Characterisation of exacerbations in non-CF Bronchiectasis to establish endpoints in measuring treatment efficacy.

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Abstract

Bronchiectasis is characterised by chronic cough productive of mucopurulent sputum and frequent exacerbations. We have aimed to validate clinical, biochemical and microbiological endpoints to aid planning of future interventional studies. We recruited fifty-eight subjects with bronchiectasis at the Lung Defence unit (Papworth Hospital, Cambridge) and studied them in stable state (no exacerbation in the preceding four weeks) and during an exacerbation over a period of two years. The results of our research are discussed in this study. Clinical symptoms: Cough chest pain, chest discomfort, colour and volume of sputum and fatigue measured by a visual analogue score are useful endpoints. Breathlessness is a reliable endpoint when measured using either a visual analogue score or modified Borg's breathlessness score. Health related quality of life measured using the Euroqol questionnaire is a sensitive marker of change during an exacerbation. The St George's respiratory questionnaire did not demonstrate a significant change during an exacerbation. Spirometry: Forced expiratory volume in the first second (actual and percentage predicted) and Forced vital capacity (percentage predicted) do not change during the course of an exacerbation. Forced vital capacity actual may be used as an endpoint. pH of exhaled breath condensate in bronchiectasis is lower than in healthy subjects but does not change during the course of an exacerbation. Sputum appearance is a valid endpoint while 24hour volume of sputum and microbial clearance and anti-pseudomonal antibody titres cannot be used. ESR and serum titres of IFN-γ, TNF-α IL-6, IL-8, IL-10, IL-17 and IL-1β and titres in sputa of IFN-γ, IL-6, IL-17 do not change during an exacerbation. C-reactive protein and titres in sputa of TNF-α, IL-8 and IL-1β are effective indicators and can be recommended for use as end points in therapeutic interventional trials.

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Chapter 1

Introduction

1.1 Non Cystic Fibrosis bronchiectasis

1.1.1 Background

This affection of the bronchia is always produced by chronic catarrh, or by some other disease attended by long, violent, and often repeated fits of coughing.

R.T.H. Laënnec [1]

Despite being regularly exposed to particulate matter containing bacteria from the environment, the healthy lung is kept “free of pathogens” by efficient defence mechanisms [2]. However structural abnormality of bronchi predisposes to recurrent infection. Bronchiectasis is a descriptive term for abnormal chronic dilatation of bronchi. Such damage is the end result of a vicious cycle of infection and inflammation arising from a number of causes either acquired or inherited [3, 4]. The age of presentation of bronchiectasis is 10-20 years after the causative incident. The initial insult results in structural damage to the airway. In time superimposition of chronic inflammation on the anatomic damage leads to further abnormalities and symptoms occur [5, 6]. This infection remains confined to the lung and has therefore also been referred to as ‘chronic bronchial suppuration’.

Bronchiectasis is the morphological definition of the disease characterised by permanent dilatation of the bronchi. It has been categorised according to the pathological appearance of the airways. This definition has remained for more than half a century. The more recent use of high resolution computed tomography (HRCT) has made the identification of this morphology only easier. Three distinct forms of bronchiectasis are described: Cylindrical or tubular, varicose and cystic bronchiectasis. Cylindrical bronchiectasis is dilatation of the airways alone, Varicose bronchiectasis is characterised by focal constrictive areas along these dilated airways. Cystic bronchiectasis is the term used for dilated airways that end in large cysts, saccules or grape like clusters [7, 8].

The disease itself can be focal and confined to a single lobe or segment of the lung. This may happen if bronchial luminal blockage occurs either due to a broncholith, foreign body [9] or a slow growing tumour. A middle lobe syndrome may cause obstruction, collapse and consolidation of the distal lung and persistent suppuration [10]. Occasionally post-operative changes may result in a narrow bronchus that may cause distal bronchiectasis in time [11]. Diffuse disease is seen in more severe cases. This kind of disease is usually associated with a systemic aetiology such as cystic fibrosis (CF) [8, 12].

Bronchiectasis is generally classified into CF and non CF bronchiectasis. In this study we refer only to non CF bronchiectasis unless otherwise specified.

1.1.2 Prevalence of bronchiectasis

Bronchiectasis unrelated to cystic fibrosis has been termed an “orphan disease”. There is little direct evidence for the incidence or prevalence of bronchiectasis. Hospital admissions and healthcare resource utilisation in the Finnish population has decreased by fifty per cent between 1972 and 1992 [13]. Following the introduction of antibiotic therapy, control of tuberculosis and effective vaccination for whooping cough and measles, there has been a decline in the incidence of bronchiectasis [3].

Currently it is estimated that more than 110,000 adults in the US have bronchiectasis [14]. This ranges from 4.2 per 100 000 persons aged 13-34 years to 271.8 per 100 000 among those aged 75 years or older [4]. The annual death rate from bronchiectasis is estimated at 970 (NHLBI 1999).

In the UK, in 2002-03, bronchiectasis accounted for 0.06% (7,605) of hospital consultant episodes. Seventy eight per cent of hospital consultant episodes for bronchiectasis required hospital admission. The mean length of stay in hospitals was 10.5 days. For hospital consultant episodes for bronchiectasis, 15-59 year olds accounted for 37%, while over 75 years for 22% (Hospital Episode Statistics, Department of Health, England, 2002-03). In addition to this, up to 29% of patients with chronic obstructive airways disease may have evidence of bronchiectasis on high resolution computed tomography (HRCT) [15] .

Bronchiectasis is more prevalent in developing countries but is not well characterised. Although with improved sanitation and nutrition, introduction of childhood immunization, and the early and frequent use of antibiotics a decline has been noted in its prevalence, the disease is still a problem in developing countries [16].

All reports from studies of adult patients confirm a predominance of women who are lifelong non-smokers. This is particularly notable in patients who have onset of symptoms in middle age and symmetrical lower lobe disease [16-21]. Mean age at presentation is between 57 and 67 years [17, 21, 22].

1.1.3 Pathophysiology of bronchiectasis

Bronchiectasis is the irreversible dilatation of those airways that have a normal diameter of >2mm. This pathological dilatation is caused by the destruction of muscular and elastic components of the airways wall. This process of bronchial wall weakening is poorly understood. Rather than being a single process, it is accepted to be a result of a number of pathophysiological processes [23].

In health there are four possible outcomes for bacteria inhaled into the bronchial tree. Firstly there is immediate clearance by mucociliary apparatus [24]. Then there is asymptomatic carriage which occurs in some bronchitis patients between exacerbations [2, 25] and lastly this may be followed by either local mucosal inflammatory reaction or invasion of bronchial wall and lung parenchyma [2].

The mucociliary system is the most important first line defence mechanism [26]. Anatomical or physiological dysfunction in the mucociliary apparatus results in impaired mucous clearance and retained secretions that are observed in bronchiectasis.

