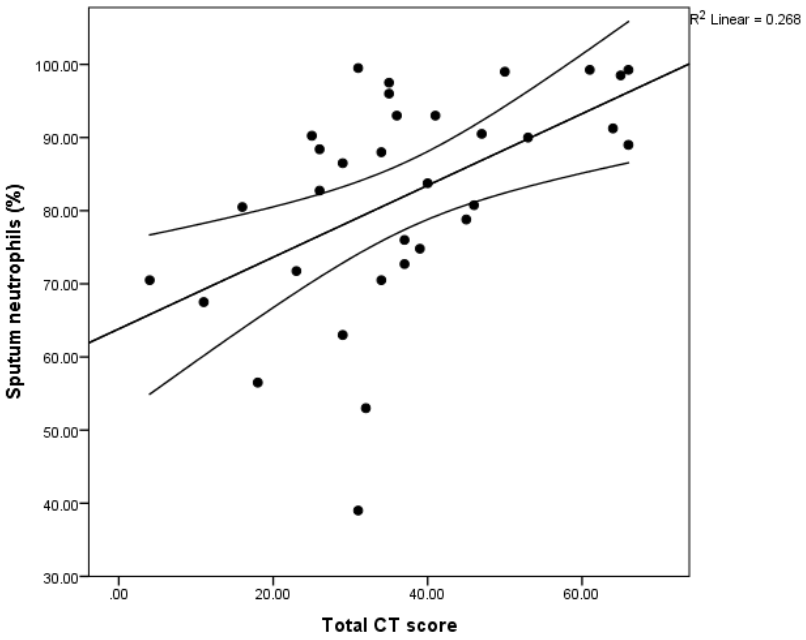


Figure 28: Scatter plot demonstrating the correlation between the CT score and the sputum neutrophil count (% predicted), p=0.002

The CT scores and sputum bacterial cultures

The box and whisker plot below demonstrates the variation in the CT score when categorised by sputum bacterial culture. The highest scores were seen in the group who cultured *Moraxella catarrhalis* and *Pseudomonas aeruginosa*. The lowest mean CT score was seen in the group who cultured *Staphylococcus aureus* and those without any significant bacterial growth.

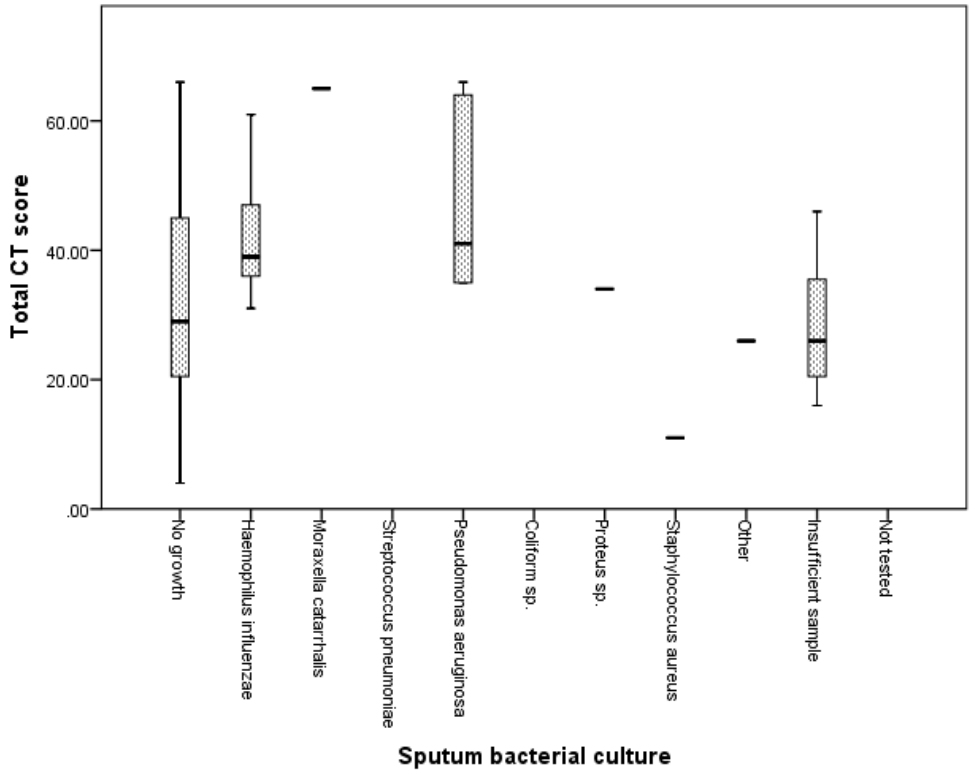


Figure 29: The mean CT score according to sputum bacterial culture

4.7.6 Safety and adverse events

Adverse events

The tables below outline the adverse events and safety aspects related to the 12 week course of erythromycin. There were 8 adverse events recorded at visit 6. The most common was GI upset which was mild in 2 participants, who continued the treatment, and more severe in another whom discontinued the treatment after 4 weeks. One participant had a sub-acute allergic reaction to the medication which resulted in discontinuation at 4 weeks. The other adverse events were: localised itching, headache, a diverticulitis flare and a non-candidal tongue coating.

Table 23: Summary of the adverse events during a 12 week course of erythromycin

Outcome	Adverse event	Number
Continued erythromycin	Mild GI upset	2
	Diverticulitis flare	1
	White tongue (not candida)	1
	Localised itching	1
	Headache	1
Stopped erythromycin	Severe GI upset	1
	Allergy	1

Effect on liver function tests, QT_c interval and non-tuberculous mycobacterial culture

There were no significant differences in either the pre- and post-erythromycin liver function tests or QT_c interval. During the observational year there were 5 positive *Mycobacterial* cultures of which 2 were a recurrent isolate of *M. abscessus* in the same participant. During the post-erythromycin period the same participant

had further positive cultures at visits 6 and 7 but remained clinically asymptomatic.

Table 24: Summary of the liver function tests, QTc interval and non-tuberculous *Mycobacterial* cultures pre- and post-erythromycin

	Pre-erythromycin	**Post-erythromycin**	***p*-value**
QT_c interval, mean (sd)	409 (12)	408 (12)	0.821
ALP, mean (sd)	83 (29)	82 (20)	0.469
ALT, mean (sd)	23 (15)	23 (11)	0.676
Bilirubin, mean (sd)	9 (3)	9 (3)	0.499
AAFB positive cultures (n)	1x *M. xenopi* 1x *M. mucogenicum* 1x M. *intracellulare* 2x *M. abscessus* (grown at visits 4 and 5)	2x *M. abscessus* (grown at visits 6 and 7)	

Microbial resistance

The effect of the erythromycin treatment on microbial resistance was reviewed. Macrolide resistance was not routinely tested for in the hospital laboratory and for financial reasons specific Macrolide resistance testing could not be performed.. The majority of the antibiotic resistance was related to amoxicillin. Microbial resistance affected a wide variety of organisms from *Haemophilus influenzae* to *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

The microbial resistance rate varied from 25.0% at visit 6 (post-erythromycin) to 50.0% at visit 2. *Haemophilus influenzae* accounted for the highest degree of resistance of around a third of all the resistant bacterial species, predominantly to amoxicillin and/or trimethoprim. In fact penicillin resistance was the most commonly seen across the bacterial species.

Table 25: The breakdown of resistant bacteria during the study period

Visit	No. samples	No. isolates	No. resistant isolates, n(%)	Breakdown	
1	19	23	9 (39.1%)	1 beta-haemolytic *Streptococcus sp.*	Tetracycline
				1 *Staphylococcus aureus*	Penicillin
				4 *Haemophilus influenzae*	2 Amoxicillin 2 Trimethoprim
				2 *Moraxella catarrhalis*	Amoxicillin
				1 *Coliform sp.*	Amoxicillin
2	16	20	10 (50.0%)	2 *Staphylococcus aureus*	Penicillin
				4 *Haemophilus influenzae*	1 Amoxicillin 2 Trimethoprim 1 Both
				2 *Moraxella catarrhalis*	Amoxicillin
				1 *Coliform sp.*	Amoxicillin
				1 *Pseudomonas aeruginosa*	Ciprofloxacin
3	14	15	6 (40%)	1 *Staphylococcus aureus*	Penicillin
				2 *Haemophilus influenzae*	1 Amoxicillin / Co-amoxyclav 1 Trimethoprim
				1 *Moraxella catarrhalis*	Amoxicillin
				1 *Coliform sp.*	Amoxicillin
				1 *Pseudomonas aeruginosa*	Ciprofloxacin
4	18	21	7 (33.3%)	1 *Staphylococcus aureus*	Penicillin
				4 *Haemophilus influenzae*	1 Amoxicillin 3 Trimethoprim
				1 *Coliform sp.*	Amoxicillin
				1 *Pseudomonas aeruginosa*	Ciprofloxacin
5	17	18	7 (38.9%)	2 *Staphylococcus aureus*	Penicillin
				3 *Moraxella catarrhalis*	Amoxicillin
				1 *Coliform sp.*	Amoxicillin
				1 *Pseudomonas aeruginosa*	Ciprofloxacin
6	10	12	3 (25.0%)	1 *Haemophilus influenzae*	Trimethoprim
				2 *Moraxella catarrhalis*	Amoxicillin
7	15	17	9 (52.9%)	1 *Staphylococcus aureus*	Penicillin
				4 *Haemophilus influenzae*	1 Amoxicillin 2 Trimethoprim 1 Amoxicillin / Co-amoxiclav
				2 *Moraxella catarrhalis*	Amoxicillin
				1 *Coliform sp.*	Amoxicillin / ciprofloxacin
				1 *Pseudomonas aeruginosa*	Ciprofloxacin

4.8 Section discussion for the clinical trial

The preceding section has involved the description of the 40 participants of the clinical trial involving a 12-week course of erythromycin in non-cystic fibrosis bronchiectasis. The most common suspected aetiology was gastro-oesophageal reflux disease and previous childhood infection such as whooping cough, pneumonia or TB. Sinusitis and otitis media were also commonly described in the cohort. The aetiology and associated factors have been discussed in more detail in the previous chapter.

Forty participants started the study. Five participants had discontinued by the end of the observational year due to various reasons: one was started on erythromycin in clinic early in the trial, two developed health problems which required medications which were contraindicated with erythromycin therapy, and one was found to have cancer which was detected at the CT scan at visit 4.

The remainder of the discussion will focus on the changes in the investigations during the one year observational period (visits 1-4), the pre- and post-erythromycin visits (visits 5 and 6) and the post-erythromycin period and final visit (visit 7). The statistical similarity of the data and the statistical differences due to the erythromycin will be reviewed along with the maintained response to the intervention.

4.8.1 Patient selection and potential bias

The patients were selected mainly from the outpatient clinics at Glenfield Hospital. It should be noted that as the main clinical trial involved a 12 week intervention with low dose erythromycin one of the inclusion criteria for the study was that the patients should not already be taking any low dose, long term antibiotic therapy. This might have resulted in a milder, less symptomatic population being recruited for the study. During the course of the study the Bronchiectasis Severity Index (154) was published and it would have been informative to have scored each patient against the system. However, despite the patients not already taking long term antibiotics it should also be pointed out that some were indeed very

symptomatic, had multiple (up to 8) exacerbations in the 18 month period of the study and several patients restarted the erythromycin post study completion. Therefore the patients recruited had a wide spread of symptoms and radiological bronchiectasis severity but by the nature of the recruitment process and the need to attend multiple appointments there may have been a bias towards the milder end of the spectrum.

4.8.2 Questionnaires

All the questionnaires demonstrated statistical similarity between stable visits and between visit 6 and 7. There were statistical improvements seen in the LCQ total score and the VAS sputum production domain. There was a statistically significant increase (worsening) in the VAS dyspnoea score between visits 5 and 6 but this did not seem to have any clinical relevance. Previous macrolide trials have reported a varied response in questionnaire scores. The EMBRACE and BLESS trials did not show any significant improvement in the SGRQ following either azithromycin or erythromycin, however the BAT trial reported a significant improvement in both the SGRQ total score and the LRTI-VAS score following a 12 month intervention of azithromycin (112,134,150). No major macrolide trial to date has used the same VAS domains as this trial, however the 24 hour sputum production was significantly reduced in the BLESS study, suggesting the improvement seen in the VAS sputum production domain was genuine.

Research in the field of Asian panbronchiolitis has shown that long-term low-dose erythromycin improved the sputum production from over 80ml/day to below 5ml/day, spirometry by as much as 750ml and total sputum neutrophil counts. There has been some suggestion that in this condition the greatest benefits are seen in the sub-group with *Pseudomonas aeruginosa* colonisation (118,119). However, this condition appears to behave quite differently and the data cannot be extrapolated in to the area of non-cystic fibrosis bronchiectasis. In non-cystic fibrosis bronchiectasis none of the major clinical trials in this area have

demonstrated a significant, sustained improvement in sputum volume, neutrophils or quality of life questionnaires (150,175).

4.8.3 Airway function investigations

Spirometry

As described in the previous section the spirometry was, on average, within normal limits although there was a large degree of variation which may be due to the range of other underlying lung conditions such as asthma and a previous smoking history in the participants. As previously discussed many bronchiectasis studies describe a cohort with largely normal lung function (29,150,175). The spirometry data demonstrated good repeatability between the stable visits. There was a significant improvement in the FEV_1 (% predicted) following the erythromycin intervention but not in the absolute values. This response was maintained after the completion of the intervention. A similar significant improvement in the FEV_1 following the 12 month course of erythromycin was seen in the BLESS trial but not the EMBRACE study (112,150). As discussed in the previous chapter there was so much variation in the FEV_1 over both the observational and interventional year within and between individuals independent of symptoms and exacerbations that I don't believe any clear conclusions can be drawn from analysing spirometry in a non-CF bronchiectasis trial.

Multiple breath washout

The lung clearance index showed statistical stability in the observation year with a significant improvement after the erythromycin intervention. At the time of writing there are not any clinical trials which evaluate the lung clearance index as a biomarker of treatment response in non-cystic fibrosis bronchiectasis. The investigation has been used with some success in cystic fibrosis (203). The lung clearance index is a marker of ventilation heterogeneity and an indirect marker of

small airway function. Non-CF bronchiectasis affects the bronchioles in addition to the bronchial branches, seen on a CT scan as tree in bud changes. This study demonstrated a significant, positive correlation with the CT severity scoring, suggesting a more severe bronchiectasis picture was associated with worse functional small airway disease.

Only 3 participants had a normal lung clearance index (<7.5). As previously discussed in more detail it is becoming increasingly clear that the lung clearance index is more sensitive to early lung pathology and has a greater association with structural changes on CT scanning than spirometry (103,107), which, as we have seen, is not a reliable biomarker in non-CF bronchiectasis.

4.8.4 Airway inflammation investigations

Exhaled nitric oxide

The exhaled nitric oxide levels were largely with the normal limits of <25ppb or the grey area of 25-50ppb, with some exceptions, such as those with known eosinophilic asthma. The levels were stable across all visits and not affected by the erythromycin intervention which was anticipated as the degree of sputum eosinophilia did not alter over the study. Macrolides have not been demonstrated to have any effect on eosinophilic inflammation (132).

Sputum differential cell counts

The sputum samples were largely neutrophilic with a baseline sputum neutrophil count of 91.0%, which is significantly higher than the level of 35% described in healthy subjects (191) and similar to the results from other bronchiectasis cohorts (175). Neutrophil recruitment has been hypothesised to a defence mechanism against the ongoing insult to the airways from colonising bacteria, inhaled particles and resulting low grade inflammation (11,13).

The sputum cell counts and proportions did not show any significant changes over the study period, even after the erythromycin intervention. Interestingly the trend for the total cell count was to climb throughout the study with the highest median value immediately after the course of erythromycin. Several large, well-conducted macrolide trials also failed to demonstrate a lack of sputum cell count response to macrolides (112,150) unlike the trials in Asian diffuse panbronchiolitis (118,119). A study of erythromycin in COPD patients demonstrated a significant reduction in both the total cell count and the sputum neutrophil count after 6 months therapy compared with the placebo group (204).

Cytokines have been used as a surrogate marker of airway inflammation with IL-8 and sputum neutrophil elastase levels significantly elevated in both neutrophilic asthma and bronchiectasis (8,13,205,206). Clarithromycin has been shown to modulate IL-8 and neutrophil levels and has been postulated as an anti-inflammatory treatment for asthma (132). However, in experimental animal models IL-8 deficient mice are very prone to pyelonephritis and septicaemia (207) suggesting a minimal protective level exists for IL-8.

4.8.5 Microbiology

The most striking finding was the increase in the number of sputum samples without any significant growth, and a reduction in the samples previously culturing *Staphylococcus aureus* and *Coliform* species following the 12-week course of erythromycin. This suggests an antibiotic effect despite the dose of the erythromycin being lower that than the minimum inhibitory concentration for antibacterial activity. The response to the antibiotic was not sustained from a microbiological point of view. The Bray-Curtis Index of similarity did not suggest any statistical changes in the culture diversity over the study period. A recent large study also did not show any changes in the microbiological profile between their azithromycin intervention group and the placebo group after a year of treatment (134). The BLESS macrolide trial group published a post-study analysis of the

microbiological data which demonstrated a significant change in the microbial diversity particularly in relation to individuals culturing *Pseudomonas aeruginosa* and *Haemophilus influenzae* (196). Their analysis utilised 16sRNA qPCR techniques which was beyond the scope of my study and possibly provided a more detailed and accurate assessment of the post-erythromycin microbiome. One of the downsides of this technique is that it cannot differentiate between live and dead organisms.

4.8.6 CT severity scoring

The three major macrolide trials did not use CT scoring as part of their study protocol (134,150,175). However there was one retrospective clinical review which has demonstrated a significant improvement in bronchial wall thickening and mucus plugging after a period of macrolide treatment (208). This was not a randomised controlled trial but it would be interesting to see if the results are seen in a trial protocol setting.

The most severely affected regions of the lungs were the lower lobes, a well-known phenomenon in non-CF bronchiectasis likely related to reduced sputum clearance exacerbated by gravitational effects. The highest mean CT scores were seen in the groups who cultured *Moraxella catarrhalis* and *Pseudomonas aeruginosa*. This has previously been demonstrated by Lynch et al. who found that the modified Bhalla CT score was significantly higher in the group who isolated *Pseudomonas aeruginosa* when compared with the group who grew other organisms (87).

My analysis demonstrated significant correlations with the VAS cough, sputum production and sputum purulence domains, FEV_1 (% predicted), the lung clearance index and the sputum neutrophil count (%). Several studies have also discovered a correlation between the CT scores and the FEV_1 (% predicted) (86–88,90). It might be anticipated that spirometric dysfunction would be associated with increasing radiographic severity. As FEV_1 is composite of airway size and muscle power the association can't be deemed to be due to the bronchial dilatation domain of the CT

score alone. The associations found in this study between the VAS cough and sputum production/purulence domains and the total CT score have not been reported elsewhere as the VAS is not as widely used as the SGRQ and the LCQ. However the associations make clinical sense. The bronchial wall thickening and mucus plugging categories are radiological surrogates of increased sputum production, and with it, cough (7). The association between CT related severity and the sputum neutrophil count has been demonstrated previously (181). My study did not demonstrate any correlation between the total CT scores and the SGRQ or LCQ total scores in keeping with some studies (91) however other authors have reported a significant correlation between these symptom scores and bronchiectasis severity (92,93).

4.8.7 Safety and adverse events

Adverse events

There were several mild adverse events and two serious adverse events related to the erythromycin intervention period. Gastro-intestinal side-effects from erythromycin are well known and due to the direct pro-kinetic effect of erythromycin on the GI tract. Gastro-intestinal side-effects are also the most commonly reported antibiotic-related events.