Coordinated ciliary motion is essential to efficient bacterial clearance. In primary ciliary dyskinesia there is inherent ciliary dysfunction. Some bacteria also produce factors that slow and disorganise the beating of cilia. Ciliary dysfunction thus facilitates bacterial binding to the epithelial surface [26].

Viscid secretions are commonly seen. The illness is related to thick retained secretions causing obstruction and mechanical distension of the airways. This distension may persist even when obstruction is cleared [8, 23]. Obstruction itself may cause parenchymal atelectasis perpetuating bronchial dilatation. Also fibrosis and scarring from parenchymal disease leads to traction on the bronchial walls [23].

The purulent secretions contain mucous glycoproteins and abundant amounts of DNA. DNA is an extremely viscous polymerised polyanion [27]. Variants of an amiloride sensitive epithelial sodium channel (ENaC) regulatory gene may also contribute to this effect [28].

The ionic content of secretions may also affect the mucociliary transport. Regulation of these features relies on active ion transport across a continuous epithelial layer and intact tight junctions. In animal studies *Pseudomonas aeruginosa* has been shown to disrupt these tight junctions. Separation of tight junctions has also been observed in human nasopharyngeal cultures infected with *Haemophilus influenzae* [29, 30].

Bacterial colonisation occurs in these secretions causing inflammation locally. This in turn causes bronchial wall damage and perpetuates infection. The most widely accepted theory is that of a vicious circle of trans mural infection and inflammation with mediator release This cyclical phenomenon is constant and results in significant morbidity [6, 31].

The bacteria that cause bronchial infections possess a wide array of potential virulence factors. They may breakdown local immunoglobulin by cleaving protease antibodies or reduce viability of circulating macrophages [2]. *Pseudomonas aeruginosa* are seen to grow as continuous sheets over the mucous surface [32] and growth in such biofilms is resistant to opsonophagocytic killing by neutrophils [33]. Another interesting aspect in

the physiology of patients infected with *Pseudomonas aeruginosa* is that it is one of the limited bacteria that can synthesise hydrogen cyanide. This may be important in its pathogenicity. Cyanide is a highly toxic compound that diffuses rapidly into tissues and can inhibit aerobic respiration through its interaction with the terminal oxidases of aerobic respiratory chains. *Pseudomonas aeruginosa* can synthesise a respiratory chain terminated by a cyanide insensitive terminal oxidase [34]. It raises the issue of which symptoms of disease are related to prolonged exposure of lung tissue to cyanide.

Many other mechanisms are implicated. Not all have been well studied to date. One such theory implicates Nitric oxide (NO) which has marked in vitro antimicrobial properties. Therefore a decrease in production of NO in bronchiectasis would result in reduced local bacterial killing, thus facilitating repeated infections and colonisation [35].

The relative contribution of host and microbial determinants need to be considered as this balance is the basis of the pathogenesis of bronchiectasis. Varied virulence factors, multiple adhesins on the bacterial surface and multiple mucosal receptors have been found for most pathogens. A greater understanding of these host-bacterial interactions should, in the future, lead to development of new treatment strategies [36].

1.1.4 Aetiology of bronchiectasis

Mucociliary clearance is the first line defence against potentially pathogenic microorganisms (PPM). Kartageners syndrome, primary ciliary dyskinesia and Young's syndrome result in defective mucociliary clearance. Ciliary immotility results

in pooled secretions that can act as a nidus for infection. Most patients will have a positive family history for a similar condition [37].

Childhood infection was a common cause for bronchiectasis. However with effective childhood vaccination strategies, measles and whooping cause are now less prevalent [38]. Yet up to 50% of patients may still have a post-infectious cause for their bronchiectasis [21]. Mycobacterial disease both typical and atypical is known to cause bronchiectasis. *Mycobacterium avium* complex in particular is known to have a subtype that affects older women usually in the right middle lobe and lingula. This is a very slow process [39]. Attributing bronchiectasis to a primary pulmonary infection is thought to be complicated as this relies on long term recall. Also patients with immune disorders are likely to suffer recurrent infections in childhood and this may cloud the history [40]. Fungal plugs and eosinophilic peribronchial inflammation seen in Allergic bronchopulmonary aspergillosis is also associated with bronchiectasis [40].

Hypogammaglobulinemia and HIV infection are also known to cause bronchiectasis [40]. Hypogammaglobulinemia can be classified into patients with low levels of Immunoglobulin G (Ig G) such as common variable immunodeficiency and Burton's disease, and other forms such as Ig A deficiency and Ig G subclass deficiency [40]. Bronchiolitis oblitrans complicating lung transplant is also known to be associated with bronchiectasis [40].

Mechanical aspiration can cause repeated insults to the lung and result in bronchial and parenchymal damage. Patients with oesophagitis and oesophageal strictures are known to have a higher incidence of bronchiectasis [41]. A higher than expected rate of *Helicobacter pylori* sero-positivity is noted in these patients [42].

Another group of patients with bronchiectasis is those with rheumatoid arthritis and inflammatory bowel disease. The bronchiectasis may precede the arthritis. It prevalence can vary between 3 – 30% in these patients [40, 43, 44]. Inherited disorders causing bronchiectasis may be genetic. An older age group at presentation makes genetic disorders less likely [5]. Consanguinity of parents is sometimes seen in up to 37.2% of patients [45].

Last but not least, a cause may never be found for the disease in many patients. This can be as high as 50% of a diseased population [45]. As treatment can be modified when a cause is found [18], these patients may miss the opportunity of optimal therapeutic benefit.

1.1.5 Clinical features of bronchiectasis

Virtually all patients with bronchiectasis have a cough [8]. In a large retrospective study from Texas, cough was reported by 90% of the cohort [5]. In a similar group of patients studied in stable state, 40% had a cough for more than 50% of days. The presence of this habitual coughing has been shown to exert a relative influence on Health related quality of life (HR-QoL) [46]. In the paediatric population reports of symptoms vary. Chronic cough has been reported as the commonest presenting symptom (83.3% of patients) in some studies [28, 45] while other workers have reported it as the second most common symptom after repeated chest infections [47]. Patients often suffer social embarrassment from their cough [40]. The aetiology of cough may be multi factorial. Neutrophilic inflammation within the airways in bronchiectasis is thought to cause an acidic environment. Endogenously reduced pH

causes potential discharge in Aδ and C fibres of airway afferent nerves in guinea pigs both of which mediate the cough reflex [48]. Also thick viscous sputum in larger airways induces cough [40].