Liver function tests and cardiac toxicity

14 and 15 member ring macrolides have been implicated as a cause of polymorphic ventricular tachycardia (torsades de pointes), monomorphic pulsed ventricular tachycardia (VT) and QT_c interval prolongation (135). A normal QTc interval is <430ms in males and <450ms in females (209). There are a number of additional risk factors: being female, increasing age, structural heart disease, genetic predisposition, co-prescription of other drugs causing QT prolongation (such as class I and III anti-arrhythmics) and electrolyte disturbance such as hypokalaemia (136,137). Only a small number of macrolide trials have monitored

ECG changes and no significant changes in the QT_c interval have been seen (114). Most papers dedicated to macrolide related ECG changes related are based around single case reports or occur in patients with underlying cardiovascular problems and contraindicated medication usage (138–141). The reported risk of sudden death has been determined to be related to a high prescribed dose, an intravenous route of administration and prolonged courses (209,210). There were no cases of QT_c prolongation in this study which is in keeping with previous studies but might have been affected by the fact that the post-erythromycin visit was carried out up to a week after the erythromycin was completed allowing any cardiac changes to have normalised. Concomitant use of other medications which might cause QT_c prolongation was a strict contra-indication for study entry.

Mycobacterial growth

The increased detection of non-tuberculous *mycobacteria* (NTM) in patients with chronic lung disease has coincided with the increased prescription of long-term low-lose macrolides for the reduction of symptoms and exacerbation frequency. Some groups have suggested that macrolide use encourages NTM culture by an inhibition of macrophage function and reduction in interferon γ both *in vitro* and in mouse models (211). This has not yet been replicated in human subjects. So far no association has been found between NTM infection and macrolide use in cystic fibrosis patients (212). Macrolides remain a first line drug in the treatment of both TB and NTM disease.

During the trial there were 4 subjects who cultured NTM organisms. Two of the cultures were isolated strains of *M. xenopi* and *M. mucogenicum* which were of uncertain significance. Another subject cultured *M. intracellulare* once during the study period and another repeatedly cultured *M. abscessus* from visit 4 onwards without any change in respiratory symptoms. This subject reported feeling well and was independently reviewed by a consultant. They did not meet the ATS criteria for treatment and so have remained under observation with ongoing good

health. There were no new cases of NTM culture after the erythromycin intervention period.

Microbial resistance

The most commonly used antibiotics in the UK currently are, in descending order: Penicillin, tetracyclines, macrolides, trimethoprim and quinolones (213). Macrolide resistant *Streptococcus pneumoniae* has risen from 8% in 1998 to 22% in 2008 as demonstrated by a Canadian study (146). The Global Resistance to Antimicrobials with *Streptococcus pneumoniae* (GRASP) project demonstrated that 29.6% of clinically important isolates were not sensitive to macrolide treatment (145). It has been hypothesized that increasing azithromycin usage is the largest cause of macrolide resistance, partly due to its long half-life of 3 days, although it has the lowest activity against *Streptococcus pneumoniae* of the macrolides (148).

This study did not demonstrate any occurrences of resistant *Streptococcus pneumoniae* unlike previous studies (134,145,148,150). While macrolide sensitivity was not routinely tested the majority of the resistance was to either amoxicillin or trimethoprim and *Haemophilus influenzae* was the most commonly resistant bacteria cultured. *Haemophilus influenzae* is a beta-lactam producing organism and the selective pressure from amoxicillin as a first line antibiotic for both upper and lower respiratory tract infections is likely to be the cause of the resistance. Amoxicillin resistance *Haemophilus influenzae* in Spain is now on the decline after prescribing measures were taken some years ago (214). Trimethoprim resistance is also common due to its use as the first-line antibiotic for uncomplicated urinary tract infections, a common condition which is treated with minimal medical input. This study also detected resistance in *Coliform* and *Pseudomonas* positive cultures, predominantly to ciprofloxacin and amoxicillin. In the UK 18% of *Escherichia coli* strains and 10% of *Pseudomonas aeruginosa* were resistant to ciprofloxacin (213). The lowest proportion of resistant organisms was seen at the post-erythromycin visit. It might be anticipated that the presence of the antibiotic would have encouraged the development of resistance however the low

numbers seen could also be due to the reduced numbers of culture-positive sputum samples and the reduced sputum production in general which characterised this visit.

Reporting of bacterial resistance and susceptibility to certain antibiotics can be subjective and related to duration of training as well as the size of the hospital (215). There is an additional subjective concept of indeterminate bacterial resistance where the bacterial colony growth is only partially inhibited by the antibiotic disc. We also know that a sputum sample will only contain bacteria from a few small areas of the lung and there have been shown to be differences between the populations in various sections of the lungs (19).

Antibiotic resistance has been shown to follow antibiotic consumption and the concern is that the increased use of long-term erythromycin will result in increasing microbial resistance which will require careful monitoring in the future.

4.9 Conclusion for the discussion of the clinical trial

The analysis of the data from the clinical trial has demonstrated that several of the investigations have shown a significant improvement following the 12 week intervention of erythromycin. Firstly the **Leicester cough questionnaire** total score demonstrated good similarity between the observational visits and a significant improvement after the erythromycin phase. The **visual analogue scale sputum production domain** also demonstrated a significant reduction in the self-reported 24 hour sputum volume. The **FEV_1 (% predicted) values** demonstrated only a very modest improvement after the erythromycin course (50ml or 2.2%) though this was statistically significant. There was not a significant improvement in the absolute FEV_1 value. Perhaps of greater clinical application was the significant improvement in the **lung clearance index** values obtained between the pre- and post-erythromycin visits. There was a striking increase in the number of **culture-negative sputum bacterial samples** following the erythromycin therapy, which returned to the previous level after a further 12 weeks. The responses seen with the erythromycin therapy were all maintained following the end of the medication period when assessed in terms of statistical similarity.

4.10 Secondary objectives

- *We hypothesize that the study participants who demonstrate the best response to erythromycin 250mg daily for 12 weeks will have neutrophilic airway inflammation and demonstrate small airways disease in the form of an increased lung clearance index on multiple breath washout testing and tree in bud changes on the CT scan.*
- *To evaluate whether the response to 12 weeks erythromycin will be sustained after the course is completed.*

4.10.1 Results

From the previous section determined a statistically significant response to erythromycin in the following investigations**: FEV_1 (% predicted), LCQ total score, VAS sputum production domain** and **lung clearance index.** There were also a larger proportion of samples **without significant bacterial growth** at the immediate post-erythromycin visit. The data has been stratified according to these investigations and the clinical data at the visit immediately prior to the erythromycin intervention (visit 5) has been described. In order to answer hypothesis number two emphasis has been placed on the results of the sputum differential cell counts, lung clearance index and CT severity scores, particularly the small airway mucus plugging domain.

Characteristics of the best responders to erythromycin therapy

How should a "responder" to the erythromycin therapy be determined? The study was powered to determine a response of greater than 200ml in the FEV_1 which was addressed in the last section. However exploratory analyses have been performed on those investigations which have demonstrated a significant improvement following a 12 week course of erythromycin. The minimum clinically important difference (MCID) between visits 5 and 6 in that particular investigation

was used to classify the data into responders and non-responders. The FEV_1 data was presented in the first hypothesis and won't be reviewed in this section.

Leicester cough questionnaire total score

The MCID for the Leicester cough questionnaire total score has been calculated to be 1.3.(216) The participants were divided into two groups – those who have had an improvement (increase) in the total score by 1.3 or more and those who hadn't. The table below demonstrates significant differences between the two groups in the SGRQ total score and the VAS cough, dyspnoea and sputum purulence domains. These investigation results were higher in the group with a response suggesting a greater degree of baseline symptoms. For the remainder of the investigations the groups were similar. The responders demonstrated a non-significantly lower sputum neutrophil count (%) at 85.13% compared to the non-responders. The lung clearance index results were similar in both groups as were the CT severity scores for small airway mucus plugging.

In summary, the participants who demonstrated a response to erythromycin in terms of a clinically important improvement in the Leicester cough questionnaire total score had more symptoms at the pre-treatment visit as suggested by the higher scores in the SGRQ total score and the VAS scores. The responders also tended towards lower sputum neutrophil counts but did not have any difference in the burden of disease in the small airways as seen in the similar CT score and LCI results.

Table 26: Comparison of the baseline characteristics between the group of participants who demonstrated the minimally important improvement in the LCQ post-erythromycin, and those who did not

Investigation at visit 5		LCQ change <1.3 n=18	LCQ change >1.3 n=17	*p*-value
SGRQ total score, mean (sd)		27.51 (17.62)	39.59 (13.24)	**0.029***
VAS cough domain, median (IQR)		28 (10-41)	46 (31-58)	**0.013***
VAS dyspnoea domain, median (IQR)		21 (6-33)	46 (22-62)	**0.010***
VAS sputum production domain, median (IQR)		40 (9-59)	56 (52-66)	0.092
VAS sputum purulence domain, median (IQR)		24 (3-40)	50 (23-65)	**0.048***
Exhaled nitric oxide (ppb), mean (sd)		30.2 (19.0)	24.1 (8.9)	0.238
Post-bronchodilator FEV_1 (l), mean (sd)		2.05 (0.70)	2.01 (0.61)	0.838
Post-bronchodilator FEV_1 (% predicted), mean (sd)		87.6 (26.0)	83.3 (23.5)	0.609
Lung clearance index, mean (sd)		10.495 (1.629)	10.491 (2.392)	0.996
Sputum total cell count (10^6/ml), median (IQR)		9.63 (1.67)	6.35 (2.21-29.90)	0.861
Sputum neutrophils (%), median (IQR)		89.0 (76.25-96.25)	83.13 (73.875-96.5)	0.632
Sputum culture (n)	**No significant growth**	5	2	
	Pseudomonas aeruginosa	1	2	
	Non-*Pseudomonas* bacteria	4	10	
	Insufficient sample	8	3	
Sputum bacterial colony forming units (x106/ml), median (IQR)		0.317 (0.220-1.115)	1.867 (0.30-86.50)	0.089
Total CT score, mean (sd)		34.24 (13.31)	38.06 (17.65)	0.481
Bronchial wall dilatation score		7.76 (4.12)	7.47 (5.27)	0.857
Bronchial wall thickening score		4.29 (3.31)	5.76 (3.70)	0.231
Extent of bronchiectasis score		10.06 (3.17)	8.71 (3.48)	0.245
Mucus plugging large airways		2.35 (2.03)	2.06 (1.75)	0.654
Mucus plugging small airways		3.18 (2.60)	3.06 (2.05)	0.884
Attenuation score		4.94 (4.76)	6.59 (4.64)	0.315
Bronchial collapse score		1.65 (3.41)	4.24 (4.32)	0.061
Tracheal collapse score		0 (0)	0.12 (0.33)	0.154

Visual analogue scale scores – sputum production domain

The minimal important difference for the improvement in the visual analogue scale for dyspnoea has been determined to be 19mm in the context of malignant pleural effusions. (217) The group who had a 19mm improvement in the VAS sputum production score following 12 weeks of erythromycin were termed responders. The only significant difference between the responders and non-responders was seen in the LCQ total score. This was lower in the responder group suggesting a greater degree of symptoms prior to the erythromycin intervention. The sputum neutrophil count was very similar between the groups at 85.250% vs. 84.250%. In terms of small airway changes there were no significant differences in either the CT total score, the small airway mucus plugging domain of the CT score or the lung clearance index. Interestingly all the participants who could not produce a sputum sample were in the non-responder group.

In summary, the participants who demonstrated a response to erythromycin with a clinically important improvement in the sputum production scores had significantly lower LCQ total scores suggesting a greater degree of pre-treatment symptoms. There was no suggestion that pre-treatment sputum neutrophil counts or small airway inflammation demonstrated by the CT scoring or the lung clearance index could be used as a biomarker for erythromycin response.

Table 27: The visit 5 investigations categorised by the minimal clinically important difference in the VAS sputum production domain

Investigation at visit 5		VAS sputum production MCID		*p*-value
		<19mm	>19mm	
SGRQ total score, mean (sd)		30.78 (16.76)	39.05 (15.45)	0.174
LCQ total score, mean (sd)		17.01 (3.13)	14.26 (2.08)	**0.012***
Exhaled nitric oxide (ppb), mean (sd)		28.8 (16.8)	23.9 (10.4)	0.376
Post-bronchodilator FEV_1 (l), mean (sd)		2.02 (0.62)	2.05 (0.75)	0.898
Post-bronchodilator FEV_1 (% predicted), mean (sd)		88.0 (26.2)	80.1 (20.7)	0.385
Lung clearance index, mean (sd)		10.854 (1.748)	9.563 (2.397)	0.148
Sputum total cell count (10^6/ml), median (IQR)		9.63 (1.58-26.40)	6.35 (3.76-15.20)	0.822
Sputum neutrophils (%)		85.250 (76.25-98.0)	84.250 (69.0-96.25)	0.464
Sputum culture (n)	**No significant growth**	4	3	
	Pseudomonas aeruginosa	2	1	
	Non-*Pseudomonas* bacteria	7	7	
	Insufficient sample	11	0	
	Not tested	0	0	
Sputum colony forming units (10^6/ml), median (IQR)		0.850 (0.220-8.833)	0.642 (0.250-86.5)	0.635
Total CT score, mean (sd)		35.37 (16.16)	36.73 (14.42)	0.810
Bronchial wall dilatation score		7.41 (4.66)	7.27 (4.96)	0.937
Bronchial wall thickening score		4.52 (4.08)	5.82 (2.04)	0.324
Extent of bronchiectasis score		8.78 (3.97)	9.36 (2.34)	0.651
Mucus plugging large airways		2.04 (1.76)	2.18 (2.14)	0.830
Mucus plugging small airways		2.93 (2.51)	2.73 (1.95)	0.816
Attenuation score		6.15 (4.83)	6.00 (4.20)	0.930
Bronchial collapse score		3.44 (4.67)	3.27 (3.29)	0.912
Tracheal collapse score		0.11 (0.32)	0 (0)	0.083

Lung clearance index scores

The MCID for the lung clearance index has been suggested to be 5%.(203) Those with a post-erythromycin improvement of >5% of the pre-erythromycin value were termed responders. The responders demonstrated significant differences in the SGRQ total score, LCQ total score, and VAS dyspnoea and sputum purulence domains. The pre-treatment SGRQ score was lower in the responder group, the LCQ higher and the VAS scores lower in the responder group paradoxically suggesting that fewer symptoms are associated with a significant improvement in the lung clearance index with erythromycin. In keeping with these findings, although not statistically significant, the responders had a significantly higher FEV_1 (% predicted) at 102.5% compared with 78.8% in the other group and lower neutrophil levels (84.75% vs. 96.25%). The total CT score was also lower in the responder group at 29 vs. 35 suggesting less severe bronchiectasis in this group. Therefore, the responders as assessed by lung clearance index had a smaller degree of sputum neutrophilia and less CT evidence of small airway changes compared to the group who did not have such a response to erythromycin.

In summary, those participants who demonstrated a significant improvement in the lung clearance index in response to a 12-week course of erythromycin will have statistically better pre-treatment symptoms as suggested by the SGRQ total score, the LCQ total scores and the VAS dyspnoea and sputum purulence domains. They are also more likely to have better spirometry results and less severe bronchiectasis on CT scoring.

Table 28: Comparison of the investigations at visit 5 categorised by the minimal clinically important difference of the lung clearance index of 5%

Investigation at visit 5		LCI change <5% n=7	LCI improvement >5% n=7	*p*-value
SGRQ total score, mean (sd)		38.66 (11.21)	21.84 (16.10)	**0.045***
LCQ total score, mean (sd)		16.12 (2.81)	18.65 (2.77)	0.536
VAS cough domain, median (IQR)		44 (10-76)	11 (4-27)	0.179
VAS dyspnoea domain, median (IQR)		46 (12-66)	10 (1-21)	**0.035***
VAS sputum production domain, median (IQR)		66 (31-76)	10 (1-19)	**0.048***
VAS sputum purulence domain, median (IQR)		50 (3-77)	10 (1-31)	0.179
Exhaled nitric oxide (ppb), mean (sd)		20.1 (4.2)	31.7 (20.6)	0.625
Post-bronchodilator FEV_1 (l), mean (sd)		1.83 (0.58)	2.42 (0.58)	0.080
Post-bronchodilator FEV_1 (% predicted), mean (sd)		78.8 (24.1)	102.5 (16.0)	0.051
Sputum total cell count (10^6/ml), median (IQR)		20.80 (5.02-30.80)	8.22 (5.31-22.08)	0.670
Sputum neutrophils (%), median (IQR)		96.250 (82.0-98.5)	84.750 (63.750-92.750)	0.583
Sputum colony forming units (10^6/ml), median (IQR)		0.640 (0.250-86.50)	1.230 (0.120-25.00)	0.796
Sputum culture (n)	**No significant growth**	1	1	
	Pseudomonas aeruginosa	1	0	
	Non-Pseudomonas bacteria	3	1	
	Insufficient sample	2	5	
Total CT score		35.00 (14.78)	29.00 (16.85)	0.492
Bronchial wall dilatation score		6.86 (5.96)	5.71 (2.50)	0.648
Bronchial wall thickening score		5.71 (3.30)	2.71 (3.20)	0.110
Extent of bronchiectasis score		7.71 (4.03)	9.00 (3.51)	0.536
Mucus plugging large airways		1.57 (1.72)	1.14 (1.46)	0.625
Mucus plugging small airways		3.43 (2.37)	2.86 (2.79)	0.687
Attenuation score		6.00 (4.28)	5.86 (5.73)	0.959
Bronchial collapse score		3.57 (3.99)	1.71 (4.54)	0.432
Tracheal collapse score		0.14 (0.38)	0 (0)	0.356

Sputum samples with bacterial clearance

At the post-erythromycin visit it was noted that there were many more sputum samples which did not culture any significant bacteria compared to the previous visit. The numbers were small as there were some participants who experienced reduced sputum production as a result of the erythromycin treatment, although as we have already seen none of these had a significant improvement in the VAS sputum production domain. The table demonstrates that there was a significant difference in the LCQ total score with the responder (sputum culture-negative) group demonstrating a lower score suggesting that those with more symptoms prior to the erythromycin saw more benefit in terms of bacterial clearance in the sputum. Although not statistically significant the sputum neutrophil count was lower in the responder group at 80.125% compared to 93.50% in the other group. There was also a large, but non-significant, difference in the sputum total cell count which was $6.59x10^6$/ml in the clearance group and $13.90x10^6$/ml in the other group. The group who achieved bacterial clearance had a higher pre-treatment bacterial colony forming unit count at $33.50x10^6$/ml ($0.250\text{-}96.70x10^6$/ml) compared to $0.932x10^6$/ml ($0.234\text{-}5.00x10^6$/ml). The lung clearance index was similar between the two groups at 11.089 in the non-responders and 10.407 in the responder group. Interestingly the total CT score was lower in the group who demonstrated bacterial clearance (43.86 in the non-responders and 33.83 in the responders) with the main score differences coming from the bronchial wall dilatation domain and the small airways mucus plugging domain.

In summary, those participants who had a response to erythromycin that involved sputum bacterial clearance had significantly more pre-treatment symptoms as suggested by the Leicester cough questionnaire total scores, and were more likely to have a lower pre-treatment colony forming unit count and less severe bronchiectasis on CT scoring.