Sputum produced has variously been described as mucoid, mucopurulent, thick, tenacious, or viscid. Blood-streaked sputum or copious haemoptysis may be seen as a result of erosive airway damage caused by infection [8]. Patients may produce mucoid sputum early in their disease, developing purulent sputum when they suffer an exacerbation [20]. Haemoptysis has been reported to be a frequent phenomenon (51.2%) with the episodes tending to recur and at least one episode when the haemoptysis exceeds a tablespoon of blood. Daily sputum producers are more frequent than patients who only produce sputum during an exacerbation [5]. Other groups have reported a lower frequency of only 20% of patients complaining of haemoptysis [46]. In the paediatric population this is less common. Young children rarely expectorate sputum [49]. The volume of sputum is thought to increase during an exacerbation. This may paradoxically reduce if the secretions are sticky and difficult to clear [50]. Sputum volume is correlated with the quality of life and decline in lung function [40, 46, 51].

Dyspnoea is another commonly reported symptom. A variety of expressions such as "out of breath," "can't breathe," or "chest tightness" can be used by patients when describing their symptoms. This suggests that each patient has a different rather than single sensory experience [52]. Aetiologically this may be related to mucous plugging or to increased inflammation within the airways. Up to 71% of patients may complain of dyspnoea in one form or another [5]. It is less common in the paediatric group and reported in much lower frequency [8.8%] [45]. Some subscales of the St Georges Respiratory Questionnaire are significantly correlated to dyspnoea, daily sputum production and wheeze [53]. A lower dyspnoea score is a predictor of mortality [21].

Chest pain may be pleuritic and present in 20-30% [5, 40]. These range from dull aches to sharp pains. Joint pains may also occur [50].

Fevers are reported as common by some [5] and rare by others [50]. Our experience is that it is an uncommon finding.

Chronic rhino sinusitis may be present. Frequency has been reported from 20-30% [20] to 60-70% of patients and ranges in severity from a mild postnasal drip to fulminant pan sinusitis [40].

Fatigue is a symptom that needs specific questioning but may be a dominant symptom for up to 70% of patients [40]. These symptoms are often out of proportion to the severity of disease and accepted by the patient as they are long term issues [50].

Recurrent lower respiratory tract infections are common [28] and patients often seek help. Children may show failure to thrive and reduced exercise tolerance [47].

Bronchiectasis may be complicated by lung abscesses, pneumothorax and empyaema [40].

Regardless of the aetiology, symptoms include chronic cough, mucopurulent sputum production, haemoptysis, dyspnoea and tiredness. Although chronic suppuration is present, clinical manifestations of sepsis are infrequent. Pyrexia is not commonly reported in bronchiectasis. It is not complained of by patients and is rarely recorded during inpatient episodes. This may be due to a tolerance that is developed by patients to persistent sepsis.

Digital clubbing was commonly described in the past. More recent reports suggest a prevalence of only 3% [5, 8]. Clubbing may be seen as part of an entity called hypertrophic osteoarthropathy which include periostitis, arthritis and sometimes

thickening and oedema of the skin around the affected joints. Pulmonary diseases such as bronchiectasis and cystic fibrosis are known to be associated with hypertrophic osteoarthropathy. Hypertrophic osteoarthropathy is due to the peripheral impaction of megakaryocytes and platelet clumps in the fingers and toes, to which this particulate matter has passed in an axial stream. The normal pulmonary vascular bed retains these large particles, which fragment before entering the systemic circulation. A right-to-left shunt allows them to bypass the pulmonary vascular bed. Platelets contain and release platelet-derived growth factor, whose known effects could explain all the pathological changes in clubbing. It may be associated with severe bronchiectasis and is less frequently reported in milder forms [54].

Adventitious breath sounds on auscultation of the chest include crackles, wheezing and ronchi [8]. A clear chest does not exclude the diagnosis.

Hemodynamic alterations due to bronchiectasis have been described [55, 56]. Left ventricular diastolic dysfunction has been described in patients with cystic fibrosis and bronchiectasis [57]. Right ventricular systolic dysfunction and pulmonary hypertension is more common than left ventricular systolic dysfunction in bronchiectatic patients [58]. Right ventricular systolic function may be impaired in both these groups which may be reflected in systemic blood pressure measurements

Most patients with bronchiectasis will have visible abnormalities on CT scanning of their nose and sinuses ranging from mild mucosal thickening to pan sinusitis. Rhino sinusitis is common and can be present in up to 70% of patients [40]. Chronic fatigue is another symptom that most patients complain of. It needs specific questioning and may be present in up to 70% of patients [40, 51].

1.1.6 Diagnosis of bronchiectasis

Ninety per cent of chest radiographs are abnormal in patients with chronic cough and phlegm who have bronchiectasis. These findings are nonspecific and may include focal pneumonitis, scattered irregular opacities, linear atelectasis or dilated thickened airways that appear as tram lines [5, 8]. However with the introduction of high resolution CT (HRCT) scanning, it has become much easier to diagnose bronchiectasis. The standard HRCT criteria are well established [40, 59]. The most specific criteria are internal diameter of bronchus wider than its adjacent artery and failure of the bronchi to taper. These criteria are based on comparisons between HRCT and subsequently resected lung samples [59].

Spirometry shows a limitation of airflow in bronchiectasis [8]. Mucous plugging and bronchial inflammation results in this moderate airway obstruction [40]. A reduced forced vital capacity is often noted but is not diagnostic by any means. Airway hyper-responsiveness is also noted in up to 40% of patients [60]. This may lead to a misdiagnosis of asthma in the early stages. This is thought to secondary to bronchial inflammation [59].

1.1.7 Therapeutics of bronchiectasis

Identifying the underlying cause of bronchiectasis helps in directing treatment [18]. Once treatable causes of bronchiectasis have been excluded, principles of management include identification and treatment of acute exacerbations, suppression of microbial load, reduction of excessive inflammatory response, promotion of bronchial hygiene,

control of bronchial haemorrhage and surgical removal of damaged segments of lung when appropriate [8].

1.1.7.1 Prevention of microbial infection and suppression of microbial load

In the majority of population, treatment is largely confined to rescue antibiotics during an exacerbation. This has a limited effect on chronic infections and disease progression. Antibiotic prophylaxis therefore is an appealing strategy [61].

The most commonly isolated pathogens are *Haemophilus influenzae, Pseudomonas aeruginosa* and *Streptococcus pneumoniae* [8]. *Pseudomonas aeruginosa* is associated with increased sputum production and poorer quality of life [8, 62]. Reduction in the sputum volume may improve the quality of life [61].

Resistance to β-lactams in subjects with *Haemophilus influenzae, Streptococcus pneumoniae* or *Moraxella catarrhalis* increases from 11% to 26% and resistance to gentamicin in subjects with *Pseudomonas aeruginosa* increases from 13% to 49% over a 5 year period when treated with these antibiotics [17] . Patients with resistant bacteria are significantly more likely to be hospitalised and have a greater number of exacerbations in a year [17]. The use of regular systemic prophylactic antibiotics therefore may be problematic due to side effects and development of antibiotic resistance [63]. It is important to encourage outpatient parental antibiotic use and use of multiple antibiotics simultaneously to try and reduce resistance [17].