Table 29: Pre-erythromycin investigations categorised by sputum bacterial clearance and bacterial persistence in response to a 12 week course of erythromycin

Baseline investigation		Non-responder (n=6)	Bacterial clearance between visit 5 and visit 6 (n=7)	*p*-value
SGRQ total score, mean (sd)		37.11 (10.54)	40.07 (6.28)	0.560
LCQ total score, mean (sd)		15.99 (1.90)	13.48 (2.13)	**0.046***
VAS cough domain, median (IQR)		47 (27-58)	40 (30-84)	0.775
VAS dyspnoea domain, median (IQR)		26 (18-66)	39 (22-62)	0.721
VAS sputum production domain, median (IQR)		56 (44-62)	57 (52-79)	0.830
VAS sputum purulence domain, median (IQR)		31 (22-65)	54 (20-79)	0.775
Exhaled nitric oxide (ppb), mean (sd)		21.9 (5.7)	36.5 (23.6)	0.194
Post-bronchodilator FEV_1 (l), mean (sd)		1.95 (0.42)	2.10 (0.93)	0.707
Post-bronchodilator FEV_1 (% predicted), mean (sd)		84.1 (25.7)	84.2 (23.9)	0.996
Lung clearance index, mean (sd)		11.089 (1.055)	10.407 (3.824)	0.788
Sputum total cell count (10^6/ml), median (IQR)		13.90 (1.58-34.40)	6.59 (4.79-9.50)	0.391
Sputum neutrophils (%), mean (sd)		93.50 (78-25-98.00)	80.125 (74.750-84.250)	0.198
Sputum colony forming units (10^6/ml), median (IQR)		0.932 (0.234-5.00)	33.50 (0.250-96.70)	0.522
Total CT score, mean (sd)		43.86 (15.74)	33.83 (9.41)	0.201
Bronchial wall dilatation score		9.43 (5.50)	5.50 (1.87)	0.116
Bronchial wall thickening score		5.86 (2.27)	5.33 (1.97)	0.668
Extent of bronchiectasis score		9.00 (3.65)	9.00 (2.19)	1.000
Mucus plugging large airways		3.14 (1.68)	2.33 (1.97)	0.439
Mucus plugging small airways		5.29 (1.50)	3.33 (1.97)	0.067
Attenuation score		6.57 (5.35)	5.67 (4.03)	0.741
Bronchial collapse score		4.43 (5.29)	2.50 (3.21)	0.454
Tracheal collapse score		0.14 (0.38)	0 (0)	0.356
Sputum culture (n)	***Pseudomonas aeruginosa***	2	1	
	Non-*Pseudomonas* bacteria	5	5	

4.11 Discussion

The purpose of this section was to answer the second hypothesis: *We hypothesize that the study participants who demonstrate the best response to erythromycin 250mg daily for 12 weeks will have neutrophilic airway inflammation and demonstrate small airways disease in the form of an increase lung clearance index on multiple breath washout testing and tree in bud changes on the CT scan.*

And to explore the secondary objective: *To evaluate whether the response to 12 weeks erythromycin will be sustained after the course is completed.*

The investigations that demonstrated a significant improvement following a 12 week course of erythromycin were: FEV_1 (% predicted), LCQ total score, VAS sputum production domain, lung clearance index. There were also a striking number of sputum samples which demonstrated bacterial clearance which were then analysed further. The response to erythromycin for the investigations and the participants was, as anticipated, heterogeneous. These investigations were analysed using the MCID for that investigation and the pre-erythromycin data was reviewed with reference to the sputum neutrophil counts and the burden of small airway inflammation.

4.11.1 Leicester Cough Questionnaire total score

The MCID for the LCQ total score was 1.3.(216) The participants who demonstrated a response to erythromycin in terms of a clinically important improvement in the LCQ total score had more symptoms at the pre-treatment visit as suggested by the higher scores in the SGRQ total score and the VAS scores. The responders also tended towards lower sputum neutrophil counts but did not have any difference in the burden of disease in the small airways in terms of CT scoring or lung clearance index.

It might be expected that a response in one quality of life questionnaire will also result in an improvement in other questionnaires simply due to the nature of the

questions. The SGRQ total scores did not change significantly with the course of erythromycin so can only be used as a surrogate marker of symptom burden. The participants who had an LCQ response to erythromycin did not have a greater degree of sputum neutrophilia or any evidence of an increased small airway inflammation on either the CT scoring or the lung clearance index. The hypothesis has not been proven for this particular group of responders.

4.11.2 Visual analogue scale – sputum production domain

There have not been any studies which calculate the minimal important difference in the VAS sputum production domain. The value used is taken from research in the VAS dyspnoea domain in the context of malignant pleural effusions and has been determined to be 19mm (217). The participants who demonstrated a response in the VAS score had significantly lower LCQ total scores suggesting a greater degree of pre-treatment symptoms. Again, changes in one quality of life questionnaire are likely to result in a similar change in others. The participants who saw a reduction in sputum production following erythromycin did not have significantly greater pre-treatment sputum neutrophil counts or a higher degree of small airway inflammation demonstrated by the CT scoring or the lung clearance index could be used as a biomarker for erythromycin response. The hypothesis could not be proven for this group of responders. Further analysis to determine the MCID for the VAS scores in the non-CF bronchiectasis population should be carried out.

4.11.3 Lung clearance index

The MCID of 5% for the lung clearance index was used (203). This value relates to research from cystic fibrosis patients and has not been validated within the non-cystic fibrosis group. Those participants who demonstrate a significant improvement in the lung clearance index in response to a 12-week course of erythromycin unexpectedly had better pre-treatment quality of life questionnaire

scores, a tendency towards better lung function and less severe bronchiectasis according to the CT scoring. These results are the opposite way around to those expected and conflict with the other investigations. However, the data has been checked several times for inaccuracies and this result appears to be true. It might be anticipated that those with a greater burden of symptoms or disease might have greater scope for improvement with any given intervention but this was not seen in this test. This particular responder group also appeared to have a lesser degree of sputum neutrophilia (84.75% vs. 96.25%) and less CT evidence of small airway inflammation.

The limited studies in cystic fibrosis which have used the lung clearance index as a measure of treatment response during exacerbations found that there was a good correlation between improved LCI and reduced symptoms according to the quality of life questionnaires used (203). My study used erythromycin in stable participants; at the time of writing there was no research assessing LCI response to low-dose prophylactic antibiotics. One of the limitations of this sub-group analysis was the small number of paired samples available for analysis. This was due to technical issues with the equipment at the time of these visits along with equipment availability in a busy research unit. Although the LCI was performed 3 times to achieve at least two FRC values within 10% the computer-generated data analysis resulted in a significant amount of unusable data. Research within a larger sample group would provide insight on whether the unexpected findings in the responder group are true.

4.11.4 Bacterial clearance from sputum

The participants who had sputum bacterial clearance in response to a course of erythromycin were found to have a higher level of pre-treatment symptoms as suggested by the LCQ total scores. They were also more likely to have a higher pre-treatment colony forming unit count, lower sputum neutrophil count, less severe

bronchiectasis on CT scoring and a similar lung clearance index compared with the non-responder group.

The numbers were small as the erythromycin treatment resulted in a reduction in sputum production. There is debate about whether a sputum sample is the most accurate method of analysing the lung microbiome. We know that a sputum sample is likely to be from one small section of the lung and that the microbiome can differ throughout the lung (19). In the case of the participants who had reduced sputum production and could not produce a sample there is insufficient evidence that there has been bacterial clearance. We also know that the majority of the bacteria with the lung microbiome cannot be cultured by standard laboratory tests and will therefore return as a sample with "no significant growth" (218). Antibiotics will result in a transient change to the lung microbiome but this quickly recovers (21). This study also saw a return in the number of positive bacterial cultures 12 weeks after the completion of the erythromycin.

The responders as determined by bacterial clearance in the sputum did not demonstrate a greater degree of small airway inflammation or sputum neutrophilia and so hypothesis number two can be rejected.

Objective

- *Are the changes induced by the erythromycin therapy maintained after the medication is stopped?*

The improvements seen in the Leicester cough questionnaire total scores and the sputum production domain of the visual analogue scale are maintained following cessation of the erythromycin. Interestingly the sputum purulence domain of the visual analogue scale continues to fall but this was not found to be significant. There was 30ml mean decline in post-bronchodilator FEV_1 but this was not significant. The mean lung clearance index increases at visit 7 but was proven to show statistical similarity. The microbiological cultures are all similar in terms of bacterial diversity as demonstrated by the low Bray-Curtis Index results, however

the number of cultures without any significant growth returned to the pre-erythromycin level after the completion of the study.

4.12 Chapter summary

This chapter has involved the presentation and discussion of the clinical trial involving 40 participants who underwent and observation year followed by a 12 week intervention of 250mg daily erythromycin with a 12 week observational period prior to the completion of the study. The primary aim was not reached – the addition of erythromycin therapy did not result in a 200ml improvement in the post-bronchodilator FEV_1. Only one participant had such a large improvement in the spirometry, largely due to recovery from a prior unexplained reduction in their spirometry results. The reason for the end-point not being met is likely to be due to several factors. Spirometry does not alter dramatically with intervention in bronchiectasis, as this is more commonly a feature of cystic fibrosis and Asian diffuse panbronchiolitis. However, significant responses to the erythromycin course were seen in other areas: the Leicester cough questionnaire total score, the visual analogue scale sputum production score, the lung clearance index and the apparent clearance of pathogens from the sputum, albeit transiently. The hypothesis stated that participants who had a response to erythromycin would have a greater degree of sputum neutrophilia and small airway changes as seen on the CT severity scores and in the lung clearance index. Unfortunately the hypothesis was disproven in all these responder sub-categories. There was no clear predictor or response from the baseline pre-treatment data.

The erythromycin course was well tolerated and the majority of the participants did not report any adverse events. There were no concerns regarding the microbial resistance or increased culture of non-tuberculous mycobacterial growth.

The exact effect of the macrolide was difficult to ascertain, but a combination of reduced sputum production and possible transient bacterial clearance appeared to exert a beneficial effect on some of the study subjects. In future studies it would be useful to ask participants to self-rate their response to the erythromycin as a marker of treatment success against which the objective investigation results could be compared against. As with all interventions the response of the person involved is complex and cannot so far be predicted by a series of investigations.

However, providing the benefits outweigh the risks the treatment should be offered and the response monitored closely.

Chapter Five: Comparison between stable visits and exacerbation visits

5.1 Introduction

Frequent exacerbations are one of the main reasons for bronchiectasis patients to be referred to secondary and tertiary healthcare services. Along with stable visits the participants from the clinical trial were seen during exacerbations where possible. Data was collected with the purpose of characterising these exacerbations in more detail, particularly during the observation year.

5.1.1 The definition of an exacerbation

There are several documented ways to define an exacerbation but the general principles include an increase in respiratory symptoms such as change in sputum colour, increased sputum production and increased breathlessness without radiological evidence of pneumonia. One study has defined an exacerbation to be 4 or more symptoms including: change in sputum (volume, colour, haemoptysis), increased breathlessness, increased cough, fever >38 degrees, increased wheeze, reduced exercise tolerance or increased lethargy, >10% reduction in FEV_1 or FVC, radiographic changes, change in chest sounds (134). Another study required more than one symptom from a list of respiratory symptoms on at least 2 consecutive days plus an increase in the mean of 3 pre-defined symptom scores by at least one point from the previous week's score (150). In the UK, the accepted definition of an exacerbation involves an increased sputum volume or viscosity, increased sputum purulence and one or more of: increased cough, wheeze, breathlessness or systemic upset (6). Many studies divide exacerbations into moderate and severe. A moderate exacerbation requires oral treatment and a severe exacerbation would require admission to hospital (204).

5.1.2 The impact of exacerbations

The rate of exacerbations per year in a study setting has been reported to be between 2.05-3.3/year (112,134,150). There is some evidence that exacerbation

frequency can adversely affect lung function decline especially in *Pseudomonas aeruginosa* colonisation (180,199).

An exacerbation of any respiratory condition can dictate quality of life, reduce productivity at work and interrupt family life; bronchiectasis is no different (179,199,219). In fact, as the recommended course of antibiotics is 10 to 14 days it could be argued that the burden of exacerbations is greater in this group of patients. If a hospital admission is required then the burden on all aspects of life is even greater. Economic analysis performed in the USA has suggested the cost to be an additional 1.1 billion US dollars each year (30). When compared to those without bronchiectasis this study estimates that the individual accrues an additional 2 in-patient days and 6 out-patient visits.

A study of bronchiectasis patients in New Zealand who had been admitted for exacerbations reported that their mean length of stay was 5.2 days (sd 5.0) compared with 4.2 days for other respiratory patients at the same hospital (220). The difference in duration could be related to the longer antibiotic course of 10-14 days recommended for an exacerbation of bronchiectasis. This study did not find any differences between the number of admissions for exacerbations and socioeconomic deprivation but there were a significantly greater number of bronchiectasis related admission in the groups of Pacific and Maaori descent and with a worse deprivation score. One study of hospital admissions over a 15-year time frame found that there was a 46% readmission rate and a 21% mortality rate over the 12 month period following a hospital admission for an exacerbation.

5.1.3 The microbiology of an exacerbation

Sputum cultures are recommended to be taken both during stable periods and at exacerbations prior to the start of antibiotics. The microbiological profile of stable bronchiectasis has demonstrated that the following pathogens are the most commonly identified: *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and *Moraxella catarrhalis*. A considerable proportion of

patients do not culture any significant pathogens despite repeated sputum sampling (20,221).

The immune system can develop a tolerance to the organisms within the current microbiome however an inflammatory cascade and subsequent exacerbation can be the result of:

1. A new inhaled pathogen (218)
2. A new viral infection (222)
3. The change from the biofilm phase to the planktonic phase, particularly for *Pseudomonas* bacteria
4. A newly acquired strain of a current pathogen either by infection or mutation, such as in the case with non-typeable *Haemophilus influenzae* (223)
5. A reduction in the host defences such as an immunosuppressant medication
6. Spread of a colonising bacteria to a new area of the lung (23)

Bacteria are usually cleared from the body via a B-cell mediated antibody response, a T-cell medicated inflammatory cascade and phagocytosis from macrophages. However bacteria have evolved mechanisms for neutralising the attack from the innate and adaptive immune systems including the formation of a biofilm to shield the bacterial colony from toxins, antibiotics and the immune system (27,224). Bacteria can avoid clearance from the airway by a variety of other mechanisms (225) such as slowing the cilia to impair mucociliary clearance by inducing ciliary paralysis and a disorganised ciliary beat, (226,227) inhibiting the action of neutrophils, lymphocytes, macrophages and immunoglobulins, inhibition of neutrophil function (228,229) and developing the ability to become an intracellular pathogen, a process called endocytosis, as demonstrated by *Haemophilus influenzae* (230). These processes enable the bacteria to colonise the airways and to continue the vicious cycle of bronchiectasis described in the introduction chapter.

5.1.4 Treatment of bronchiectasis exacerbations

The BTS guidelines for non-cystic fibrosis bronchiectasis describes the recommended antibiotic therapy for the most common organisms in the event of an exacerbation (6). Fourteen days of antibiotic treatment are recommended. Along with antibiotic therapy, the optimal treatment for an exacerbation involves optimisation of airway clearance using physiotherapy techniques and aids such as hypertonic saline and acapella devices. Successful treatment of an exacerbation has been demonstrate to involve a reduced CRP and improved quality of life scores. There is not necessarily a significant change in sputum microbiology or sputum differential cell count between the start and completion of treatment (231).

5.2 Methods

5.2.1 Identifying an exacerbation

Participants who felt they were becoming unwell with an exacerbation were encouraged to contact the department at the point that they would usually start their stand-by antibiotics or see their GP. They were assessed for a change in symptoms including: increased cough, increased or greatly reduced sputum production, increased sputum purulence, fever, lethargy, increased wheeze, increased breathlessness and reduced exercise tolerance. The presence of two or more new symptoms suggested an exacerbation. The participant was invited into the department to undergo further investigations, but if they were unable to attend the subject would commence their stand-by antibiotics.

5.2.2 Exacerbation visit protocol

The following investigations were carried out: quality of life questionnaires (St George's respiratory questionnaire, Leicester cough questionnaire and the visual analogue scale for cough, dyspnoea, sputum production and sputum purulence), exhaled nitric oxide levels, pre- and post-bronchodilator spirometry, routine blood tests (full blood count, electrolytes and c-reactive protein) and induced sputum to obtain samples for the differential cell count, colony forming unit count, microbiology and fungal culture. Participants also underwent a physical examination and were prescribed antibiotics and/or steroids where necessary according to previous bacterial sensitivities and allergy history, in keeping with the University Hospitals of Leicester NHS Trust prescribing policy. If the exacerbation occurred between visits 5 and 6 the erythromycin was omitted for the period of the acute antibiotic. The duration of treatment was 10-14 days as per national recommendations. Exacerbations were treated as per usual clinical practice so chest x-ray was carried out if there was clinical concern. Physiotherapy was optimised by the specialist bronchiectasis physiotherapist as indicated at these visits.

The next stable visit was postponed until at least 6 weeks after the exacerbation visit to ensure the symptoms had settled and any inflammatory changes had returned to baseline. If a further exacerbation occurred before the next stable visit could be completed the visit was postponed once more.

This chapter will focus on the secondary objective as outlined below.

Objective

- *To evaluate the changes in sputum inflammation, microbiological and fungal cultures both when stable and during exacerbations.*

5.3 Results

5.3.1 Numbers of exacerbations

Where possible all patients with exacerbations were reviewed. Over the course of the study there were 104 exacerbations from 33 participants, meaning 7 did not have an exacerbation, of which one left the study after the first visit. The highest number of exacerbations in any participant was 8. The table below demonstrates the number of participants who had exacerbations and the number who were able to attend the research unit for further investigations.

There were 80 exacerbations during the observational year with a mean number of 2 (sd. 1.60) the observational year and 2.63 (sd. 2.06) for the whole study period (range 0-8). The study duration ranged from 18 months to 26 months depending upon the number of exacerbations.

Table 30: The breakdown of exacerbations during the study and the number of patients attending the research unit for their exacerbations

Exacerbation number	Number of patients n=40 (%)	Number attending the research unit n=33 (%)
1	33 (82.5%)	23 (69.7%)
2	26 (65.0%)	11 (42.3%)
3	18 (45.0%)	7 (38.9%)
4	12 (30.0%)	7 (58.3%)
5	7 (17.5%)	6 (85.7%)
6	5 (12.5%)	2 (40.0%)
7	2 (5.0%)	2 (100.0%)
8	1 (2.5%)	1 (100.0%)
Total, n (%)	**104 (100%)**	**59 (56.7%)**

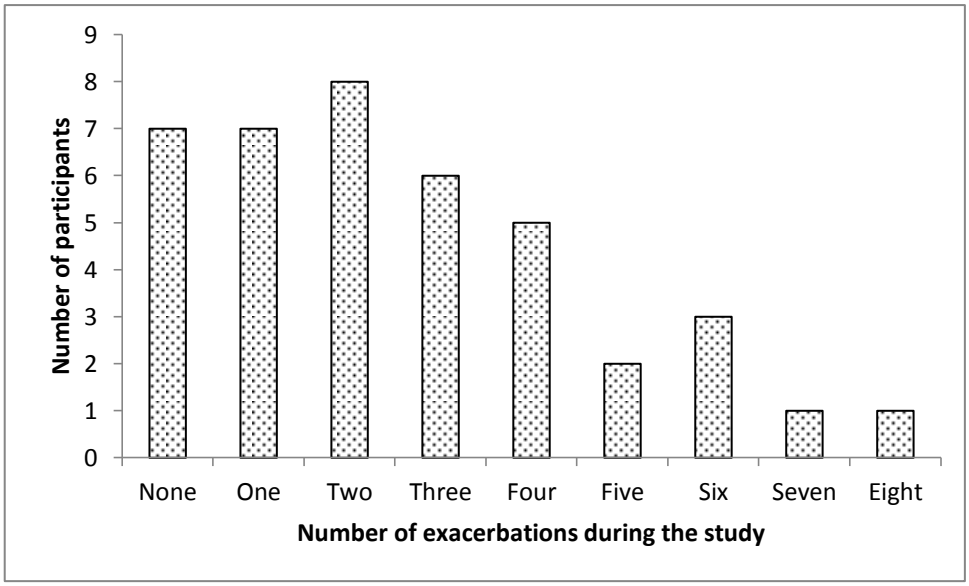


Figure 30: The number of participants who had exacerbations

There were 79 exacerbations prior to visit 5, the visit where the erythromycin intervention was started, and 24 exacerbations after visit 5 until the end of the study at visit 7. This means there 1.98 exacerbations per participant during the observation year and 0.65 exacerbations per participant during and after the erythromycin. However, it should be noted that the study was not powered to look for a statistical difference in the number of exacerbations pre- and post-erythromycin therapy.

5.3.2 Characteristics of stable and exacerbation periods. Symptoms from exacerbations

As with the baseline visit the current symptoms at the time of the exacerbation were recorded and have been shown together in the following table for comparison. During periods of good health 85% of the participants complained of a daily cough with an estimated daily sputum volume of 15ml. The presence of a cough affected 97.5% of those attending for an exacerbation and their daily estimated sputum production had increased to 40ml. The majority (77.5%) of the participants were Medical Research Council (MRC) class 1 and 2 when stable. An

exacerbation resulted in a reduction in functional status and a subsequent increase in the MRC class. Only 29.5% had an MRC class of 1 or 2 during an exacerbation. Six participants reported feeling so unwell during the exacerbation they were largely confined to a chair or bed (MRC 5). None of the participants reported haemoptysis when they were stable but this number increased to 37.5% during an exacerbation.

Table 31: The self-reported symptoms during stable periods and exacerbations

Self-reported characteristics			Value
Stable	**Self-reported cough, n (%)**		34 (85.0)
	Self-estimated sputum volume (ml), median (IQR)		15 (20)
	Self-reported haemoptysis, n (%)		0 (0)
	Medical Research Council dyspnoea scale, n (%)	**1**	9 (22.5)
		2	22 (55.0)
		3	5 (12.5)
		4	4 (10.0)
Exacerbations	**Self-reported cough, n (%)**		39 (97.5)
	Self-estimated sputum volume (ml), median (IQR)		40 (32)
	Self-reported haemoptysis, n (%)		15 (37.5)
	Medical Research Council dyspnoea scale, n (%)	**1**	1 (2.5)
		2	11 (27.5)
		3	10 (25.0)
		4	12 (30.0)
		5	6 (15.0)

5.3.3 Comparison of investigations between the baseline visit and the first exacerbation visit

The table below outlines all of the investigation results for both the baseline visit and the first exacerbation visit. There were 33 exacerbations with 23 participants attending the research unit to undergo investigations.

Table 32: The comparison of the investigations at the baseline visit and the first exacerbation

Questionnaire domain	Visit 1 n=23	Exacerbation 1 n=23	*p*-value
St George's Respiratory Questionnaire Total score, mean (sd)	33.96 (16.67)	48.52 (14.25)	**0.007***
Leicester Cough Questionnaire Total score, mean (sd)	15.95 (3.87)	12.12 (2.54)	**0.002***
VAS cough domain (mm), median (IQR)	29 (19-54)	71 (56-73)	**0.000^**
VAS dyspnoea domain (mm), median (IQR)	25 (9-29)	63 (28-76)	**0.001^**
VAS sputum production domain (mm), median (IQR)	36 (15-52)	72 (47-76)	**0.000^**
VAS sputum purulence domain (mm), median (IQR)	18 (4-49)	60 (49-77)	**0.001^**
Post bronchodilator FEV_1 (l), mean (sd)	2.04 (0.73)	1.96 (0.55)	0.204
Post bronchodilator FEV_1 (% predicted), mean (sd)	86.1 (26.1)	83.9 (22.0)	0.117
Post bronchodilator FVC (l), mean (sd)	2.90 (0.84)	2.75 (2.75)	0.195
Post bronchodilator FVC (% predicted), mean (sd)	97.0 (22.6)	95.8 (18.4)	0.189
Post bronchodilator ratio (%), mean (sd)	70.2 (11.9)	70.3 (10.6)	0.442
Exhaled nitric oxide (ppb), mean (sd)	25 (14)	29 (23)	0.663
Total cell count ($x10^6$/ml), median (IQR)	6.93 (3.16-14.80)	16.800 (5.490-30.450)	0.794
Neutrophil count (%), median (IQR)	91.00 (70.28-96.53)	94.78 (87.40-98.00)	0.546
Eosinophil count (%), median (IQR)	1.33 (0.50-3.38)	0.75 (0.25-2.25)	0.913
Absolute neutrophil count ($x10^6$), median (IQR)	6.515 (2.313-12.815)	15.907 (5.185-29.902)	1.000
Absolute eosinophil count ($x10^6$), median (IQR)	0.068 (0.024-0.246)	0.295 (0.091-0.444)	0.477

Quality of life questionnaires

There were significant differences between the baseline and exacerbation visit with all domains of the questionnaires. The total score for the St George's respiratory questionnaire increased from 38.96 (sd. 16.67) at visit 1 to 48.52 (sd. 14.25) at exacerbation 1, with a higher score indicating more symptoms. The total score for the Leicester cough questionnaire fell from 15.95 (sd. 3.87) at the baseline visit to 12.12 (sd. 2.54) at the first exacerbation, with a lower score indicating more symptoms. The Visual analogue scale scores increased in all domains at the exacerbation visit by 2-3 fold indicating an increase in cough, breathlessness, sputum production and purulence.

Airway function investigations – spirometry

There were no significant differences between the post-bronchodilator spirometry results at the baseline visit and the first exacerbation visit.

Airway inflammation investigations

Exhaled nitric oxide

The mean exhaled nitric oxide level increased from 25ppb (sd. 14ppb) at visit 1 to 29ppb (sd. 23) at the exacerbation visit but this was not significant.

Sputum differential cell counts

The mean total cell count was higher at the exacerbation visit than the baseline visit at 16.800×10^6/ml compared to 6.93×10^6/ml although this difference was not significant. The median neutrophil count was greater in the exacerbation group, due to the higher total cell count, but the percentages at the baseline and exacerbation visit were similar with the majority of the neutrophil counts falling between 90-95%. These differences were not statistically significant. As expected the eosinophil counts also varied between study participants with the majority of

the values lying around 1%. Again, there were no significant differences between the exacerbation and baseline groups.

Sputum bacterial culture results

Of the twenty-three patients with an exacerbation who attended the research unit for further investigations, twenty-one were able to expectorate sufficiently to enable a sample for bacterial culture to be sent. The sputum culture results were statistically similar in each group. The sputum bacterial colony forming units were lower at the exacerbation visit at $0.57x10^6$/ml compared to $0.77x10^6$/ml but this difference was not significant (p=0.859).

Table 33: The result of sputum microbiological culture during the first exacerbation (analysis based upon percentages)

		Visit 1	Exacerbation 1	*p*-value
No. samples		30	21	
No. bacterial isolates		22	16	
No. participants		40	23	
Sputum bacterial culture, n (%)	**No significant growth**	11 (36.7)	7 (33.3)	0.859
	Pseudomonas aeruginosa	3 (10.0)	2 (9.5)	
	Non-*Pseudomonas* bacteria	19 (63.3)	14 (66.7)	
	Insufficient sample	11 (27.5)	2 (8.6)	
Sputum colony forming units (10^6/ml), median (IQR)		0.770 (0.3-2.6)	0.57 (0.27-4.15)	0.507

5.3.4 The "non-exacerbators"

During the study period of 7 visits (18 months' minimum duration) there were 7 participants who did not have an exacerbation. One of these left the study very early so has been excluded from the analysis. The characteristics of this group were analysed against those who did have an exacerbation to identify any

differences which might have led to fewer exacerbations. The participants were divided into the categories of "no exacerbations", "one to three exacerbations" during the study period and "four or more exacerbations".

There were no significant differences between the groups except in the VAS sputum purulence domain results which was at its lowest level in the middle group who had one to three exacerbations. There was a tendency towards lower scores in the VAS cough, dyspnoea and sputum production groups but due to the wide interquartile range these results were not significant. The sputum bacterial colony forming units were highest in the non-exacerbation group but this was not significant.

Table 34: The comparison data at the baseline visit of those who did not have any exacerbations during the study period and those who had multiple exacerbations

	No exacerbations	One to three exacerbations	Four or more exacerbations	*p*-value
SGRQ total score, mean (sd)	37.09 (13.56)	38.52 (19.20)	36.92 (16.00)	0.965
LCQ total score, mean (sd)	15.03 (4.94)	14.58 (4.18)	16.09 (3.64)	0.625
VAS cough domain, median (IQR)	20 (4-54)	34 (20-54)	44 (15-48)	0.724
VAS dyspnoea domain, median (IQR)	11 (3-69)	24 (4-36)	39 (14-45)	0.634
VAS sputum production domain, median (IQR)	33 (13-53)	46 (19-67)	51 (36-68)	0.447
VAS sputum purulence domain, median (IQR)	51 (18-65)	34 (5-51)	55 (50-66)	**0.029**
Exhaled nitric oxide (ppb), mean (sd)	24.5 (13.4)	25.9 (13.1)	23.9 (12.0)	0.913
Post-bronchodilator FEV_1 (l), mean (sd)	2.22 (0.64)	2.12 (0.62)	1.92 (0.75)	0.617
Post-bronchodilator FEV_1 (%), mean (sd)	82.5 (22.2)	86.3 (24.6)	83.8 (25.8)	0.930
Lung clearance index, mean (sd)	9.492 (2.029)	9.448 (1.631)	10.327 (2.021)	0.436

Sputum total cell count (x10^6/ml)	6.350 (3.06-10.60)	8.245 (3.20-13.70)	7.730 (1.670-16.60)	0.978
Sputum neutrophils (%), median (IQR)	87.13 (64.0-97.0)	87.80 (69.80-95.25)	91.00 (75.0-97.0)	0.547
Sputum eosinophils (%), median (IQR)	1.00 (0.25-1.75)	1.50 (0.50-3.75)	1.00 (0-4.0)	0.826
Sputum neutrophils (x10^6/ml), median (IQR)	5.078 (1.805-10.388)	6.430 (2.125-11.970)	7.826 (2.955-15.106)	0.701
Sputum eosinophils (x10^6/ml), median (IQR)	0.041 (0.028-0.060)	0.069 (0.020-0.30)	0.070 (0-0.259)	0.742
No significant growth	1	7	2	
Positive culture	3	9	6	
Pseudomonas aeruginosa	0	0	2	
Non-*Pseudomonas* bacteria	3	7	4	
Insufficient sample	2	4	3	
Sputum bacterial colony forming units (x10^6/ml)	2.316 (1.100-2.510)	0.770 (0.440-1.385)	0.620 (0.460-3.500)	0.759
Total CT score	36.17 (14.19)	35.19 (14.65)	36.64 (18.87)	0.961
Bronchial wall dilatation score	5.50 (4.28)	7.57 (4.75)	8.00 (4.92)	0.563
Bronchial wall thickening score	5.50 (4.37)	4.43 (3.22)	5.45 (4.18)	0.691
Extent of bronchiectasis score	7.00 (4.65)	9.33 (3.15)	9.27 (3.66)	0.353
Mucus plugging large airways	1.33 (1.75)	2.00 (1.95)	2.64 (1.69)	0.376
Mucus plugging small airways	2.33 (2.25)	2.71 (2.33)	3.45 (2.50)	0.591
Attenuation score	8.83 (4.40)	5.86 (4.54)	5.09 (4.64)	0.264
Bronchial collapse score	5.50 (4.72)	3.19 (4.52)	2.64 (3.47)	0.407
Tracheal collapse score	0.17 (0.41)	0.05 (0.22)	0.09 (0.30)	0.645

5.4 Discussion

Many antibiotic trials in non-cystic fibrosis bronchiectasis use exacerbation frequency as an end-point, but do not characterise the exacerbations as part of the analysis. There are relatively few papers that have focused on exacerbations. Of these a large proportion have either recruited their participants during hospital admission or are comprised of a retrospective review of hospital notes, meaning the data will be automatically biased towards a more severe exacerbation.

5.4.1 Exacerbation frequency and symptoms

There were 104 exacerbations in 33 patients during the course of the study. The highest number of exacerbations in a single patient was 8 and the mean number of exacerbations was 2 (sd. 1.60) for the observational year and 2.63 (sd. 2.06) for the whole study period of 18-26 months. The duration of the study period was dependent upon the number of exacerbations. The exacerbation frequency was similar but slightly lower than the figure reported in the recent macrolide clinical trials of 2.05-3.3/year (112,134,150). The slightly lower exacerbation frequency might be explained by the fact that the participants of this study were recruited predominantly from clinics rather during a hospital admission, although this was not a contraindication to study entry. Only one participant required admission and one required self-administered intravenous antibiotics.

A proportion of the exacerbations did not yield any data as some participants did not feel well enough to attend the research unit for investigations. It was surprisingly common for participants to fall ill either while on holiday or over bank holiday weekend. In this instance antibiotics had been taken for some time before a visit could be arranged. There was a risk of obtaining data from a partially treated exacerbation so if antibiotics had been taken for more than 3 days prior to contact with the research unit the visit was not carried out.

The symptom burden obviously reflected the per-protocol definition of an exacerbation. Almost all had an increased cough and sputum production with

lethargy and reduced functional level, as reflected in the worsening MRC class proportions. There were a small group who felt very unwell but had a reduction in their usual sputum production. The definition of an exacerbation of bronchiectasis is not consistent between studies or guidelines but the principles are similar. The protocol for defining an exacerbation in this study was in keeping with the national bronchiectasis guidelines (6).

5.4.2 Visit investigations

Quality of life questionnaires

The SGRQ total score, LCQ total score and visual analogues scale domains of cough, dyspnoea, sputum production and purulence all changed significantly between the baseline and exacerbation visits suggesting a deterioration in symptoms. This reflects the symptom-related nature of the questionnaires, particularly the visual analogues scales which specifically address changes in the cough, breathlessness and sputum production, all of which are markers of an exacerbation. Previous studies have demonstrated a similar increase in the SGRQ score during an exacerbation (179) and significant improvement after completion of antibiotic treatment (231).

Airway investigations – spirometry

There were no significant differences between the mean spirometry results between the baseline visit and the exacerbation visit. In contrast to research in cystic fibrosis, the FEV_1 in non-CF bronchiectasis is neither a useful measure of the onset of an exacerbation nor a marker of an adequately treated exacerbation. However, spirometry reporting is variable between studies with half demonstrating some deterioration at the time of exacerbation (179) and half unable to demonstrate any significant change (21). The reasons behind this phenomenon are unclear and have neither been commented upon nor explored in

the literature. In the previous chapter I demonstrated that spirometry in bronchiectasis can vary considerably between individuals even during stable periods, in that some will have an increase in the FEV_1 and some will show a reduction in the FEV_1 over any given time period. It is possible that by chance some studies happen to have a greater number of those individuals with spirometric deterioration and other studies will have an equal mix of those who have a reduction and those who show an improvement in the FEV_1 during the study period with the net change being minimal.

Exhaled nitric oxide

This biomarker is a surrogate for eosinophilic airway inflammation rather than a bacterial or viral driven exacerbation (186,232). It is, therefore, unsurprising that there were no significant changes between the stable and exacerbation visit.

Sputum differential cell counts

The sputum neutrophil count is highly neutrophilic in non-CF bronchiectasis even during the stable state (112). Sputum purulence has been used as a surrogate marker for sputum neutrophilia and airway bacterial load (233) and has also been demonstrated to increase along with other sputum inflammatory markers during exacerbations (11,234). However, the sputum inflammatory cell counts did not alter significantly at the time of an exacerbation in my study population despite the assumption that the neutrophil count would rise. This has been supported by the findings in previous studies (179).

Sputum bacterial culture

The most commonly identified bacteria at the time of an exacerbation in non-CF bronchiectasis are *Haemophilus influenzae, Pseudomonas aeruginosa* and *Streptococcus pneumoniae* (220,231).

My study demonstrated a non-significant reduction in the sputum bacterial colony forming units between the baseline and first exacerbation visit. This mirrors the findings from a detailed microbiological study by Tunney et al. where both the aerobic and anaerobic bacterial numbers fell during an exacerbation independently of antibiotic treatment (21). Another study of stable bronchiectasis patients did not find any change between the sputum bacterial culture rates when stable and during exacerbations, which prompted the authors to question whether bacteria may not be solely responsible for the exacerbations (179). However, some studies have found that exacerbations have been demonstrated to be associated with a high bacterial load and a higher degree of sputum inflammation (223,233).

My study has shown that the bacterial culture proportions are similar in the baseline and exacerbation visit. Unfortunately we could not carry out detailed microbiome analysis using culture-independent methods which would have enabled us to comment on the bacterial diversity within the samples at various visits. Interestingly the bacterial population the lung has been shown to become much less diverse during an exacerbation than in the stable state (21). However, there is also evidence that each individual has their own microbiological profile which is largely maintained over time even during exacerbations (19,21).

5.4.3 The "non-exacerbators"

There were 6 participants who did not have an exacerbation during the study period. On reviewing the baseline characteristics there were no significant differences between those who had an exacerbation and those who did not. They had a tendency to lower (better) scores on the visual analogues scales for cough, sputum production and dyspnoea but this was not statistically significant due to the wide interquartile ranges.

Antibiotic trial studies don't pass any comments on those subjects who don't experience an exacerbation during the study period, although this may be due to the inclusion criteria specifying a certain exacerbation frequency in the year prior

to the entry to the study. However, in my analysis those who did not have an exacerbation still had a similar degree of baseline symptoms, airway dysfunction and inflammation. Interestingly there were no differences in the CT derived bronchiectasis severity scores. This does not answer the question of why some patients exacerbate frequently and some don't but further research around this subject is required.

5.5 Conclusion

This chapter describes the changes in the quality of life questionnaires, airway inflammation, airway function and sputum microbiota between periods of stable health and acute exacerbations. The anticipated deterioration in the quality of life questionnaires was partly due to the symptom driven definition of an exacerbation. There were no significant differences seen in any of the other investigations – the sputum was similarly neutrophilic, the spirometry remained stable and the sputum colony forming units fell during an exacerbation although this was not significant. These findings have been demonstrated in previous research studies although the body of research is conflicting in all these areas. The sole aspect of exacerbations that has not received any attention involves the bronchiectasis patients who do not have frequent exacerbations. This study looked at these patients in detail but interestingly they were not physiologically different to their fellow participants who suffered recurrent exacerbations, even in terms of bronchiectasis severity. This is an area which merits further investigation. Perhaps improved understanding of why some bronchiectasis patients have fewer exacerbations than others will aid better methods of infection reduction for future patients.

Chapter Six: Filamentous and non-filamentous fungi and non-cystic fibrosis bronchiectasis

6.1 The role of fungi in bronchiectasis

Fungi are eukaryotic micro-organisms genetically more similar to animals than plants. Fungal cell walls contain chitin, rather than cellulose or glycoproteins, which is a hard structure that is more recognised as part of the exoskeleton of crustaceans and insects. Fungi are ubiquitous within the indoor and outdoor environments. The characterisation of the fungal airway microbiome is less well understood than the bacterial microbiome. Fungal detection by culture has historically yielded small numbers of organisms but improved culture-dependent and independent techniques are being developed with exciting results (235–238). Where we now know the lungs are not sterile of bacteria, the transient presence and colonisation of fungi within the lungs is also becoming increasingly recognised.

6.1.1 Overview of the most common fungi in humans

The air around us contains many small particles including fungal spores which are inhaled and grow within the nutrient rich environment of the lungs. Thermophilic fungi, such as *Aspergillus* species, are able to germinate at body temperature which can lead to non-invasive fungal growth within the bronchial lumen (fungal bronchitis), mycetoma formation and semi-invasive or invasive lung infection. Patients with chronic lung diseases have a greater chance of encouraging increased fungal growth for the same reasons that they are more prone to bacterial infections – impaired innate immunity and disordered mucociliary clearance (239–243). The increased use of inhaled and oral corticosteroids and antibiotics encourage the local lung environment to become more favourable for fungal growth by reducing the number of organisms competing for nutrients (241). This section introduces the most common fungal pathogens in respiratory disease and the growing body of evidence regarding fungal growth in specific chronic respiratory conditions.

Candida species

Candida is the most common non-filamentous fungus to colonise oral, respiratory, genital and gastro-intestinal mucosa and epithelial surfaces in healthy humans, of which *Candida albicans* is the most prevalent. Invasive candidiasis is the fourth most common hospital acquired sepsis (244) with the risk factor for development being neutropaenia, especially caused by haematological malignancies, critical illness, prolonged steroid use, intensive care treatment and multiple indwelling lines (245). Candida bronchitis has been proposed as an under-recognised and under-treated condition in patients with chronic sputum production and respiratory symptoms (246).