In an attempt to reduce the use of systemic antibiotics, inhaled or aerosolised antibiotics have been tried. Inhaled aerosol produces a high concentration of the drug locally and reduces systemic absorption. They have a better side effect profile and systemic complications are fewer. Side effects include unpalatable taste, expense and a hoarse voice. There is a small risk of bronchospasm with inhaled antibiotics. The most important drawback is that aerosolised antibiotics do not penetrate the deep lung. As a result there is a concentration gradient between the proximal and distal airways. This gradient encourages antibiotic resistance [64]. Coughing and haemoptysis are other limiting factors in the adoption of aerosolised antibiotics in bronchiectasis [64].

Aerosolised Tobramycin has also shown to increase significantly the time to exacerbation in patients with cystic fibrosis [65]. A statistically significant fall in bacterial density has been reported at 7 days after therapy with inhaled Tobramycin [3, 66, 67]. Nebulised aminoglycosides improve sputum *Pseudomonas aeruginosa* density and myeloperoxidase levels in bronchiectasis [68]. The regular use of inhaled Tobramycin or Colistin can decrease exacerbations of lung disease, decrease bacterial counts, and improve pulmonary function in patients colonised with *Pseudomonas aeruginosa* [3]. Significantly fewer admissions were noted in a randomised control trial where the active group received aerosolised Ceftazidime and Tobramycin for 12 months [69].

Various regimes including a single antibiotic on and off for alternate months and different antibiotics for alternate months have been used. The safety and effectiveness of these strategies are yet to be established [64].

1.1.7.2 Anti-inflammatory therapy.

Inflammatory mediators are implicated in the pathogenesis of bronchiectasis. It would therefore follow that suppression of this inflammation locally with inhaled corticosteroid therapy may be beneficial. Indeed in stable state bronchiectasis, inhaled corticosteroids improve 24hour sputum volume. The improvements in spirometric indices are small or none at all. Sputum purulence remains unchanged. Symptomatically cough is improved but exacerbation frequency is not altered [70, 71].

Daily sputum producers became unproductive when treated with long term Azithromycin. The mechanism of action of Azithromycin is not fully known but immune-modulatory effects seen in lung transplant patients along with antimicrobial effects are thought to be responsible [61]. The anti-pseudomonas effect of Azithromycin may represent an inhibitory effect on quorum sensing, biofilm formation, production of immune-stimulatory exoproducts and the inflammatory response to the organism [72, 73]. Low dose erythromycin is also known to down regulate glycoconjugate release and improves lung function and reduces sputum production. However the long term effects are unknown [74].

1.1.7.3 Broncho-pulmonary hygiene and bronchiectasis

Airway clearance is an integral component in the management of bronchiectasis. Physical therapy and mechanical aids in chest physiotherapy are used. The traditionally used method of postural drainage (PD) is now replaced by the active cycle of breathing technique (ACBT). ACBT comprises breathing control, thoracic expansion exercises

and forced expiratory technique and postural drainage. Some of these can be done independently while percussion and vibration need more than one person. The flutter valve is a simple mechanical device that produces oscillating pressure on exhalation, which helps loosen secretions and prevent airway closure. Acapella is a handheld device which combines positive expiratory pressure with high-frequency oscillation therapy.

While efficacy is vital, tolerability of the procedure is an important consideration. PD is less tolerated when patients suffer with gastro-oesophageal reflux. Discomfort and lack of time also give mechanical aids an edge over physical therapy. Early acceptability and tolerability are likely to be influential in preference and ultimately adherence [22]. While ACBT-PD has been perceived as significantly more useful in clearing sputum, it also causes more discomfort and is time consuming. Patients prefer a flutter valve to ACBT-PD. Sputum volume is also more when patients use ACBT-PD [22].

Airway clearance technique prescriptions are for life. Those that can be performed independently in the domiciliary setting are likely to influence adherence.

1.1.7.4 Surgery and bronchiectasis

The role of surgery is limited in the post antibiotic era. The goals of surgery include removing foreign bodies, obstructing tumours, elimination of segments that may be contributing to acute exacerbations and removal of lobes if suspected to cause haemorrhage [8].

Lung transplant (LT) can be successfully performed on patients with advanced bronchiectatic lung disease with subsequent good post-transplant quality of life. Despite the presence of chronic lower respiratory tract infection with bacterial pathogens in these patients pre-transplant and post-transplant infections do not generally have significant impact on survival, although infection with antibiotic-resistant bacteria may complicate post-transplant management [75].

1.1.8 Prognosis in non CF bronchiectasis

Bronchiectasis is commonly disabling but not a fatal disease. The prognosis of patients with bronchiectasis remains unclear. While quality of life may be compromised fatality is uncommon. Survival rates are as high as 97%, 89%, 76% and 58% at 1, 2, 3 and 4 years respectively. The mean survival time is estimated as 44±1.6 years [21].

Comorbidities increase the suffering in patients with bronchiectasis. It is often difficult to assess if these comorbidities are the cause or result of the bronchiectasis. Sinusitis is seen in up to 31% of patients while gastro-oesophageal reflux is reported in 17% of patients [22].

No significant radiological deterioration is seen to occur over periods almost as long as a decade. The annual fall in FEV_1 may be small but some patients go on to develop respiratory failure [5]. The average age at death has increased in the last few decades and the cause of mortality is now mostly related to respiratory failure [76].

In cystic fibrosis it is recognised that patients with chronic infection with *Pseudomonas aeruginosa* have reduced survival and a more rapid decline in lung function than

patients with chronic infection with S*taphylococcus aureus* [77]. The evidence in non CF bronchiectasis is limited and conflicting. It is possible that Pseudomonas aeruginosa in non-CF bronchiectasis can cause accelerated decline in lung function but this can be modified by the use of nebulised antibiotic therapy [4].

Long term mortality is thought to be significantly associated with age, body mass index, MRC dyspnoea scale, vaccination, radiographic extent, hypoxemia, hypercapnia, and functional parameters [21].

1.2 Acute infective exacerbations in bronchiectasis

1.2.1 Defining an acute exacerbation in bronchiectasis

A patient may identify an acute exacerbation in bronchiectasis. The clinician or researcher is usually guided by this to initiate treatment. No standard definition has been proposed or accepted as yet for an exacerbation in bronchiectasis. However in an attempt to understand, describe and measure an exacerbation various definitions have been used.

A glance at practice in other respiratory diseases may be useful. In chronic obstructive pulmonary disease, increasing dyspnoea, sputum production and worsening sputum purulence is sometimes used whilst describing an exacerbation [78]. In Cystic fibrosis, a significant fall in lung function (measured as >200 mL reduction in the forced expiratory volume in 1st second (FEV_1), together with three or more of: increased cough or shortness of breath; increased sputum volume; increased sputum purulence; haemoptysis; fever (>37.5°C); new or increased crackles heard in the chest; new shadowing on chest X-ray and weight loss is often used [79].