Aspergillus species

Members of the *Aspergillus* genus are found growing in both nutrient-rich (food) and nutrient-poor environments (walls) as well as in damp and decaying material such as compost and leaf litter. Inhaled spores develop into fungal hyphae within the lungs. The most common species involved in lung disease is *Aspergillus fumigatus*. *Aspergillus* related lung diseases include: Allergic Broncho-Pulmonary Aspergillosis (ABPA) or Mycosis (ABPM), fungal ball formation with a pre-existing cavity (Aspergilloma), semi-invasive *Aspergillus* infection and invasive disease. Invasive disease occurs in immunosuppressed individuals. Defects found within the IL-17 signalling pathway (247,248), T-cell mediated immunity (249), hyper-IgE syndrome with signal transducer and activation of transcription gene 3 (STAT3) related immunodeficiency (250) and Chronic Granulomatous Disease (CGD) (251) predispose to invasive *Aspergillosis*.

Penicillium species

These fungi are abundant in the environment, present both indoors in dust and outdoors in soil. They are responsible for rotting food, the most easily recognised

form being bread mould, but have role in cheese manufacture and the penicillin producing pharmaceutical industry.

Scedosporium species

This saphrophytic fungus, often found in the soil from potted plants and stagnant water, is the second most commonly identified filamentous fungus seen in cystic fibrosis with a prevalence of 6.5-10% (252). Successful culture of this organism requires a selective media in order to eradicate *Aspergillus* growth, such as SceSel+ culture medium.

6.1.2 The role of fungus in chronic lung conditions

Asthma

Fungi play an important role in the pathogenesis and overall disease control of asthma. Fungal sensitisation affects up to 10% of the healthy population and up to 70% of individuals with severe asthma. Fungal detection and colonisation has been associated with a lower FEV_1 in affected asthmatics when compared to asthmatics who are not colonised with fungi (239). Another study has detected that asthmatic lungs cultured a different population of fungi than non-asthmatic lungs, although the clinical relevance of this is as yet unknown (253).

Chronic obstructive lung disease

The role of fungal sensitisation and colonisation in COPD is increasingly being investigated (242,254). A study of 128 patients with all stages of COPD demonstrated that *Aspergillus fumigatus* was the most prevalent fungus identified, seen in 36.7%, using enhanced culture techniques, while 12.5% cultured other filamentous fungi, principally *Penicillium* species (240). The group with a positive fungal culture had a sputum total cell count double that from the culture negative

group and this was linked to the significantly higher doses of inhaled corticosteroid use in this group. However, there were no significant differences in the quality of life questionnaires, *Aspergillus* sensitisation, spirometry, GOLD classification and blood eosinophilia between the two groups. Exacerbation data was also captured and there was no association between bacterial positive exacerbations and fungal culture.

Cystic fibrosis

As with the bacterial microbiome, multiple fungal species are present in the lungs of cystic fibrosis patients in large numbers. The most commonly cultured fungal species are *Aspergillus* species, particularly *fumigatus*, *Scedosporium* species, *Exophialia* species and *Candida* species with indoor potted plants proposed as a significant risk factor in this group (252). Cystic fibrosis patients have been found to have an increased prevalence of fungal sensitisation compared to the general population (255–257).

Bronchiectasis

There is very little in the way of research in fungal presence in non-cystic fibrosis bronchiectasis, unlike in cystic fibrosis and asthma. Bronchiectasis can exist as a consequence of prolonged inflammation caused by allergic bronchopulmonary mycosis but this is an uncommon cause.

6.1.3 Fungal hypersensitivity

Fungi, along with pollens, are one of the main aeroallergens causing sensitisation in up to 10% of the population. This sensitisation is not always symptomatic or clinically important but has been found to be far more common in asthma, bronchiectasis and cystic fibrosis without meeting the diagnostic criteria of allergic broncho-pulmonary mycosis (ABPM) (258). The diagnostic criteria for ABPM are

as follows: (1) History of asthma, (2) Positive fungal IgE or skin prick test, (3) Positive fungal IgG precipitins, (4) Proximal bronchiectasis, (5) Blood or sputum eosinophilia, (6) Total serum IgE >1000ng/ml, (7) Flitting pulmonary infiltrates.

Fungal spores are sufficiently small to be inhaled into the airways, sinuses and alveoli where they provoke an IgE-mediated allergic response. Most of the spores identified in outdoor air belong to the *Ascomycota* and *Basidiomycota* phyla, which include *Aspergillus*, *Penicillium*, *Alternaria* and *Cladosporium* species (258). *Aspergillus* species and *Penicillium* species form the majority of the spores identified from indoor air with levels peaking during the autumn months in the UK (259).

Patients with a history of atopy are most likely to become sensitised to *Aspergillu*s above other fungi, although sensitisation to *Penicillium* species and *Candida* are also a cause of ABPM (255,260,261). Fungal sensitisation is distinct from the development of the condition allergic broncho-pulmonary mycosis which involves the development of IgG antibodies, mucus plugging, flitting consolidation and possible evolution to bronchiectasis. The distribution is typically upper lobe rather than lower lobe (262).

Aeroallergen sensitisation also depends upon repeated or prolonged exposure to the allergen. We know that patients with severe lung disease have deficiencies in their innate immunity including the mucociliary escalator mechanism for lung clearance, allowing for colonisation and sensitisation. It is not understood why the prevalence of ABPA is higher in cystic fibrosis (an IgE-independent condition) than COPD, where both conditions demonstrate reduced airway clearance due to a variety of mechanisms. Several genetic factors have been demonstrated to increase the likelihood of ABPA developing. These are polymorphisms in IL-4, IL-10, SP-A, CFTR in cystic fibrosis and Toll-like receptors (263–267).

6.1.4 Methods of fungal culture

Traditionally it has been felt that a positive sputum fungal culture is an unusual occurrence and almost certainly indicates a pathological condition. However, it has become clear that improved culture methods and culture-independent techniques can dramatically improve yield (236).

Culture-dependent methods

Routine laboratory sputum culture follows national protocols and bacterial detection is favoured over fungal yield. Standard laboratories follow the Health Protection Agency B57 (2.5, 2014) protocol using Sabouraud dextrose agar (SDA) inoculated with homogenised and diluted sputum and a culture period of 48 hours (238). Specific fungal culture methods are employed in the setting of cystic fibrosis in specialised centres but not generally in other respiratory conditions. Research centres specialising in fungal diseases have developed a protocol which greatly improves fungal culture growth rates plating concentrated sputum plugs on favourable culture medium such as Potato Dextrose Agar containing 16mcg/ml chloramphenicol, 4mcg/ml gentamicin and 5mcg/ml fluconazole (PGCF) and an increased culture period of up to 6 weeks (236,268). As *Aspergillus* species have a tendency to overshadow other fungal culture, selective media to inhibit *Aspergillus* culture and promote *Scedosporium* culture have been devised such as SceSel+ (*Scedosporium* selective) culture medium.

Culture-independent methods

Further insight into the fungal microbiome has been enabled by the development of culture-independent methods using polymerase chain reaction (PCR) based techniques. This process amplifies a fragment from the 18s RNA of the fungus and allows the identification of several species in one sample (269).

6.2 Methods

The induced sputum sample from the participants at the stable visit was collected in a sterile container and stored on ice in the University of Leicester sputum processing laboratory. Both PGCF culture medium (potato dextrose agar (PDA) containing 16mcg/ml chloramphenicol, 4mcg/ml gentamicin and 5mcg/ml fluconazole) and SceSel+ culture medium were used.

As per protocol the samples were processed within 2 hours within a class II hood. The sputum sample was divided into two equal parts. One part was used to obtain a homogenized sample, the other to obtain sputum plugs.

The sputum plug was removed and placed on a culture medium made of potato dextrose agar (PDA) containing 16mcg/ml chloramphenicol, 4mcg/ml gentamicin and 5mcg/ml fluconazole (PGCF).

The homogenised sputum sample was mixed with an equal volume of 0.1% DL-dithiothreitol (DTT) and incubated at 37°C for 15 minutes. After incubation, 10µlitres of homogenized sputum were diluted to a 1:500 concentration in sterile water. Aliquots (10µlitres and 100µlitres) of both homogenized and diluted-homogenized sputum were inoculated in parallel onto both PGCF and SceSel+ plates.

In addition approximately 150mg of sputum plug was inoculated onto PGCF and SceSel+ plates. As an additional control, 100µlitres of 0.1% DTT was inoculated onto both media. All plates were sealed with Nescofilm™ and then transferred to the University fungal laboratory where they were incubated for seven days at 37°C.

Plates were inspected, without opening, three times at 40-48 hours, at 5 days and on day 7. The number of visible colonies was recorded at each time point. After seven days, filamentous and non-filamentous colonies were examined and identified based on macroscopic and microscopic features.

6.3 Results

Induced sputum samples collected at the stable visits were plated and sent to the University of Leicester mycology laboratory for culture and species detection. The results have been divided into filamentous and non-filamentous (candida) fungal cultures.

6.3.1 Filamentous fungi

Only four participants did not have any positive fungal cultures from the entire study. For this reason it is very difficult to divide participants into positive and negative fungal cultures. The results presented below have been categorised by the number of visits during the study with a positive fungal culture for that participant. The investigations analysed are from the baseline visit.

Associated conditions, steroid use and fungal sensitisation

There was no statistical difference in the number of participants with asthma or ABPA in terms of frequency of fungal culture when compared to the participants without. Steroid use, either inhaled or oral, also did not cause an increase in the number of fungal positive samples when compared to the participants who did not take any steroid treatment. The total IgE levels significantly reduced as the number of positive sputum fungal cultures increased. There was a non-significant increase in the *Aspergillus* specific IgG as the number of fungal cultures increased. While the differences in the specific fungal IgE levels and the *Aspergillus* IgG levels were not significant across the groups there were no positive test values for any of these investigations in the group who did not culture any fungus during the study.

Table 35: The effect of fungal culture frequency and asthma, ABPA, steroid use and fungal sensitisation.

		Number of visits with positive fungal cultures			*p*-value
		None (4)	1-2 (20)	3 or more (12)	
Asthma (n)	**No**	2	13	10	0.380
	Yes	2	7	2	
ABPA (n)	**No**	4	16	12	0.174
	Yes	0	4	0	
Inhaled steroids (n)	**No**	2	10	4	0.646
	Yes	2	10	8	
BDP equivalent (mcg), median (IQR)		400 (0-1200)	200 (0-1200)	650 (0-1600)	0.718
Oral steroids (n)	**No**	4	19	10	0.428
	Yes	0	1	2	
Oral steroid dose, median (IQR)		0 (0)	0 (0)	0 (0)	0.394
Total IgE , median (IQR)		102.4 (43.4-279.0)	66.7 (18.0-279.5)	17.0 (11.0-33.4)	**0.038***
Total IgE	**Negative**	2	12	12	**0.032***
	Positive	2	8	0	
Aspergillus IgE, median (IQR)		0.06 (0.03-0.13)	0.04 (0.03-1.55)	0.03 (0.02-0.17)	0.560
Aspergillus IgE (n)	**Negative**	4	14	10	0.368
	Positive	0	6	2	
Aspergillus IgG, median (IQR)		4.0 (4.0)	27.1 (15.8-43.7)	35.8 (12.4-36.0)	0.236
Aspergillus IgG (n)	**Negative**	1	12	9	0.566
	Positive	0	6	2	
Penicillium IgE, median (IQR)		0.02 (0.01-0.02)	0.01 (0.01-0.59)	0.01 (0-0.02)	0.175
Penicillium IgE (n)	**Negative**	3	14	11	0.321
	Positive	0	5	1	
Candida IgE, median (IQR)		0.02 (0-0.30)	0.02 (0-0.97)	0.00 (0-0.1)	0.076
Candida IgE (n)	**Negative**	3	13	12	0.061
	Positive	0	6	0	

Quality of life questionnaires, airway function and bronchiectasis severity

The most significant differences between the groups were seen in the visual analogue scale sputum purulence domain, where the scores increased steadily as the number of culture positive visits increased from 29mm (4-54mm) with no positive samples to 53mm (50-70mm) at 3 or more positive samples (p=0.049). The other visual analogue scale domains also demonstrated an increase in symptoms with increased frequency of sputum fungal growth. The Leicester cough questionnaire also demonstrated a reduction in the scores with increased fungal detection suggesting more frequent fungal culture is associated with an impact on quality of life and respiratory symptoms.

The absolute value of the FEV_1 (l/min) fell as the number of sputum fungal cultures increased, however, the percentage predicted value demonstrated the reverse trend – a lower predicted value was seen in the group who did not culture any fungi in the sputum. The lung clearance index steadily increased as the number of positive samples increased although this was not significant.

There was no difference between any of the CT scoring domains apart from the bronchial wall thickening domain which was higher (worse) as the number of positive samples increased (p=0.034).

Table 36: The effect of number of positive fungal cultures on the quality of life questionnaires, spirometry, lung clearance index and CT scoring.

	Number of visits with positive fungal cultures			*p*-value
	None (4)	1-2 (20)	3 or more (12)	
SGRQ Total, mean (sd)	41.93 (25.84)	36.09 (15.92)	38.86 (11.68)	0.910
LCQ Total, mean (sd)	16.02 (5.52)	15.40 (3.46)	13.90 (3.94)	0.483
VAS Cough domain (mm), median (IQR)	14 (6-55)	30 (16-50)	44 (24-55)	0.343
VAS Dyspnoea domain (mm), median (IQR)	22 (4-63)	21 (7-39)	33 (18-47)	0.543
VAS Sputum Production domain (mm), median (IQR)	26 (11-63)	38 (19-56)	61 (50-73)	0.069
VAS Sputum Purulence domain (mm), median (IQR)	29 (4-54)	42 (7-53)	53 (50-70)	**0.049***
Post bronchodilator FEV_1 (l), mean (sd)	2.19 (0.78)	2.02 (0.61)	1.91 (0.54)	0.317
Post bronchodilator FEV_1 (%), mean (sd)	78.9 (21.3)	82.0 (24.7)	82.4 (22.6)	0.141
Post bronchodilator FVC (l), mean (sd)	2.95 (0.86)	2.89 (2.89)	2.82 (0.59)	0.793
Post bronchodilator FVC (%), mean (sd)	83.6 (14.5)	94.4 (21.3)	99.5 (21.1)	0.197
Post bronchodilator ratio (%), mean (sd)	72.8 (8.5)	69.4 (13.7)	67.1 (9.7)	0.267
Total CT score	26.50 (10.54)	40.44 (17.61)	34.75 (12.54)	0.224
Bronchial wall dilatation score	3.75 (2.63)	9.17 (5.60)	6.33 (3.31)	0.108
Bronchial wall thickening score	2.75 (3.20)	5.72 (3.85)	5.75 (2.86)	**0.034***
Extent of bronchiectasis score	6.00 (4.69)	10.11 (3.46)	7.83 (3.16)	0.097
Mucus plugging large airways	1.25 (1.89)	2.50 (1.98)	2.08 (1.56)	0.390
Mucus plugging small airways	0 (0)	3.44 (2.23)	3.08 (2.15)	0.055
Attenuation score	6.75 (4.35)	6.28 (4.61)	6.08 (4.78)	0.935
Bronchial collapse score	5.75 (6.02)	3.06 (3.75)	3.58 (4.74)	0.637
Tracheal collapse score	0.25 (0.5)	0.11 (0.32)	0 (0)	0.381
Lung clearance index	8.716 (1.204)	9.953 (1.831)	10.370 (2.119)	0.280

Airway inflammation and bacterial culture

The nasal nitric oxide levels were significantly higher in the group who did not culture any fungi at 523.1ppb (sd. 398.7) falling to 178.6ppb (sd. 124.2) in the group who had 3 or more positive samples. The sputum total cell count was at its lowest in the group without any fungal growth at $3.500x10^6$/ml cells. The sputum neutrophil percentage climbed from 67.5% in the group without fungal growth to 92.3% in the group who cultured fungi on 3 or more occasions. A similar picture was seen in the absolute neutrophil counts. There was a fall in the eosinophil percentage as the number of fungal cultures increased. There was a non-significant decrease in the bacterial colony forming unit counts as the number of occasions the fungal cultures increased.

Table 37: The effect of frequency of sputum fungal culture on sputum inflammation.

	Number of visits with positive fungal cultures			
	None (4)	**1-2 (20)**	**3 or more (12)**	**p-value**
Exhaled Nitric Oxide (ppb), mean (sd)	18.0 (8.9)	28.3 (12.8)	22.4 (11.2)	0.295
Nasal Nitric Oxide level (ppb), mean (sd)	523.1 (398.7)	321.3 (163.3)	178.6 (124.2)	**0.009***
Sputum total cell count ($x10^6$/ml), median (IQR)	3.500 (2.060-6.585)	9.300 (2.380-19.150)	8.245 (4.725-20.050)	0.379
Sputum neutrophils (%), median (IQR)	67.50 (61.25-84.25)	85.00 (70.28-95.38)	92.30 (82.00-97.00)	0.300
Sputum eosinophils (%), median (IQR)	1.50 (0.75-4.00)	1.45 (0.50-3.88)	1.00 (0.50-2.25)	0.955
Sputum neutrophil count, ($x10^6$/ml), median (IQR)	2.153 (1.282-5.727)	6.630 (1.695-13.538)	7.782 (3.974-24.540)	0.228
Sputum eosinophil count ($x10^6$/ml), median (IQR)	0.060 (0.025-0.330)	0.072 (0.019-0.266)	0.070 (0.041-0.259)	0.942
Sputum bacterial colony forming units ($x10^6$/ml), median (IQR)	1.598 (0.483-3.005)	1.035 (0.280-2.600)	0.634 (0.440-1.085)	0.773

Fungal culture results

The results of the sputum fungal cultures are listed in the table below. The most commonly cultured fungi were the *Aspergillus* species – *fumigatus, flavum, niger* and *nidulantes. Aspergillus fumigatus* was cultured on 56 occasions, followed by *Aspergillus niger* on 7 occasions, *Aspergillus flavia* on 4 occasions and *Aspergillus nidulantes* on 2 occasions. *Penicillium* species were cultured from 5 sputum samples and other fungi from 15 samples. The "other" group consisted of: unidentified white filamentous fungus, *Phanerochaete sordida, Rhizomucor pusillus, Rhizopus microspora, Paecilomyces species, Thermoascus crustaceous, Ceriporia* species, *Corticiaceae* species and *Phlebia suberialis.*

The SceSel+ cultures consisted of the fungi listed in the table below. They included: *Gloeophyllum trabeum*, *Coprinellus xanthotrix*, *Hyphodontia palmae*, *Coprinopsis cinerea*, *Rhizomucor meihei*, *Talaromyces* species and *Agaricaceae* species. They were all isolated findings on a single occasion apart from *Rhizomucor meihei* and *Hyphodontia palmae* which were cultured on two separate occasions.

The median colony forming units were between 0 and 1 but the interquartile range was from 0-29 for the PGCF and SceSel+ combined data.

During exacerbations (table below) the fungal growth was reduced in terms of colony forming units and variation. Aspergillus accounted for the majority of the species identified. Non-filamentous fungi in the form of candida were cultured in similar proportions to the stable visits.

Table 38: The sputum fungal culture results.

		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	n
Number attending visit		40	39	36	38	35	35	35	
Number of sputum samples suitable for fungal culture		36	32	25	23	26	17	19	
Fungal isolates / samples		14/13	17/16	10/7	12/11	17/15	11/9	8/7	
Result from PGCF culture medium (n)	***Aspergillus fumigatus***	8	11	5	7	11	9	5	56
	Aspergillus niger	1	0	2	1	2	1	0	7
	Aspergillus flavia	2	0	1	0	1	0	0	4
	Aspergillus nidulantes	1	0	0	0	0	0	1	2
	***Penicillium* species**	1	1	1	0	0	1	1	5
	Other	1	5	1	4	3	0	1	15
	No growth	23	16	18	12	11	8	12	
SceSel+ culture (n)		*Gloeophyllum trabeum* (1) *Coprinellus xanthothrix* (1) White filamentous (1)	*Hyphodontia palmae* (1) White filamentous (2) *Rhizomucor miehei* (1) *Coprinopsis cinerea* (1)	*Phanerochaete chrysosporium* (1) White filamentous (1) x2 *Talaromyces stollii* (1)	None	*Rhizomucor miehei* (1) *Phanerochaete sordida* (1)	*Agaricaceae* species (1) *Talaromyces funiculosus* (3) *Hyphodontia palmae* (1)	None	
Fungal colony forming units, median (range)		0 (0-4)	1 (0-29)	0 (0-8)	0 (0-3)	1 (0-21)	1 (0-26)	0 (0-10)	
Yeast colony forming units, median (range)		7 (0-200)	4 (0-150)	0 (0-130)	3 (0-300)	5 (0-401)	7 (0-163)	2 (0-130)	

Table 39: Filamentous and non-filamentous fungal culture during exacerbations

		Exac 1	Exac 2	Exac 3	Exac 4	Exac 5	Exac 6	Exac 7	Exac 8
Number of exacerbations (n)		33	26	18	12	7	5	2	1
Attended (n)		23	11	7	7	6	2	2	1
No. sputum samples suitable for fungal culture (n)		20	7	5	4	6	0	2	1
No. fungal isolates / samples (n)		5/5	6/5	4/3	0/0	2/2	0/0	1/1	0/0
Result (n) from PGCF culture medium	***Aspergillus fumigatus***	5	5	2	0	2	0	1	0
	Aspergillus niger	0	0	0	0	0	0	0	0
	Aspergillus nidulantes	0	0	0	0	0	0	0	0
	Aspergillus flavia	0	0	1	0	0	0	0	0
	Penicillium sp.	0	1	1	0	0	0	0	0
	Other	0	0	0	0	0	0	0	0
Additional isolates from SceSel+ culture medium		0	0	0	*Coprinellus xanthothrix* CFU=1	0	0	0	0
No growth		15	2	2	4	4	0	1	1
Fungal colony forming units, median (range)		0 (0-7)	2 (0-5)	0 (0-3)	0 (0)	0 (0-9)		1 (0-1)	0 (0)
Yeast colony forming units, median (range)		6 (0-250)	45 (0-200)	15 (0-400)	1 (0-60)	5 (0-200)		60 (13-106)	14 0)

6.3.2 Non-filamentous fungi

Four participants (10%) cultured *Candida* at all seven visits and 4 participants (10%) did not culture any *Candida* at all during the visit although 2 of these did not produce any sputum samples so this means 2 participants (5%) never cultured *Candida* species in the sputum. Therefore 95% of the study participants cultured yeast at some point during the trial.

The table below is the comparison between the participants who are colonised with non-filamentous fungi and those who were not. If the sputum sample was positive for *Candida* growth on 2 or more occasions throughout the study period this was counted as colonisation, as all the stable study visits were 12 weeks or further apart. This is in keeping with the BTS definition of bacterial colonisation in bronchiectasis (6). The only significant differences between the two groups were seen in the total scores of the SGRQ and the LCQ scores.

Table 40: Comparison of investigations and baseline data between participants who were colonised with Candida species and those who were not.

		Not colonised with *Candida* species n=16	Colonised with *Candida* species n=24	*p*-value
Asthma (n)	**No**	12	17	0.775
	Yes	4	7	
Allergic bronchopulmonary aspergillosis (n)	**No**	16	20	0.089
	Yes	0	4	
Inhaled steroids (n)	**No**	9	9	0.249
	Yes	7	15	
BDP equivalent (mcg), median (IQR)		0 (0-1200)	650 (0-1600)	0.484
Oral steroids (n)	**No**	13	23	0.137
	Yes	3	1	
Oral steroid dose (mg), median (IQR)		0(0)	0 (0)	0.145
***Candida* IgE level**		0.01 (0-0.03)	0.01 (0-0.08)	0.538
***Candida* IgE**	**Negative**	12	20	0.848
	Positive	2	4	
SGRQ total score, mean (sd)		30.99 (16.69)	41.68 (15.95)	**0.048***
LCQ total score, mean (sd)		16.96 (3.56)	14.02 (3.95)	**0.022***
VAS cough domain (mm), median (IQR)		21 (12-39)	43 (19-59)	0.057
VAS dyspnoea domain (mm), median (IQR)		14 (4-38)	27 (13-45)	0.163
VAS sputum production domain (mm), median (IQR)		21 (14-48)	54 (35-67)	0.062
VAS sputum purulence domain (mm), median (IQR)		35 (10-59)	51 (18-54)	0.498
Post-bronchodilator FEV_1 (l/min), mean (sd)		2.05 (0.80)	2.07 (0.53)	0.943
Post-bronchodilator FEV_1 (% predicted), mean (sd)		87.6 (27.0)	82.9 (22.5)	0.552
Post-bronchodilator FVC (l/min), mean (sd)		2.94 (0.73)	2.88 (0.59)	0.771
Post-bronchodilator FVC (% predicted), mean (sd)		104.2 (19.2)	92.2 (20.7)	0.078
Lung clearance index, mean (sd)		10.365 (2.026)	9.430 (1.638)	0.137
Exhaled nitric oxide level (ppb), mean (sd)		24.3 (14.3)	24.8 (11.3)	0.897
Sputum total cell count (10^6/ml), median (IQR)		6.535 (3.430-10.60)	8.915 (2.415-19.150)	0.868
Sputum neutrophil count (%), median (IQR)		93.40 (70.28-97.40)	89.40 (69.13-95.38)	0.513
Sputum eosinophil count (%), median (IQR)Sputum eos% V1		0.88 (0.25-3.00)	1.45 (0.50-3.50)	0.511
Total CT score, median (IQR)Total CT score		30.50 (25.0-37.0)	35.50 (30.0-50.0)	0.173

6.4 Discussion

6.4.1 Filamentous fungi

All but 4 participants cultured fungus in their sputum at some point during the study period. For the most part the fungi were cultured in low numbers.

Quality of life questionnaires

There were no significant differences in the SGRQ and LCQ total scores between the fungal culture groups. It has been suggested that sputum fungal detection is linked with lower quality of life scores (240) but that was not suggested by my data. The visual analogue scale scores for the cough, sputum production and sputum purulence domains were progressively higher as the number of positive sputum samples increased. This was only significant in the sputum purulence domain, a finding which has been discussed in previous research (270). The trend suggested an association between sputum production and purulence and fungal culture. It might be that an increased sputum volume provides a more nutrient rich environment for fungal culture, or rather that fungal growth stimulates sputum production.

Steroid use

It has previously been found that inhaled and oral steroid use predisposes to fungal growth (240,241). Indeed it is well known that oral candida is a common side-effect of inhaled steroid treatment in asthma. However, my data did not demonstrate any difference in fungal culture frequency between those who were prescribed inhaled or oral steroids.

Fungal sensitisation

The total IgE levels significantly reduced as the number of positive sputum cultures increased. There was a non-significant increase in the *Aspergillus* specific

IgG as the number of fungal cultures increased. While the differences in the specific fungal IgE levels and the *Aspergillus* IgG levels were not significant across the groups there were no positive test values for any of these investigations in the group who did not culture any fungus during the study. *Aspergillus* species were the most commonly cultured fungi hence *Aspergillus* sensitisation was reviewed.

Lung function

My data demonstrated a non-significant reduction in the absolute FEV_1 (l/min) as the number of positive fungal samples increased. However, despite the reduction in the absolute FEV_1 the percentage predicted value increased, suggesting those who cultured fungi in the sputum more frequently actually had better lung function. This was also reflected in the FVC (% predicted) which also improved as the number of positive samples increased. This is different from the findings in a group of asthmatics where sputum fungal detection was associated with reduced lung function (239).

Sputum inflammation

The group who did not culture any fungus during the entire study had the lowest sputum total cell count, which was three times lower than the groups who repeatedly cultured fungus. However there was no difference between the culture positive groups so the number of positive sputum samples does not seem to be important. Previous research has also suggested that the sputum total cell count is higher in those who have a positive sputum culture (240). The total cell count was predominantly neutrophilic in my study cohort with the neutrophil count getting progressively, but not significantly, higher with an increasing number of culture positive samples.

Fungal culture

The most commonly cultured fungi in my study using standard culture techniques revealed that *Aspergillus* species and *Penicillium* species were the most commonly identified, in keeping with previous studies in patients with COPD and cystic fibrosis (240,254,271). These are common environmental fungi which thrive in the indoor environment. Sputum culture using a SceSel+ medium inhibits *Aspergillus* growth and revealed a wide variety of other fungal organisms. Some studies have detected *Scedosporium* species using SceSel+ selective media in cystic fibrosis patients however, this fungus was not cultured from my samples (271).

Thermoascus crustaceous is found in the outdoor environment particularly orchards where it can infect fruit juice. It is one of the most heat resistant fungi making it difficult to kill by pasteurisation (272). However, it is not a common pathogen to humans. *Rhizomucor pusillus* is a pathogen to both humans and animals and is found in the outdoors, particular decaying matter such as compost heaps. *Rhizopus microsporus* is a plant fungus commonly found in maize and rice but is also used in the fermentation of soy to make soy sauce and tempeh. It has been known to cause lung infection in humans but only rarely in immunocompetent hosts (273). *Paecilomyces* species are common environmental organisms found both indoors in soft furnishings and outdoors. *Ceriporia* species are one of the many corticoid fungi which grow on rotting wood and are widely found in the outdoors environment. *Corticiaceae* species are a family of fungi which are also predominantly wood-rotting saprotrophs. Another wood decaying fungus is *Phlebia subserialis* which causes white rot.

The SceSel+ culture results revealed the following fungi: *Gloeophyllum trabeum* (a very common plant fungus causing brown rot), *Coprinellus xanthotrix* (also known as the Inkcap mushroom), *Hyphodontia palmae* (a tree fungus), *Coprinopsis cinera* (another mushroom), *Rhizomucor meihei* (used commercially to produce rennet from milk (274)), *Talaromyces* (a common indoor fungus (275)) species and *Agaricaceae* species (another mushroom).

During exacerbations the number of colony forming units and the number of varieties of filamentous fungi were reduced compared to the exacerbation visits. *Aspergillus* species accounted for the vast majority of the positive cultures and the other fungi identified on the SceSel+ plates at the stable visits were not seen. The non-filamentous fungi results were similar during exacerbations and stable visits. There is very little data collected on fungal identification during exacerbations. It is possible that a change in the microbiome such as from an external viral or bacterial infection might reduce the fungal culture numbers due to competition for nutrients.

Fungi have been detected more commonly than previously thought due to improved culture techniques. In a study of CF patients 27% were found to be *Aspergillus fumigatus* culture positive but this number increased to 74% when RT-PCR techniques were employed (256). In addition, fungal detection can be dependent upon the culture medium, sample dilution and incubation time. None of the participants in the study had invasive or semi-invasive fungal lung disease.

6.4.2 Non-filamentous fungi

Almost all of the study population demonstrated a growth of *Candida* in their sputum at some point during the study. I initially reviewed the data based on those who had >100 colony forming units of non-filamentous fungi detected at the baseline visit versus those who had <100 colony forming units detected however there were only three subjects in the >100 CFU group and the data was not significant. I reviewed the culture results for the entire study and reported the data according to which subjects were colonised with yeast and which were not using the standard definition of bacterial colonisation in bronchiectasis of two or more cultures of the same organism three or more months apart. Analysing this data revealed significant differences in the quality of life questionnaires. Those who were colonised had significantly worse scores in the St George's Respiratory Questionnaire and Leicester Cough Questionnaire and worse, albeit not significant,

in the visual analogue domains when compared with those who were not colonised. There were no significant differences between the two groups for the spirometry results, lung clearance index, sputum inflammation and CT scores.

Similar results have previously been reported in cystic fibrosis patients (257). There is minimal research available in the area of yeast culture and non-cystic fibrosis bronchiectasis. A case series of *Candida* bronchitis has been published involving patients often with underlying lung disease and recurrent *Candida* culture in the sputum. The study reports good resolution of symptoms following treatment with oral and nebulised anti-fungal medication and steroids (246). Unfortunately there are no randomised controlled trials available in this area.

6.5 Conclusion

The sputum filamentous fungal cultures were carried out using the protocol devised by the University of Leicester fungal laboratory which uses a method that results in a greater fungal culture rate. This meant that of those in the study who were able to provide a sample to send for fungal culture the number of positive cultures is high and the range of fungi identified very broad. The most striking findings were that an increased number of positive fungal culture results throughout the study period were significantly related to a fall in the IgE level which was unexpected. The quality of life questionnaires demonstrated a positive trend towards worsening symptoms as the number of positive sputum cultures increased. This was significant in the VAS sputum purulence score. The changes in the quality of life questionnaires were both reflected in any change in the spirometry, small airways function or CT total scores although the bronchial wall thickening score increased with the number of cultures. Interestingly the nasal nitric oxide levels showed a significant reduction with an increasing number of cultures despite the lack of association with nasal symptoms or recurrent sinusitis (chapter 3). This has never been studied previously and the reasons for this finding are unclear. The exhaled nitric oxide levels were unchanged. An increasing number of fungal cultures saw a trend to a reduction in the sputum bacterial colony forming unit numbers. While the total cell count increased the eosinophil count remained stable (with a resultant reduction in eosinophil %) with increasing fungal cultures which was unexpected. However the neutrophil count increased despite the bacterial colony forming unit count reducing. Although neutrophils are traditionally associated with bacterial infection it might be that they also have a role in fighting fungal infection. It is also possible that the bacteria and fungi are competitors and as one population increases the other decreases.

The colonisation of non-filamentous fungi was also reviewed. Colonisation was associated with significantly worse St Georges Respiratory and Leicester cough questionnaires. There was no change with any other parameter. Yeast colonisation

is often overlooked but might be an important cause of respiratory symptoms as captured by the questionnaires.

Chapter Seven: Thesis Summary

7.1 Background

Recurrent chest infections and daily symptoms of breathlessness, fatigue and sputum production are the most common features of non-cystic fibrosis bronchiectasis. Low-dose, long-term macrolides can have an impact on the clinical picture. This clinical study was commenced prior to the publication of the large BLESS study which used low-dose erythromycin as part of a placebo-controlled trial in non-CF bronchiectasis patients with a reduction in exacerbations as the primary end-point. Due to time and financial constraints neither a large, nor a placebo-controlled, trial could not be carried out for the purposes of this thesis. However, my study involved a wide range of investigations which highlighted some previously unknown findings.

The clinical trial had an observational-interventional structure and 68 participants with CT proven non-cystic fibrosis bronchiectasis were recruited from the Glenfield Hospital in Leicester. The aetiology of the bronchiectasis was characterised and further investigations to assess airway inflammation, lung function, quality of life, CT severity and airway micro- and mycobiology were performed.

7.2 Main findings

7.2.1 Cohort characteristics

The study population were predominantly female, had post-infectious or idiopathic bronchiectasis and a higher than expected degree of autoimmune disease. The spirometry was unremarkable in a large proportion of the group but the lung clearance index was abnormal in all but two participants. Lung clearance had been used to detect early lung damage in cystic fibrosis but had not been used clinically in non-cystic fibrosis bronchiectasis. The population had neutrophilic airway inflammation regardless of bacterial colonisation or infection; in fact the sputum neutrophil count increased with fungal colonisation. As expected the most

commonly cultured bacterial were *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae*. The most commonly identified filamentous fungi were the *Aspergillus* species, but the additional use of SceSel+ plates to inhibit *Aspergillus* and bacterial growth resulted in the detection of a large variety of environmental fungi which were cultured in low numbers. The majority of the study population who provided sputum samples cultured fungus during the study and those who had 3 or more positive cultures were found to have significantly lower total IgE levels, significantly lower nasal nitric oxide levels and significantly worse scores on the VAS sputum purulence scale. Increasing number of positive fungal cultures showed a trend towards increasing symptoms in the quality of life questionnaires, worsening lung clearance index and an increase in bronchial wall thickening. There was also a trend to a reduction in the sputum bacterial colony forming unit numbers but an increase in the sputum neutrophil count. The presence of bacterial colonisation was not associated with a higher burden of symptoms as detected on the quality of life questionnaires.

A higher burden of symptoms as determined by the SGRQ and the LCQ were associated with a worse FEV_1 and lung clearance index along with the presence of non-filamentous fungal airway colonisation. A higher burden of symptoms as demonstrated by the VAS scores were correlated with a worsening radiological severity, a lower FEV_1 (% predicted), a higher lung clearance index, the presence of filamentous fungi and a higher sputum neutrophil count (%). A higher radiological severity score was not associated with the scores from the SGRQ or LCQ questionnaires, only the VAS scores.

7.2.2 Exacerbations

Where possible, participants with an exacerbation were seen in the research unit in order to complete quality of life questionnaires, spirometry and sputum culture and inflammation testing. The quality of life questionnaires accurately detected an increase in symptoms at the time of exacerbation. Interestingly, there were no significant changes with the spirometry and sputum differential cell counts.

Counter-intuitively the sputum colony forming units showed a trend towards a reduction during an exacerbation. Similarly, the amount and variety of non-filamentous fungi cultured during the exacerbations was reduced compared to the stable periods. The participants who did not have any exacerbations during the study period were also analysed but did not demonstrate any physiological differences to their fellow participants who suffered recurrent exacerbations.

7.2.3 Clinical trial

The main research hypothesis stated that the 12 week course of erythromycin would bring an improvement to the FEV_1 of 200ml. This was not proven by the study and only a single participant was found to have an improvement in FEV_1 greater than 200ml between the pre- and post-erythromycin visits. Both the observational year and the intervention period demonstrated a degree of variation in the FEV_1 readings which were not attributable to any factors such as an exacerbation or change in symptoms. Spirometry in non-CF bronchiectasis is unlikely to be a useful end-point in a study. The lung clearance index correlated with the CT-related disease burden and VAS symptom scores and was also demonstrated to have a significant improvement after a 12 week course of erythromycin.

Another of the study hypotheses stated that participants who had a greater degree of sputum neutrophilia and small airway inflammation as demonstrated by the CT severity scores and the lung clearance index would have a better response to erythromycin would have. While improvements were seen in the lung clearance index it wasn't necessarily the participants with the highest neutrophil counts or worst lung clearance index that demonstrated the benefits. The vast majority of participants had a sputum neutrophilia.

One of the challenges of the data analysis was to determine how a response to erythromycin could be objectively determined. There were many participants on the study who reported feeling a lot better on the drug but identifying the reasons

behind the improvement were more difficult. The main areas which reflected an improvement were the lung clearance index, the Leicester cough questionnaire total score, the visual analogue scale sputum production score and the apparent clearance of pathogens from the sputum, albeit transiently. The exact effect of the macrolide was difficult to ascertain but a combination of reduced sputum production and possible transient bacterial clearance appeared to exert a beneficial effect on some of the study subjects.

The erythromycin treatment was well tolerated and the majority of the participants did not report any adverse events. There were no concerns regarding the microbial resistance or increased culture of non-tuberculous mycobacterial growth.

7.3 Limitations of the study

This study was started prior to the publication of the large BLESS trial which used low-dose erythromycin in non-cystic fibrosis bronchiectasis. My trial was much smaller than the BLESS trial. We had initially planned to use a placebo drug and run a randomised-controlled trial but unfortunately the cost of this was too high to be feasible. The study was run on a very small scale and I completed the majority of the investigations along with all of the admin, filing, recruitment and data entry. I was able to consult a statistician at the start of the study but there was not a statistician allocated specifically to my study. The participants recruited to the study were not already taking a long-term antibiotic so it could be argued that they belonged to the milder end of the spectrum. The Bronchiectasis Severity Index was published towards the end of my study but it would have been helpful to rate the bronchiectasis severity for each subject to aid data interpretation. In reality there was a wide variety of participants recruited to the study. Some have re-started the erythromycin following the trial completion, some continue to feel better post-erythromycin and have not needed to restarted the drug and some participants did not derive a lot of benefit from the intervention perhaps as they felt fairly well at

the start of the study. However, the patient selection represents the real-world clinic patients who do not always meet the specific requirements of studies.