In a previous study testing the therapeutic effect of Deoxyribo nuclease, authors chose a variety of symptoms of which patients have only had to achieve 4 of 9 to achieve a protocol defined exacerbation. These included (i) change in sputum production (consistency, colour, volume or haemoptysis); (ii) increased dyspnoea (chest congestion or shortness of breath); (iii) increased cough; (iv) fever (38°C) (v) increased wheezing (vi) decreased exercise tolerance, malaise fatigue or lethargy; (vii) FEV_1 or

FVC decreased by 10% from a previously recorded value; (viii) radiographic process indicative of a new pulmonary process; or (ix) changes in chest sounds [27].

Duration of symptoms can occasionally guide the definition. In a trial of inhaled corticosteroids, an exacerbation was defined as deterioration for more than 24 hours in at least three respiratory symptoms (including cough, dyspnoea, haemoptysis, increased sputum purulence or volume, and chest pain) with or without fever (≥37.5°C), radiographic deterioration, systemic disturbances or deterioration in chest signs [71].

Strict criteria can make patient recruitment to a project difficult. Simpler definitions are sometimes used in an attempt to include more patients. In a prospective adult cohort study assessing the effect of 2 weeks of intravenous therapy, increasing cough, sputum production and sputum purulence was adopted as a definition [80].

Choosing a symptom and sign along with an optimal duration of these parameters appears critical. An increase in the sputum volume usually over a 24 hour period remains invariable in most definitions [21, 46, 61]. Change in sputum colour, viscosity or purulence seems to be common and is frequently included [21, 46, 61]. Breathlessness, chest discomfort and haemoptysis are less often added. All these symptoms are experienced by patients and may be transient. Therefore the duration of symptoms is important. Most groups stress the need for symptoms to persist for more than 24hours [21, 46, 61].

Our group has previously studied the therapeutic use of inhaled Tobramycin in the treatment of an exacerbation of bronchiectasis [3]. The criteria used to define an 'acute exacerbation' was the presence of at least two of the following symptoms: increased cough, increased volume of sputum produced, increased sputum purulence, increased dyspnoea or increased wheezing; and at least one of the following: fever ≥38°C, malaise. This definition has been adhered to for the purpose of this study.

There is continuing difficulty in both research and clinical practice in distinguishing exacerbations from day-to-day symptom variation [81, 82]. Further research with symptom diaries would be required to validate definitions of acute exacerbations in bronchiectasis. This is beyond the scope of this study.

1.2.2 Pathophysiology of an acute exacerbation in bronchiectasis

Morbidity associated with bronchiectasis is related to recurrent exacerbations and HR-QoL [80, 83]. While our understanding of the pathophysiology of stable state bronchiectasis has progressed, little is known about the changes that occur during an acute exacerbation [2]. These episodes are probably related to a complex relationship between host defence and airway microbiology that impacts on sputum production and airflow obstruction.

Knowledge from the world of CF may provide us with clues. For instance it is possible that viral infections trigger these episodes when bacterial colonisation is already present [84, 85]. Perhaps, as in chronic obstructive airways disease, acquisition of new strains of a colonising organism alters the fine immune balance [86]. Bacterial

clearance at the end of antibiotic treatment is seen to be associated with reduced sputum volume [80].

More work remains to be done to provide evidence to support these assumptions for patients with bronchiectasis [84]. Borrowing theories from CF has previously not worked. While deoxyribonuclease 1 is an effective treatment in patients with CF, these benefits are not directly transferable to patients with bronchiectasis [27] Most studies of preventive and treatment strategies of respiratory exacerbations in bronchiectasis have been undertaken at a single centre and have included small patient numbers where power calculations have not been reported and where the definitions of exacerbations have been limited [84]. Table 1.1 lists the possible causes of an exacerbation in bronchiectasis. This is by no means an exhaustive list.

Table 1.1: Some possible causes of an acute exacerbation in bronchiectasis

Possible cause
1. New bacterial infection 2. New strain of existing bacteria causing infection 3. Change in bacterial density i.e. sudden growth in numbers 4. Viral infection

1.2.3 Therapeutics of exacerbations in bronchiectasis

1.2.3.1 Antibiotic treatment of acute exacerbations of bronchiectasis.

Antibiotic therapy is complex in bronchiectasis and includes short-term empirical treatment for acute exacerbation [87]. The choice of antibiotics is usually guided by previous isolates of potentially pathogenic microorganisms (PPM) in sputa. The optimum period of treatment is also unknown but patients often require longer courses of antibiotics. While therapy has been administered for between 5 and 28 days, a period of 14 days is common practise [80].

It is unknown if there is benefit from stopping prophylactic antibiotics at the time of an exacerbation. Most published studies have included patients if they were already on prophylactic antibiotics [80]. Two studies have shown clear benefit in 24 hour volume, microbial clearance and HR-QoL when patients are treated for a period of 14 days based on previous microbial sensitivities. We are therefore guided by these at the time of the study [80, 83]. Since then the British thoracic society have published guidelines that recommend 14 days of antibiotic treatment [88].

1.2.3.2 Active chest physiotherapy in exacerbations of bronchiectasis

Limited information is available on exercise training in patients suffering an acute exacerbation of bronchiectasis Postural drainage and percussion represent the physiotherapist's mainstay in dealing with exacerbations of chronic obstructive disease. Similar manoeuvres may increase production of sputum and produce improvements in pulmonary function in bronchiectasis [89].

Effective physiotherapy could influence the frequency and outcome of an acute exacerbation. This has not been previously systematically studied.

1.2.3.3 Other modalities of treatment of an exacerbation in bronchiectasis.

Patients with bronchiectasis have a chronic low grade infection and inflammation, punctuated by exacerbations. These are characterised by increased inflammation locally often triggered by infection, exposure to aero-irritants, or gastric contents [64]. It would therefore be reasonable to trial systemic or inhaled anti-inflammatory agents in the acute phase. However this study remains to be done and for the purpose of this thesis patients did not require adjunctive therapy in addition to antibiotics.

1.3 Measuring outcome and setting end points for therapeutic trials in bronchiectasis

"*How will I know when my treatment has been clinically successful*?" From an uninitiated observer the answer to this would be "*when an increase in life expectancy and/or quality of life as measured by treatment burden, time in hospital, or similar has been reached*" [90].

Markers of disease activity in bronchiectasis fall into the categories of inflammation, infection, imaging and lung physiology. With regard to stable state bronchiectasis a large cohort of patients would need to be studied for long periods of time to show significant benefits in treatment [90]. Currently a successful outcome relies predominantly on the subjective assessment of symptom resolution [80].

Spirometry - FEV_1 (forces expiratory volume in the first second), FVC (forced vital capacity) has traditionally been used in lung disease to measure change. Longitudinal studies have been used to predict disease progression or the natural history of lung disease [91, 92]. It has historically also been used to study acute exacerbation in lung disease [91]. It is therefore not surprising that it has been frequently employed within studies of bronchiectasis to assess functional state and measure change.

FEV_1 and FVC reflect changes in the calibre of bronchi. In bronchiectasis the bronchial walls may be inflamed. Anti-inflammatory medication such as inhaled corticosteroid may reduce this inflammation. FEV_1 and FVC were therefore useful end point to use in assessing response to inhaled fluticasone in a randomised controlled trial. However there was no significant change noted on treatment in these indices [71]. Secretions may accumulate in saccules of the wall within the bronchi. Also active bacterial invasion is thought to happen at the mucociliary surface. Trialling antibiotics by measuring change in spirometric indices would then be reasonable. Both aerosolised and long term oral antibiotics have been tested for efficacy in bronchiectasis. Spirometric indices failed to show an improvement but were used as end points in these studies [3, 61, 64]. It could be assumed that cleaving viscous sputum may decrease obstruction and improve compliance of lungs. In a study of the efficacy of DNase as a mucolytic in bronchiectasis, a fall of 1.7% in FEV_1 was noted in the placebo arm including 176 patients. The therapeutic arm however noted an even greater fall in the FEV_1 [27]. Although used frequently, spirometric indices need to be studied systematically in bronchiectasis. Their usefulness as end points is yet to be proven.

Frequency of exacerbations, counting episodes of hospitalisation, and time to exacerbation may be end points. Inhaled corticosteroids and additional antibiotic regimes have also failed to decrease the frequency and time to exacerbation [3, 93]. Indirect measurements such as requirement of intravenous antibiotics per month have also been used as end points [94].

Twenty four hour sputum volume is an attractive end point. Dyspnoea as measured on the Medical Research Council scale, forced spirometry functional variables, daily sputum volume, short-acting β2- agonist dosage, and HR-QoL are all significantly related to sputum volume [46]. Inhaled fluticasone and prophylactic oral Azithromycin reduce sputum volume significantly [61, 71]. Also in acute exacerbations of bronchiectasis 24 hour sputum volume is significantly reduced on treatment with antibiotics [80]. Twenty-hour sputum volume appears useful as an end point.

Microbial presence in sputum is the hallmark of this disease. Microbiological culture of sputum is therefore critical to any study in bronchiectasis. Sputum appearance alone is useful and a purulence score has been successfully used as a marker of disease [71]. Sputum bacterial density [64], sputum microbiology and microbial clearance [61, 80, 83] have also been used as markers of disease to study outcome in therapeutic trials. Any study in bronchiectasis would be incomplete without a microbiological analysis of sputum.

Whilst symptoms may appear difficult to quantify, attempts have been made to measure them scientifically. Dyspnoea has been measured on the Medical Research Council scale [46]. Indirectly HR-QoL questionnaires can assess patient's symptoms and a specific cough questionnaire was designed in Leicester that has been validated in bronchiectasis [95]. The St George Respiratory questionnaire is validated in bronchiectasis [53].

Improvements in inflammation may be associated with improvement in bronchiectasis symptoms. Biochemical measures of inflammation in sputum or serum can be measured to assess outcomes [80]. Functional outcomes are also important and although lung function parameters may not be helpful, a walk test such as the incremental shuttle walk is worth exploring [80]. Finally arterial blood gas changes may also prove useful [21].

Outcome-based measurements are important to guide health care delivery [96]. Respiratory tools therefore can play an important role in maximizing positive outcomes from therapy [97]. These outcome measures need to be validated, easily accessible and relevant [98]. In an attempt to understand which markers of lung disease are sensitive in predicting the outcome of treatment we have conducted a prospective longitudinal study that we hope will add more information to the current understanding of exacerbations in non CF bronchiectasis.

1.4 Quality of life in bronchiectasis

1.4.1 Background

In patients with chronic lung disease, the relationship between changes in symptomatic and functional state and conventional physiological indices is often weak [99, 100]. This is seen particularly in interventions such as respiratory rehabilitation programmes, in which patients are taught to cope with their physiological limitations [101]. Direct measurement of the impact on patient's lives is therefore necessary to assess whether interventions are of benefit [100].

Health-related quality of life (HR-QOL) is therefore a relevant and quantifiable outcome of care. HR-QoL is impaired in stable state bronchiectasis and deteriorates during an acute exacerbation [83, 102]. HR-QoL is a potentially important marker for evaluating existing and new therapies in bronchiectasis.

1.4.2 Determinants of quality of life in bronchiectasis

Most aspects of bronchiectasis impact on the quality of life. Symptoms are experienced in mild disease, even in stable state. These deteriorate during an exacerbation and exert a greater impact. Appropriate management of symptoms on identifying the underlying aetiology has a positive impact on the quality of life in these patients.

Demographically, age does not impair the quality of life, but women with bronchiectasis report poorer quality of life scores on physical functioning and social

functioning than males. A gender-related difference in response to chronic disease is important in tailoring an education and management plan to each individual patient. Evidence indicates that males and females perceive their health status differently, with females having a more accurate perception of objective clinical health status [102, 103].

The amount of sputum produced by patients with bronchiectasis is a fundamental symptom that interferes decisively with HR-QoL. In stable state bronchiectasis, on analysis of variables influencing the different questionnaire subscales, daily sputum production explains a greater per centage of the variance of the symptoms subscale than dyspnoea alone. The amount of sputum produced accounts for over one fourth of the variance of the symptoms subscale (27%), while on adding dyspnoea to the analysis the explanation of variance increases by only 11%, while the cumulative effect accounts for 38% of the variance of the symptoms scale [53]. In the activity sphere, dyspnoea as measured on the Medical research council scale is an important variable that exerts an influence. A poorer activity score has been implicated as an independent variable among patients who have received repeated courses of steroid treatment [46, 104].

Chronic productive cough is a common symptom in patients with bronchiectasis that is associated with a reduction in HR-QoL. Bronchopulmonary hygiene physical therapy (BHPT) is widely prescribed for patients with bronchiectasis, although the evidence for its efficacy is limited. As little as a 4 week outpatient-based BHPT regime can lead to a significant improvement in cough-related quality of life [105].

Upper airway symptoms are also frequent in patients with bronchiectasis. Associations between upper and lower airways diseases have been demonstrated in other respiratory

conditions including asthma (allergic rhinitis & nasal polyposis) and chronic obstructive lung disease (chronic rhinosinusitis). However while bronchiectasis itself has a considerable impact on the quality of life, upper airway symptoms have no additional impact on the quality of life of patients with bronchiectasis [102].

Patients with bronchiectasis often complain of abnormal tiredness, difficulty in concentrating or low spirits. Moderate-severe anxiety is more frequent than equivalent levels of depression. Anxiety and depression scores are associated with perceived health status. Up to 34% of patients have elevated scores for anxiety, depression or both. Therefore, treatment aimed at reducing symptoms and improving exercise capacity alone will not reduce levels of anxiety which requires alternative therapy [106].