7.4 Future work

The primary end-point of this study was a 200ml improvement in the FEV_1 which was demonstrated to be less sensitive to improvements following erythromycin than a visual analogue scale for sputum production and purulence and the lung clearance index. Future trials involving non-CF bronchiectasis patients could use an improvement in the lung clearance index of around 5% as an objective end-point. Another possibility would be a 24 hour sputum weight or officially documented sputum purulence / colour using a colour chart as the visual analogue scale is subjective. It would also be useful to ask participants to self-rate their response to the erythromycin as a marker of treatment success against which the objective investigation results could be compared against. Another CT scan following erythromycin treatment could be performed to determine whether the macrolide treatment had any impact on the tree-in-bud changes seen.

A third of the sputum samples collected did not reveal any significant growth on standard laboratory culture. Future studies could involve bacterial RNA PCR analysis in order to provide more microbiological data.

The erythromycin therapy was well-tolerated with minimal side-effects at the dose it was given. Provided care is taken to avoid drug interactions this treatment could have a beneficial effect on the daily symptoms experienced by bronchiectasis patients.

Appendix

Study Protocol

Phenotyping bronchiectasis based on aetiology, exacerbation characteristics and response to erythromycin.

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1.0 Introduction

1.1 Background

Non-cystic fibrosis bronchiectasis is a persistent, usually progressive and potentially disabling respiratory condition which is characterised by chronic sputum production and recurrent chest infections. The gold standard method of diagnosing the condition is Computed Tomography (CT) but the diagnosis was previously based upon a suggestive history and chest x-ray findings of thick-walled bronchi. The pathological changes in bronchiectasis have been described by Lynne Reid in the 1950s who described a classification of cylindrical, varicose and saccular appearances on the bronchogram of the posterior basal segment of the lower lobe of resected lungs which correlated to a description made by Ewart in A System of Medicine published in 1898. Lynne also noted the striking appearance of

luminal obstruction from mucus casts, luminal dilatation, wall thickening and infiltration with inflammatory cells and fibrosis.[1] This histology is recognised as bronchiolitis which is increasingly recognised as contributing significantly to the clinical symptoms of bronchiectasis. The area of non-invasive small airway testing is emerging and could provide useful information in bronchiectasis. In the 1990s Bhalla derived a CT scoring system to assess disease progression in cystic fibrosis.[2] This scoring system is based on the histological findings in bronchiectasis, is widely used and has been modified for non-cystic fibrosis bronchiectasis.[3]

There is increasing evidence that bronchiectasis is a diverse condition in terms of its aetiology, distribution, inflammatory characteristics, sputum microbiology, lung function and effect on quality of life. CT scans are being performed more frequently with a resultant increase in the detection of early bronchiectasis. GP surgeries can have as many as 12 patients per practitioner and a rise in the prevalence is anticipated.[4] World-wide prevalence varies greatly and is influenced by childhood infections, healthcare resources and the increasing availability of CT. A study of the CT scans of asymptomatic patients aged 23-86 undergoing health screening in Korea the prevalence in the study population was found to be 9.1% with the condition being more common in females than males and increasing with age.[5] In contrast a review of 5.6 million healthcare claims in the United States found and overall prevalence of 0.25%.[6] Historically bronchiectasis was felt to be due to pulmonary infections such as whooping cough, pneumonia and TB but the widespread use of vaccinations and antibiotics have resulted in a decline in these causative factors. More commonly disorders of the immune system and genetic defects in mucociliary clearance are attributed. There is an association between bronchiectasis, connective tissue disorders and inflammatory bowel disease. Patients are routinely investigated for antibody and sub-class deficiencies as effective immunoglobulin treatments are available. A large proportion of patients don't have an identifiable underlying cause for the disease, although some conditions such as primary ciliary dyskinesia are not routinely tested for. In a review of 65 cases of bronchiectasis in children in New Zealand only 8% were

tested for ciliary dysfunction. Even when an entire clinic cohort is tested for ciliary dysfunction only a small proportion are positive.[7,8]

There is no consensus regarding long-term antibiotics in bronchiectasis but there is growing interest in the macrolide group of antibiotics which are used with increasing frequency in the long term in COPD, cystic fibrosis and non-CF bronchiectasis, where they have been shown to significantly reduce exacerbation frequency and improve lung function.[9,10] A small study conducted to produce sufficient information to power a large on-going randomised double-blind placebo controlled trial was carried out in Australia. 24 patients were given 250mg erythromycin daily for a year with a greater than 50% reduction in exacerbation frequency by the end of the year.[11] This was shown again in a large randomised placebo-controlled trial using erythromycin 250mg BD for 12 months. A 70% reduction in exacerbation frequency was demonstrated.[12] Macrolides are widely used to treat diffuse panbronchiolitis with a proven improvement in survival and symptoms and are being researched for COPD and asthma.[13] As a group they are widely available, required in low doses for the anti-inflammatory effects and well tolerated. Their mechanisms of action include the bacteriostatic inhibition of RNA dependent bacterial protein synthesis, immunomodulatory and anti-inflammatory effects without causing immunosuppression.[14] In the acute setting the host defence is augmented by increasing interleukin and nitric oxide production.[15] In the longer term there has been proven suppression of inflammatory mediators such as TNF-α and IL-8.[16] Macrolides reduce airway secretion by blocking mucin production and have been proven to inhibit pseudomonas motility and biofilm formation.[17]

While research has been performed regarding the aetiology in a clinic cohort little is known about how lung function, bacterial colonisation and airway inflammation change over time and during exacerbations in patients with bronchiectasis. While we know that erythromycin is proven to reduce the exacerbation frequency in bronchiectasis there is likely to be a heterogenous response to the macrolide. These biomarkers will be studied with a view to identifying one or several which are characteristic of the cohort of patients who have had the best response to

erythromycin. Glenfield hospital have performed trials which have resulted in the phenotyping of asthma and proven the beneficial effect on targeted treatments.[18,19] In another study the most impressive response to macrolide treatment was seen in the group with neutrophilic airway inflammation, a phenotype which is most commonly seen in chronic bacterial infection.[20] A retrospective review of 57 clinic patients who had received 3 months of erythromycin over the last 6 years was conducted. The data was arranged into tertiles according to change in FEV_1 pre- and post-erythromycin. Overall there was a 115.3ml (s.e.m 37.5) increase in FEV_1 after 3 months treatment. The best improvement was 900ml and the mean delta FEV_1 in the higher tertile was 422ml. There was a significant improvement in FEV_1 post treatment in the patients with a bronchiolitis / bronchiectasis overlap ($p<0.05$). Patients in the higher tertile of response tended to have a larger sputum neutrophil count and were more likely to have bronchiectasis and tree in bud changes on CT.

This study aims to identify a biomarker which can be measured easily in order to tailor our treatment to a specific phenotype. This means patients who are likely to respond to a macrolide in the future will receive the treatment earlier and patients who are less likely to respond will not receive unnecessary medication.

1.2 Study Design

The study is divided into two phases. The first year is an observational cohort study. In the second year we will give each of the participants low dose erythromycin for 12 weeks followed by a period of observation for 3 months.

1.3 Study Aims

1.3.1 Primary Aim

We anticipate an improvement in the FEV_1 of at least 200ml following a 3 month course of erythromycin at 250mg once a day.

1.3.2 Primary Hypothesis

We hypothesize that the most common phenotype in our study population will have neutrophilic airway inflammation.

We hypothesize that the study participants who demonstrate the best response to erythromycin 250mg daily for 12 weeks will have neutrophilic airway inflammation and demonstrate small airways disease in the form of an increase lung clearance index on multiple breath washout testing and tree in bud changes on the CT scan.

1.3.3 Secondary Objectives

To evaluate whether the response to 12 weeks erythromycin will be sustained after the course is completed.

To create phenotypes based on biomarkers including blood and sputum inflammation, aetiology, radiological severity scores and lung clearance index.

To evaluate the changes in sputum inflammation, microbiological and fungal cultures both when stable and during exacerbations.

2.0 Target subject population

Potential participants will be largely recruited from out-patient clinics where a diagnosis of bronchiectasis based upon symptoms of recurrent chest infections and cough with sputum production and a confirmatory CT scan. The study has been powered to find an improvement in FEV_1 rather than altering the number of exacerbations. All patients will take the three month course of erythromycin.

The number of participants has been determined by a sample size calculation. We have used data from a retrospective review of clinics for patients with bronchiectasis over the last 6 years who received a 3 month course of erythromycin. We have demonstrated that the difference in the change in FEV_1 (delta FEV_1) pre- and post-treatment between the patients who had the best and worst improvement in symptoms overall was 336mls with a standard deviation of 0.255l. Using 80% power and a type 1 error rate of 0.05 9 participants are required. This can be increased to 12 participants to account for a 20% drop out rate. A delta FEV_1 value of 156ml is 2 standard deviations of the variability of spirometry. Using a 200ml difference in delta FEV_1 pre- and post-study between the best and worst responders the same calculation generates a sample size of 32 participants with a 20% drop out rate. We would be looking to recruit around 40 participants to increase the study power.

Cohort phenotyping and cluster analysis will require at least 200 sets of baseline data. During the study period we will continue to recruit a maximum of 50 patients to be seen for one baseline visit only. Drs Range and Ohri have kept a database containing demographic data and investigation results from the patients attending their bronchiectasis clinics. Baseline data will be obtained from this database and will be added to the cohort for the purposes of cluster analysis.

2.1 Inclusion and Exclusion Criteria

Group 1 consists of 40 patients who will be recruited for the entire study and receive the erythromycin intervention. Group 2 will consist of 50 patients who will attend for one baseline visit only.

2.1.1 Inclusion Criteria (Group 1)

- Bronchiectasis proven on CT scan and presence of symptoms (cough, sputum production, recurrent infections sufficient to indicate a diagnosis of bronchiectasis to the referring consultant)
- Aged 18 to 100 inclusive
- Ability to give valid consent
- Willingness to attend the hospital every 3 months for the study duration.

2.1.2 Inclusion Criteria (Group 2)

- Bronchiectasis proven on CT scan and presence of symptoms (cough, sputum production, recurrent infections sufficient to indicate a diagnosis of bronchiectasis to the referring consultant)
- Aged 18 to 100 inclusive
- Ability to give valid consent
- Willingness to attend the hospital for one visit

2.1.3 Exclusion Criteria (Group 1)

- Active TB
- Aged under 18 and over 100
- Unable to perform procedures such as pulmonary function tests, spiromtery or unable to attend visits due to ill health
- Patients with known cystic fibrosis
- Patients with traction bronchiectasis due to fibrosis as a primary diagnosis

- Patients who are unable to consent
- Patients already on long term antibiotics
- Patients with macrolide allergy / severe intolerance / long QT interval on ECG
- Patients on medications with a proven interaction with erythromycin, with the exception of simvastatin.

2.1.4 Exclusion Criteria (Group 2)

- Active TB
- Aged under 18 and over 100
- Unable to perform procedures such as pulmonary function tests, spirometry or unable to attend visits due to ill health
- Patients with known cystic fibrosis
- Patients with traction bronchiectasis due to fibrosis as a primary diagnosis
- Patients who are unable to consent

3.0 Methodology

The participant will receive verbal and written information regarding the nature, purpose and possible risks / benefits of the study. They will be informed that they are free to discontinue the study at any time. The participant will be free to ask questions and will be given sufficient time to consider the information and provide written consent. The information will be received no fewer than 24 hours before signing a consent form, one copy of which will remain with the participant. Written consent will be obtained before any invasive procedure for the study and the volunteer will retain their own copy of the consent form. The participant will be assigned a Unique Study Number and this will be entered into the study file. If consent is obtained the patient will undergo the baseline investigations and this will be counted as Visit 1. This will need to occur 6 or more weeks after an exacerbation. At this visit the patient will answer detailed questions regarding:

- Age of symptom onset
- Age of diagnosis
- Potential causes – ear nose and throat symptoms, gastrointestinal symptoms, childhood infections, previous TB, fertility problems, joint, eye and skin symptoms that may suggest a connective tissue disease or rheumatoid arthritis.
- Family history
- Smoking history, alcohol consumption, occupation and pets
- Past medical history
- Concomitant medication. The medication, dose and frequency will be recorded and checked against the list of contraindicated medications. These are as follows:

<u>Avoid use</u>

- Amiodarone (Cordarone X) and Dronedarone (Multaq) – atrial fibrillation
- Reboxetine (Edronax) – antidepressant
- Mizolastine (Mizollen) – anti-histamine
- Artemether / Lumefantrine (Riamet) – anti-malarial
- Tolterodine (Detrusitol) – anti-muscarinic
- Droperidol (Xomolix), Zuclopenthixol (Clopixol), Pimozide (Orap), Quetiapine (Seroquel) and Amisulpride (Solian) –anti-psychotic
- Saquinavir (Invirase) – anti-viral
- Lercandipine (Zanidip) – calcium-channel blocker
- Colchicine (only in hepatic / renal impairment)
- Vinblastine (chemotherapy)
- Ergotamine(Cafergot, Migril) and Methysergide (Deseril) – ergot alkaloid (migraines)
- Eletriptan (Relpax) – $5HT_1$ receptor agonist (migraine)
- Ivabradine (Procoralan) – intractable angina

Caution advised

- Alfentanil (Rapifen) – opiate analgesic, increased plasma toxicity
- Disopyramide (Rythmodan) – anti-arrhythmic, increased risk of toxicity
- Rifabutin (Mycobutin) – anti-bacterial, increased toxicity so reduce Rifabutin dose
- Coumarins (Warfarin, Sinthrome, Phenindione, Dibigatran, Rivaroxaban) – anti-coagulation
- Carbemazepine (Tegretol, Carbagen), Valproate (Epilim, Episenta, Epival) – anti-epileptic, increased plasma concentration
- Loratadine (Loratadine) – anti-histamine, increased plasma concentration
- Clozapine (Clozaril, Denzapine, Zaponex) – anti-psychotic, possible increased risk of convulsions
- Ritonavir (Norvir), Telaprevir (Incivo) – anti-viral, increases plasma concentration of erythromycin
- Midazolam, Zopiclone (Zimovane) – sedatives, anxiolytics, inhibits metabolism so increases sedation
- Calcium-channel blockers (Amlodipine/Istin, Diltiazem/Tildiem, Slozem, Viazem, Zemtard, Felodipine/Plendil, Isradipine/Prescal, Lacidipine/Motens, Nicardipine/Cardene, Nifedipine/Adalat, Adipine, Nimodipine/Nimotop, Verapamil/Cordilox, Securon, Univer, Verapress, Vertab) – angina and hypertension, increased risk of side effects
- Digoxin (Lanoxin) – atrial fibrillation, increased risk of side effects
- Ciclosporin – immune suppression, erythromycin inhibits metabolism
- Cilostazol (Pletal) – intermittent claudication, increased plasma concentration, reduced dose of cilostazol recommended
- Clopidogrel (Plavix) – anti-platelet, possible reduction of effect
- Methylprednisolone – possible reduction in metabolism
- Everolimus (Afinitor, Votubia), Docetaxol (Taxotere), Arsenic Trioxide (Trisenox) – cytotoxics, possible increased risk of arrhythmia
- Eplerenone (Inspra) – diuretic, dose reduction of eplerenone needed

- Domperidone (Motilium) – anti-emetic and pro-motility, possible increased risk of ventricular arrhythmia
- Bromocriptine (Parlodel), Cabergoline (Dostinex) – dopaminergics for acromegaly, increased risk of toxicity
- Zafirlukast (Accolate) – asthma, reduction of plasma concentration
- Atorvastatin (Lipitor), Pravastatin (Lipostat), Rosuvastatin (Crestor) – increased risk of myopathy and variable plasma concentrations
- Estradiol (OCP: Qlaira, HRT: lots) – increased plasma concentration
- Galantamine (Reminyl, Galsya) – alzhiemer's, icreased plasma concentrations
- Dienogest (Progesterone, Qlaira) – increased plasma concentration
- Sildenafil (Viagra, Revatio), Tadalafil (Cialis), Vardenafil (Levitra) – reduced dose of sildenafil required
- Sirolimus (Rapamune) and Tacrolimus (Adoport, Modigraf, PrografTacni, Vivadex, Advagraf) – immune suppression, increased plasma concentration of both drug and erythromycin
- Theophylline (Uniphylline, Slo-phyllin, Nuelin SA) – increased plasma concentration and possible reduction in erythromycin absorption
- Ticagrelor (Brilique) – used with aspirin as an anti-platelet
- Cimetidine (Tagamet) – stomach ulcers, increased risk of toxicity and deafness

The patient will fill in the Leicester Cough Questionnaire, St George's Respiratory Questionnaire and VAS assessment of cough and sputum as part of our quality of life measures. They will also undergo blood tests to include:

- Full blood count, urea and electrolytes, c-reactive protein and liver function tests.
- Immunology tests to include immunoglobulin levels (IgA, IgE, IgG, IgM and aspergillus specific IgE), autoantibodies (ANA, ANCA, RhF, ENA), alpha 1 anti-trypsin levels and pneumococcal serotypes. If any of the pneumococcal serotype levels are low the standard clinical procedure we will follow is to

repeat the pneumococcal and meningitis vaccinations and repeat the levels by blood tests 3 months later to assess the response.

- Full pulmonary function tests.
- Non-invasive small airway tests – multiple breath washouts and impulse oscillometry.
- Induced sputum collection. The sample can be divided into microbiological tests [qualitative microbiological culture including mycobacterial and fungal growth, viral titres, biofilm presence and quantitative bacterial load using polymerase chain reaction (qPCR)] and inflammatory investigations (differential cell counts).
- Exhaled oral and nasal nitric oxide (NO) leveles will be collected as a marker of airway inflammation and as a screen for Primary Ciliary Dyskinesia (PCD). If the nasal NO is low we will refer the patient to the PCD clinic for further investigations. These are part of normal clinical practice.

The patients will be seen at scheduled visits at 3, 6 9 and 12 months after the initial first visit. All of these scheduled visits will be performed greater than 6 weeks after an exacerbation.

If a patient takes simvastatin as part of their medication the option of withholding the medication, in the case of primary prevention, or substituting the medication with an alternative statin, in the case of secondary prevention, for the three months of the erythromycin treatment will be discussed with both the patient's GP and cardiologist. If there is an agreement for this process the changes will be made at visit 5. In the event of non-agreement from either the GP or cardiologist the patient will have the option of leaving the study, or continuing to provide samples.

<u>Visits 2-4 (months 3, 6 and 9)</u>

The following investigations will be performed:

- Blood tests to include full blood count, c-reactive protein and urea and electrolytes.

- Induced sputum for differential cell counts, bacterial growth and qPCR, biofilm presence colony forming units and mycobacteria.
- Exhaled nitric oxide to assess airway inflammation.
- Quality of life questionnaires (Leicester Cough Questionnaire, St George's Respiratory Questionnaire and Visual Analogue Scale for cough and sputum).
- Spirometry.
- Multiple breath washout test to assess baseline small airway disease (if not performed at visit 1).
- During the first year of the study we will perform a low-dose CT scan to allow the severity of the bronchiectasis to be graded using Roberts criteria.[3]

Visit 5 (month 12)

The second phase is an interventional study followed by a period of observation. Participants will receive 3 months of erythromycin at 250mg OD. Drugs will be supplied at visit 5. The following investigations will be performed:

- Blood tests to include full blood count, c-reactive protein and urea and electrolytes.
- Induced sputum for differential cell counts, bacterial culture and qPCR, colony forming units, biofilm, presence of fungi, viral titres and mycobacteria.
- Exhaled nitric oxide to assess airway inflammation.
- Quality of life questionnaires (Leicester Cough Questionnaire, St George's Respiratory Questionnaire and Visual Analogue Scale for cough and sputum).
- Spirometry and non-invasive small airway tests (multiple breath washouts).
- ECG to exclude prolonged QT interval.