The number of exacerbations in the previous year also seems to influence the quality of life. Some studies suggest that this is an important contributor while others have found hospital patient admissions due to exacerbation of the disease to be only weakly related to HR-QoL score as measured by the SGRQ [46, 53].

However after the onset of chronic respiratory failure, life expectancy and quality of life is poor in patients with bronchiectasis [107].

Bacterial colonisation of sputa has been shown to directly impact on the quality of life in patients with bronchiectasis. Patients in whom the sputa is colonised by *Pseudomonas aeruginosa* have a significantly worse quality of life than those who are colonised by other potentially pathogenic microorganisms or not at all [62, 108, 109].

There is conflict in evidence as to whether other microorganisms affect the quality of life in bronchiectasis. In one study, identification of *Haemophilus influenzae* in the sputum meant a better quality of life than if no organism was isolated. However

isolation of *Haemophilus influenzae* did affect other parameters of disease activity, such as CT scan bronchiectasis scores and the frequency of exacerbations [62]. Others suggest that patients having microorganisms other than pseudomonas have a worse quality of life than those without microorganism [102, 108, 109].

Functional variables such as FEV_1 do not affect the symptoms subscale of HR-QoL significantly. However, the FEV_1 following bronchodilator therapy, is a relevant HR-QoL conditioning variable. As in COPD, this should not be used as the sole severity marker of stable bronchiectasis [46, 104]. Inflammatory markers in exhaled breath have not been found to impact on the quality of life as measured by the SGRQ [110].

Appropriate treatment with antibiotics improves infection related inflammation and thus improves cough, wheeze and sputum volume. A significant improvement between onset of exacerbation and completion of treatment has been reported in dyspnoea scores, mastery and emotional domains of the Chronic Respiratory disease questionnaire [83]. Similarly the use of inhaled colistin in the treatment of bronchiectasis with recurrent Gram-negative infections is shown to improve quality of life and slow decline in forced expiratory volume in 1s and forced vital capacity [111]. Treatment with inhaled steroids in patients with non-CF steady-state bronchiectasis results in improvement in daily sputum production, dyspnoea, days without cough, and number of doses of short-acting β_2 agonists required weekly. It is suggested that this improvement is seen as soon as within 1 month of commencement of treatment and persists for at least 6 months whilst on the treatment. A significant improvement is seen in the HR-QoL of these patients. Although the number of adverse events are greater, they are local and reversible [112]. Patients with chronic respiratory failure due to bronchiectasis, treated at home with nocturnal non-invasive for at least 12 months have also reported an improvement in their quality of life [107].

In the longer term, pulmonary rehabilitation programmes may be of some benefit. In a prospective study to determine the effects of a pulmonary rehabilitation programme some patients with bronchiectasis completed a 6-week outpatient pulmonary rehabilitation programme that included education, physical and respiratory care instruction and supervised exercise training. Outcome assessment was performed at baseline, on completion of the programme and 3 months after the programme. Disease-specific quality of life was assessed using the Chronic Respiratory Disease Questionnaire (CRDQ). All domains of the CRDQ improved significantly and patients who completed a comprehensive pulmonary rehabilitation programmes showed an improvement in their health status [113].

If disease is localised, surgical treatment of bronchiectasis yields immediate resolution of symptoms, better quality of life and no mortality [114]. The amount of sputum produced, colonisation with *Pseudomonas aeruginosa* and appropriate medical or surgical management seem to be the most important determinants of the quality of health in bronchiectasis.

1.4.3 HR-QoL and acute exacerbations in bronchiectasis.

A significant improvement has been noted in all individual domain scores of the SGRQ following completion of treatment with antibiotics during the course of an exacerbation [80]. In another prospective cohort study patients completed the questionnaire at the onset of an exacerbation, on completion of antibiotics at day 14 and four weeks after the completion of antibiotics. A significant improvement was noted in three of the four domains (dyspnoea, emotional and mastery) of the CRDQ.

All the improvement was seen by Day 14 and there was no further change in 4 weeks [83]. No study has assessed quality of life in stable state and followed it through an exacerbation to date.

1.5 Measuring lung function in bronchiectasis

1.5.1 Background

Spirometric indices have traditionally been used to stratify lung disease as mild moderate or severe. Variability in theses indices have been used as surrogate markers of inflammation. Assessing treatment effects and clinical outcomes therefore would seem a natural progression.

Annual physiological decline in lung function is well known. The structural changes include chest wall and thoracic spine deformities which impairs the total respiratory system compliance leading to increased work of breathing. The lung parenchyma loses its supporting structure causing dilation of air spaces: "senile emphysema". Respiratory muscle strength decreases with age and can impair effective cough, which is important for airway clearance. The lung matures by age 20-25 years, and thereafter aging is associated with progressive decline in lung function. [115].

Lung function has been well studied in patients with Cystic Fibrosis. Measurement of lung function in these patients is known to reflect both acute and chronic changes [90]. The Forced expiratory volume in one second (FEV_1) has been used as a surrogate marker in Cystic Fibrosis. An acute change in FEV_1 caused by mucous plugging has

been clearly demonstrated and provides a short term endpoint [116]. The lung function declines no more than 1% per year in these patients [117]. This may be useful in longitudinal studies that occur over prolonged periods of time.

1.5.2 Spirometry in non-CF bronchiectasis

Most patients with bronchiectasis have a varying degree of airflow obstruction [28, 118]. There is also an increase in pulmonary flow resistance in a significant number of patients [119]. The mechanism of airway obstruction in bronchiectasis is thought to be multi factorial. Bronchial plugging by secretions, mucosal oedema, bronchospasm, distortion, kinking of bronchi or excessive dynamic collapse of airways on expiration may all contribute to this. The decrease in dynamic pulmonary compliance could be attributed to a non-uniform distribution of airway resistance as well as increased stiffness of the lungs due to patchy fibrosis and atelectasis. The pulmonary transfer factor may be reduced or normal [120].

1.5.3 Use of spirometry as a biomarker in bronchiectasis

FEV_1 has been used as an endpoint in studies to assess efficacy of treatment. Long term treatment with Azithromycin has shown no significant improvement in actual and % predicted FVC [61]. In another therapeutic trial of Azithromycin as a prophylactic antibiotic in bronchiectasis there was a trend of improvement in all lung function parameters but this was not statistically significant [94].

1.5.4 Spirometry and prognosis in bronchiectasis

Impaired pulmonary function is of prognostic importance in bronchiectasis. Physiological decline in lung function is said to be exacerbated in bronchiectasis. The majority of patients have impaired spirometry with airflow obstruction but normal carbon monoxide diffusing capacity. Multivariate analysis has shown that factors significantly associated with worse lung function are bronchial hyper responsiveness, concomitant asthma, higher serum globulin, higher peripheral leukocyte count, lower serum albumin, greater sputum volume, diffuse disease and older age [121].