Visits 6and 7 (months 15 and 18)

The following investigations will be performed:

- Blood tests to include full blood count, c-reactive protein, liver function tests, urea and electrolytes.
- Blood samples for theophylline levels if applicable.
- Induced sputum for differential cell counts, bacterial culture and qPCR, colony forming units, biofilms, fungal culture and viral titres and mycobacteria.
- Exhaled nitric oxide to assess airway inflammation.
- Quality of life questionnaires (Leicester Cough Questionnaire, St George's Respiratory Questionnaire and Visual Analogue Scale for cough and sputum).
- Spirometry
- Small airway tests (multiple breath washouts)
- ECG to exclude prolonged QT interval (visit 6 only)
- Adverse symptom profile.

Compliance will be checked at visit 6. The participant will be asked to keep the erythromycin packaging and return all unused tablets along with the packaging at visit 5 for assessment at visit 6.

Exacerbations

If a study participant is having an exacerbation we will schedule them to attend the hospital as soon as possible so investigations can be performed and treatment initiated. This is likely to comprise of antibiotics either with or without the addition of steroids. The erythromycin would be omitted during the duration of the antibiotic treatment. An exacerbation is characterised by a change in baseline respiratory symptoms for 2 or more days requiring additional antibiotic and/or steroid medication. Many studies divide exacerbations into moderate and severe. A moderate exacerbation requires oral treatment and a severe exacerbation would

require admission to hospital.[21] Identical investigations to those performed at the stable visits will be performed with the addition of sputum viral and fungal culture and small airway tests.

Physiotherapy

Physiotherapy will be optimised for each patient both in the stable state and during exacerbations. This follows the best care model offered by our bronchiectasis clinic and complies with current BTS guidelines for non-cystic fibrosis bronchiectasis.[22]

CT Scoring

Each CT scan will be scored by two independent radiologists according to the Roberts scoring system which has been validated for non-cystic fibrosis bronchiectasis.[3] This scoring system is used against each lobe and the maximum score is 10 for each lobe and 60 overall as the lingula is counted.

	GRADE 0	**GRADE 1**	**GRADE 2**	**GRADE 3**
EXTENT	No disease	Up to 1 pulmonary segment	>1 pulmonary segment	Generalised cystic changes
AIRWAY DILATATION	None	100-200% of arterial diameter	200-300% of arterial diameter	>300% of arterial diameter
BRONCHIAL WALL THICKNESS	None	<50% of arterial diameter	50-100% arterial diameter	>100% arterial diameter
MUCUS IN AIRWAYS	Absent	Present		

The end of the trial will be the last visit of the last subject undergoing the trial.

4.0 Sample Collection

4.1 Blood Sampling

Blood will be collected in the appropriate blood sample bottles in an aseptic manner. The samples will be labelled and sent to the laboratory on foot or via the pod system.

4.2 Sputum Sampling

The patient will undergo collection of induced sputum using a standard protocol and 6% hypertonic saline to obtain the best quality sample possible. Standard infection control measures will be observed. The sputum samples will be collected for microscopy, culture and qPCR, sensitivity, fungal testing, viral PCR, mycobacterial culture and differential cell counts for evidence of inflammation. The samples will be collected at each visit and during an exacerbation.

4.3 Pulmonary Function Tests

Full pulmonary functions tests will be carried out using a standard protocol in the Respiratory Physiology Department at the baseline visit.

4.4 Spirometry

Spirometry will be performed at every visit. The test will be carried out in the department by the investigator or a delegate using a validated and uniform technique.

4.5 Small Airway Tests

These investigations will be performed in the Respiratory Physiology Department following a standard protocol at the baseline visit and visits 5,6 and 7.

4.6 Exhaled Nitric Oxide

This will be performed in the department using a standard technique. This will be performed at each visit both when stable and during an exacerbation.

4.7 Nasal Nitric Oxide

This investigation will be carried out using our exhaled nitric oxide machine and a nasal adaptor. The test will be performed at the baseline visit as a screening tool for primary ciliary dyskinesia.

4.8 Nasal Epithelium Sampling

Nasal epithelial sampling will be carried out after consent if the nasal nitric oxide level suggests the possibility of ciliary dyskinesia. The procedure is carried out under local anaesthetic using a standard protocol. Nasal sampling will be performed in a specialist clinic.

5.0 Discontinuation of Subjects

5.1 Criteria for Discontinuation

Subjects will be discontinued from the study if they no longer wish to take part and withdraw consent. Participants may choose to discontinue the study for a variety of reasons including drug intolerance. Participants who are commenced on any medication whose use is contraindicated with erythromycin will be withdrawn from the study. Participants who are no longer able to give valid consent will also be withdrawn from the study. Any data collected from the withdrawn participants will be included in the final analysis.

6.0 Subject Safety

6.1 Recording and Reporting of Adverse Events

Information about study outcomes and adverse events will be collected only during the period of erythromycin administration, ie: from visits 5 to 6. Serious adverse events which are related to bronchiectasis will be collected for the duration of the study during each visit.

6.2 Definition of an Adverse Event

6.2.1 Adverse Event (AE)

This is defined as any untoward medical occurrence.

6.2.2 Serious Adverse Event (SAE)

A Serious Adverse Event is defined as an untoward medical occurrence which is life-threatening or results in death, hospitalisation, persistent or significant disability or birth defects.

6.2.3 Suspected Unexpected Serious Adverse Reactions

A serious adverse reaction of a nature which is unexpected from the summary of product characteristics (SmPC)

6.2.4 Reporting Adverse Events and Serious Adverse Events

All SAEs will be reported within 24 hours of becoming aware of them and a report will be faxed to the study sponsor. Only SAEs related to study procedures or adverse events causing discontinuation in the study (DAEs) will be recorded in the participant's notes and the database. SAEs related to study procedures will be collected and a root cause analysis will be performed. Investigators will be required to identify if the event is related or expected. Upon receipt, SAEs will be reviewed by the Principal Investigator or a designated colleague to assess

expectedness and causality. A summary of safety will be included in the annual progress report to the Ethics committee.

7.0 Source data, data management and data processing

7.1 Source data

For the purposes of this study the source data is comprised of the hospital records and the centralised investigation reporting for radiology (IMPAX) and blood and sputum results (iLAB). Source data will be used to populate the case record form (CRF) where appropriate.

7.2 Data collection and storage

Data will be collected on the paper CRF initially and then transcribed to the online CRF which directly populates the database. The online database is designed by RedCAP and is secured by a password. The database is backed up multiple times a day by the University Hospitals of Leicester computer servers. The paper CRFs and the site file are kept in an office secured by a keypad.

7.3 Data management

The data will be inputted by the collaborators or chief investigator and will be validated by a member of the management team. Data queries will be managed by the chief investigator or collaborators. The database will be locked after all the data is inputted and validated for each participant on a sequential visit basis. Data release for each visit will occur after the database is locked.

8.0 Biological sampling procedures

8.1 Handling, storage and destruction of biological samples

The samples will be used up or disposed of after analyses.

8.2 Chain of custody of biological samples

A full chain of custody will be maintained for all samples throughout their lifecycle. The principal investigator will keep full traceability of collected biological samples from the subjects whilst in storage at the Glenfield Hospital.

8.3 Withdrawal of informed consent for donated biological samples

If a subject withdraws consent to the use of donated biological samples, the samples will be disposed of if not already analysed and documented.

9.0 Statistical Management

The Respiratory Research Unit employs a statistician who will be involved in the analysis of the data. Analysis will be performed using SPSS and GraphPad. The response in sputum inflammation, cultures, spirometry, exacerbation frequency and well-being will be analysed and compared to the longitudinal data collected from the previous year. We will use a variety of validated statistical methods to analyse the clusters. The questionnaire data will be analysed using the Wilcoxon test, the spirometric values will be compared using the Student's T-test and the ANOVA test, and correlation will be calculated using the Spearman's rank test.

10.0 Publication of data

The data will be written up as an MD higher degree. The study information will be disseminated to study participants by means of an informal gathering. The overall

findings and the impact on future treatment and research will be addressed. Written information in the form of a patient leaflet will be provided if required.

Visual Analogue Scale, COPD Symptoms **VAS**

Regard the line as representing the full range of each dimension. Rate your symtoms as they are at the moment.

University Hospitals of Leicester NHS

NHS Trust

Leicester Cough Questionnaire (LCQ)

This questionnaire is designed to assess the impact of cough on various aspects of your life. Read each question

1. In the last 2 weeks, have you had chest or stomach pains as a result of your cough?

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

2. In the last 2 weeks, have you been bothered by sputum (phlegm) production when you cough?

1. Every time ☐
2. Most times ☐
3. Several times ☐
4. Some times ☐
5. Occasionally ☐
6. Rarely ☐
7. Never ☐

3. In the last 2 weeks, have you been tired because of your cough?

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

4. In the last 2 weeks, have you felt in control of your cough?

1. None of the time ☐
2. Hardly any of the time ☐
3. A little of the time ☐
4. Some of the time ☐
5. A good bit of the time ☐
6. Most of the time ☐
7. All of the time ☐

5. How often during the last 2 weeks have you felt embarrassed by your coughing?

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

6. In the last 2 weeks, my cough has made me feel anxious.

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

7. In the last 2 weeks, my cough has interfered with my job, or other daily tasks.

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

8. In the last 2 weeks, I felt that my cough interfered with the overall enjoyment of my life.

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

9. In the last 2 weeks, exposure to paints or fumes has made me cough.

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

10. In the last 2 weeks, has your cough disturbed your sleep?

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

11. In the last 2 weeks, how many times a day have you had coughing bouts?

1. All the time (continuously) ☐
2. Most times of during the day ☐
3. Several times during the day ☐
4. Some times during the day ☐
5. Occasionally through the day ☐
6. Rarely ☐
7. None ☐

12. In the last 2 weeks, my cough has made me feel frustrated.

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

13. **In the last 2 weeks, my cough has made me feel fed up.**

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

14. **In the last 2 weeks, have you suffered from a hoarse voice as a result of your cough?**

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

15. **In the last 2 weeks, have you had a lot of energy?**

1. None of the time ☐
2. Hardly any of the time ☐
3. A little of the time ☐
4. Some of the time ☐
5. A good bit of the time ☐
6. Most of the time ☐
7. All of the time ☐

16. **In the last 2 weeks, have you worried that your cough may indicate a serious illness?**

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

17. **In the last 2 weeks, have you been concerned that other people think something is wrong with you, because of your cough?**

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

18. **In the last 2 weeks, my cough interrupted conversation or telephone calls.**

1. Every time ☐
2. Most times ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

19. **In the last 2 weeks, I feel that my cough has annoyed my partner, family or friends.**

1. Every time I cough ☐
2. Most times when I cough ☐
3. Several times when I cough ☐
4. Some times when I cough ☐
5. Occasionally when I cough ☐
6. Rarely ☐
7. Never ☐

Thank you for completing this questionnaire.

Designed by MEDICAL ILLUSTRATION
at LEICESTER ROYAL INFIRMARY
Birring/RESPIRATORY MEDICINE/11.02/18447VY

ST. GEORGE'S RESPIRATORY QUESTIONNAIRE
ORIGINAL ENGLISH VERSION

ST. GEORGE'S RESPIRATORY QUESTIONNAIRE (SGRQ)

This questionnaire is designed to help us learn much more about how your breathing is troubling you and how it affects your life. We are using it to find out which aspects of your illness cause you most problems, rather than what the doctors and nurses think your problems are.

Please read the instructions carefully and ask if you do not understand anything. Do not spend too long deciding about your answers.

Before completing the rest of the questionnaire:

Please tick in one box to show how you describe your current health:

Very good	Good	Fair	Poor	Very poor
☐	☐	☐	☐	☐

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continued...

St. George's Respiratory Questionnaire
PART 1

Questions about how much chest trouble you have had over the past 3 months.

Please tick (✓) *one box for each question*:

		most days a week	several days a week	a few days a month	only with chest infections	not at all
1.	Over the past 3 months, I have coughed:	☐	☐	☐	☐	☐
2.	Over the past 3 months, I have brought up phlegm (sputum):	☐	☐	☐	☐	☐
3.	Over the past 3 months, I have had shortness of breath:	☐	☐	☐	☐	☐
4.	Over the past 3 months, I have had attacks of wheezing:	☐	☐	☐	☐	☐

5. During the past 3 months how many severe or very unpleasant attacks of chest trouble have you had?

Please tick (✓) *one*:

- more than 3 attacks ☐
- 3 attacks ☐
- 2 attacks ☐
- 1 attack ☐
- no attacks ☐

6. How long did the worst attack of chest trouble last?
(Go to question 7 if you had no severe attacks)

Please tick (✓) *one*:

- a week or more ☐
- 3 or more days ☐
- 1 or 2 days ☐
- less than a day ☐

7. Over the past 3 months, in an average week, how many good days (with little chest trouble) have you had?

Please tick (✓) *one*:

- No good days ☐
- 1 or 2 good days ☐
- 3 or 4 good days ☐
- nearly every day is good ☐
- every day is good ☐

8. If you have a wheeze, is it worse in the morning?

Please tick (✓) *one*:

- No ☐
- Yes ☐

continued…

St. George's Respiratory Questionnaire
PART 2

Section 1

How would you describe your chest condition?

Please tick (✓) *one*:

- The most important problem I have ☐
- Causes me quite a lot of problems ☐
- Causes me a few problems ☐
- Causes no problem ☐

If you have ever had paid employment.

Please tick (✓) *one*:

- My chest trouble made me stop work altogether ☐
- My chest trouble interferes with my work or made me change my work ☐
- My chest trouble does not affect my work ☐

Section 2

Questions about what activities usually make you feel breathless <u>these days</u>.

Please tick (✓) in ***each box*** that applies to you ***these days***:

	True	False
Sitting or lying still	☐	☐
Getting washed or dressed	☐	☐
Walking around the home	☐	☐
Walking outside on the level	☐	☐
Walking up a flight of stairs	☐	☐
Walking up hills	☐	☐
Playing sports or games	☐	☐

St. George's Respiratory Questionnaire
PART 2

Section 3

Some more questions about your cough and breathlessness <u>these days</u>.

	Please tick (✓) in ***each box*** that applies to you ***these days***: True	False
My cough hurts	☐	☐
My cough makes me tired	☐	☐
I am breathless when I talk	☐	☐
I am breathless when I bend over	☐	☐
My cough or breathing disturbs my sleep	☐	☐
I get exhausted easily	☐	☐

Section 4

Questions about other effects that your chest trouble may have on you <u>these days</u>.

	Please tick (✓) in ***each box*** that applies to you ***these days***: True	False
My cough or breathing is embarrassing in public	☐	☐
My chest trouble is a nuisance to my family, friends or neighbours	☐	☐
I get afraid or panic when I cannot get my breath	☐	☐
I feel that I am not in control of my chest problem	☐	☐
I do not expect my chest to get any better	☐	☐
I have become frail or an invalid because of my chest	☐	☐
Exercise is not safe for me	☐	☐
Everything seems too much of an effort	☐	☐

Section 5

Questions about your medication, if you are receiving no medication go straight to section 6.

	Please tick (✓) in ***each box*** that applies to you ***these days***: True	False
My medication does not help me very much	☐	☐
I get embarrassed using my medication in public	☐	☐
I have unpleasant side effects from my medication	☐	☐
My medication interferes with my life a lot	☐	☐

continued…

St. George's Respiratory Questionnaire
PART 2

Section 6

These are questions about how your activities might be affected by your breathing.

Please tick (✓) in ***each box*** that applies to you ***because of your breathing***:

	True	False
I take a long time to get washed or dressed	☐	☐
I cannot take a bath or shower, or I take a long time	☐	☐
I walk slower than other people, or I stop for rests	☐	☐
Jobs such as housework take a long time, or I have to stop for rests	☐	☐
If I walk up one flight of stairs, I have to go slowly or stop	☐	☐
If I hurry or walk fast, I have to stop or slow down	☐	☐
My breathing makes it difficult to do things such as walk up hills, carrying things up stairs, light gardening such as weeding, dance, play bowls or play golf	☐	☐
My breathing makes it difficult to do things such as carry heavy loads, dig the garden or shovel snow, jog or walk at 5 miles per hour, play tennis or swim	☐	☐
My breathing makes it difficult to do things such as very heavy manual work, run, cycle, swim fast or play competitive sports	☐	☐

Section 7

We would like to know how your chest <u>usually</u> affects your daily life.

Please tick (✓) in ***each box*** that applies to you ***because of your chest trouble***:

	True	False
I cannot play sports or games	☐	☐
I cannot go out for entertainment or recreation	☐	☐
I cannot go out of the house to do the shopping	☐	☐
I cannot do housework	☐	☐
I cannot move far from my bed or chair	☐	☐

St. George's Respiratory Questionnaire

Here is a list of other activities that your chest trouble may prevent you doing. (You do not have to tick these, they are just to remind you of ways in which your breathlessness may affect you):

Going for walks or walking the dog

Doing things at home or in the garden

Sexual intercourse

Going out to church, pub, club or place of entertainment

Going out in bad weather or into smoky rooms

Visiting family or friends or playing with children

Please write in any other important activities that your chest trouble may stop you doing:

. . . . . .

. . . . . .

. . . . . .

. . . . . .

Now would you tick in the box (one only) which you think best describes how your chest affects you:

It does not stop me doing anything I would like to do ☐

It stops me doing one or two things I would like to do ☐

It stops me doing most of the things I would like to do ☐

It stops me doing everything I would like to do ☐

Thank you for filling in this questionnaire. Before you finish would you please check to see that you have answered all the questions.

Change in FEV_1 pre- and post-erythromycin <200ml and >200ml

The table below has been moved to this section a there was only one participant who demonstrated an improvement in FEV_1 post-erythromycin >200ml, which was the primary aim of the study. Statistical analysis was not possible on this data although it has been described below.

The table below shows that this participant had worse quality of life scores for the SGRQ, LCQ and all VAS domains. Unsurprisingly the FEV_1 (% predicted) was also abnormal at 61.9% compared to the normal mean value in the other group. The sputum total cell count was much lower at $1.12x10^6$/ml compared to the rest of the group and the sputum neutrophil percentage was similar at 82%. The bacterial colony forming units were very different – $125x10^6$/ml for the responder compared with $0.700x10^6$/ml for the remainder of the group. The lung clearance index was worse in this individual at 11.335 compared to the mean value of 10.458 for the remainder. The CT scores were worse for this participant at 65 compared 34.97 for the rest of the group. The largest differences were seen in the extent of the bronchiectasis, the bronchial wall dilatation and thickening categories.

Table 41: The baseline characteristics categorised by delta FEV_1 <200ml and >200ml pre- and post-erythromycin

Investigation at visit 5	Change in FEV_1 pre-and post-erythromycin	
	<200ml n=34	>200ml n=1
SGRQ total score, mean (sd)	33.00 (16.68)	46.38
LCQ total score, mean (sd)	16.22 (3.11)	13.77
VAS cough domain, median (IQR)	32 (18-57)	57
VAS dyspnoea domain, median (IQR)	26 (13-53)	68
VAS sputum production, median (IQR)	53 (16-63)	70
VAS sputum purulence, median (IQR)	28 (10-61)	71
Post-bronchodilator FEV_1 (% predicted), mead (sd)	86.2 (24.6)	61.9
Sputum total cell count (10^6/ml), median (IQR)	8.16 (2.99-23.54)	1.12
Sputum neutrophils (%), median (IQR)	85.250 (74.750-96.250)	82.000
Sputum bacterial colony forming units (10^6/ml), median (IQR)	0.700 (0.234-5.000)	125.000
Lung clearance index, meadn (sd)	10.458 (2.022)	11.335
Total CT score, mean (sd)	34.97 (14.91)	65.00
Mucus plugging large airways, mean (sd)	2.05 (1.87)	3.00
Mucus plugging small airways, mean (sd)	2.81 (2.34)	5.00
Bronchial wall dilatation score, mean (sd)	7.08 (4.39)	18.00
Bronchial wall thickening score, mean (sd)	4.76 (3.58)	10.00
Extent of bronchiectasis score, mean (sd)	8.86 (3.57)	12.00

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