Normal adults of may lose up to 30mls of their ventilatory capacity in a year. In bronchiectasis twenty per cent of patients show no decline or an improvement in the FEV_1 [122]. But up to 60% of patients show a decline of some volume. This has been variously put at 39mls/year [123] , 49mls/year [5] , 50mls/year [122] and 54-55mls/year [5, 51]. The most important factor linked with an accelerated decline in FEV_1 in stable patients with non-CF bronchiectasis is chronic colonization with Pseudomonas aeruginosa. This loss can be as much as 123 mls/year [124]. Some reports place it at a smaller value with the overall decline in FEV_1 in patients infected with Pseudomonas aeruginosa as 23mls. In those never infected with Pseudomonas aeruginosa it was recorded as 24mls [125]. The lung damage is thought to progress at the same rate in both sexes [18, 21]. In attempt to identify a relationship between inflammation and spirometry, a paediatric study was unable to find any correlation between Leukotriene B4 and FEV_1 [126]. Patients with more advanced disease and moderate obstruction (FEV1 <65%) have more inflammation within the airway. Levels of TNF-α , IL-1B and IL-10 are higher in bronchial lavage fluid of these patients [127]. FEV_1 and FVC have been found to be weak predictors of mortality [21].

1.5.5 Impulse Oscillometry (Forced oscillation technique) and lung disease

Conventional methods of lung function testing provide measurements obtained during specific respiratory actions of the subject. In contrast, the Impulse Oscillometry technique (IOS) determines breathing mechanics by superimposing small external pressure signals on the spontaneous breathing of the subject. IOS utilises the externally applied pressure signals and their resultant flows to determine lung mechanical parameters. These external forcing signals, may be mono- or multi-frequency, and are applied either continuously or in a time-discrete manner.

These pressure–flow relationships are largely distinct from the natural pattern of individual respiratory flows, so that measured IOS results are, for the most part, independent of the underlying respiratory pattern. Therefore, Oscillometry minimises demands on the patient and requires only passive cooperation of the subject. Potential applications of Oscillometry include paediatric, adult and geriatric populations. Oscillometry is also useful in veterinary medicine and hence in animal studies. Diagnostic clinical testing, monitoring of therapeutic regimens, and epidemiological evaluations, are easily done using IOS and importantly are independent of the severity of lung disease [128].

The most obvious relationship with other pulmonary function tests concerns the use of spirometry. Spirometry measures maximal forced respiratory efforts, while IOS measures quiet breathing. In bronchiectasis, IOS has not been compared to spirometric techniques as yet.

IOS has been used to monitor response to interventions in asthmatic children. IOS has been reported to show greater sensitivity to inhaled corticosteroid or to beta-agonist inhalation than spirometry. Both inhaled corticosteroids and beta-agonists improve small airways function, and IOS responses manifest prominent changes in indices of peripheral airway obstruction. In contrast, spirometric sensitivity to small airways function is less prominent. Accordingly, it is expected that IOS might provide useful indices of peripheral airway change in response to therapeutic interventions [129, 130]. In patients with chronic obstructive airways disease IOS is able to detect significant change after bronchodilator therapy and FEV_1 is less sensitive [131].

In addition to its simplicity and non-invasiveness, IOS may be an useful clinical tool not only for detecting pulmonary functional impairment, but also to some extent in estimating the patient's quality of daily life and well-being [132].

1.6 Exhaled breath condensate and bronchiectasis

1.6.1 Exhaled breath condensate

Exhaled breath consists of a gaseous phase that contains volatile substances, such as nitric oxide, carbon monoxide and hydrocarbons, and a liquid phase termed exhaled breath condensate (EBC) [133]. It is assumed that the airway surface liquid is aerosolized during turbulent airflow such that the contents of the condensate reflect the composition of the airway surface liquid [134]. Exhaled breath condensate (EBC) was first reported as a human body fluid in 1980 in the context of studies of surface active properties/surfactant [135]. Naturally EBC contains mostly water vapour (>99.99%) The fraction of droplets are thought to contain analytes of interest [136]. These include a large number of mediators including adenosine, ammonia, hydrogen peroxide, isoprostanes, leukotrienes, nitrogen oxides, peptides and cytokines. Although EBC contains mainly metabolic products from the airways and the lung, it also contains products of other organs that reach the lung via the blood stream. As a consequence, the identification of products released from a particular site in the body may provide organ-specific information of oxidative stress [133]. Concentrations of these mediators are influenced by lung diseases and modulated by therapeutic interventions.

1.6.2 Exhaled breath condensate and lung disease

The lung exists in an oxygen rich environment which together with its large surface area and extensive blood supply, makes the organ susceptible to injury mediated by reactive oxygen species [ROS] [133]. Increased production of ROS has been directly

linked to lipid oxidation which may cause direct lung injury [137, 138]. Oxidative stress is associated with a range of inflammatory lung diseases, including asthma, adult respiratory distress syndrome, idiopathic pulmonary fibrosis, pneumonia, and chronic obstructive pulmonary disease [137]. It is thought that the various biomarkers identified in EBC may be indicative of different aspects of oxidative stress and that this non-invasive approach may be useful in categorising pulmonary disease.

Hydrogen peroxide in exhaled breath is a direct measure of oxidant burden in air spaces, is soluble and equilibrates with air [139]. Increased concentrations of H_2O_2 in EBC has been demonstrated in asthma, ARDS, CF and lung cancer [134]. This is however complicated by confounding factors. EBC H_2O_2 is also elevated in smokers and during a common cold. Therefore more sensitive and specific assays will be required [133].

Nitric oxide [NO] is another marker of airway inflammation and indirectly a measure of oxidative stress. Stable NO derived products can be measured in the EBC. 3-nitrotyrosine is one such product, concentrations of which are increased in association with worsening symptoms and deteriorating lung function in asthma [133, 140]. Total Nitrite and Nitrate concentrations are also elevated in cystic fibrosis patients and asthmatics compared to control subjects [137].

In addition pro and anti-inflammatory cytokines can be measured in exhaled breath. Interleukine-1β, Interleukine-6, Interleukine-8 have all been reported in EBC from human subjects [134]. Concentrations of IL-6 are raised in EBC of patients with cystic fibrosis [133, 141].

EBC can be exploited to assess a spectrum of potential biomarkers, thus generating a fingerprint characteristic of the disease. By assessing the nature of oxidative stress in this manner, the most appropriate therapy can be selected and the response to treatment monitored [133].

1.6.3 Monitoring lung disease by measuring pH of exhaled breath condensate.

Airway pH homeostasis is maintained by a balance of different buffer systems and the production of acids and bases in the airways [142]. The assay used to determine EBC pH is easy and measurements are highly reproducible [133]. pH levels are also related to eosinophilic or neutrophilic inflammation of the airways [143]. The pH of EBC has been found to be low in stable asthma, COPD, bronchiectasis, cystic fibrosis, and acute respiratory distress syndrome [142]. The pH of EBC collected from patients with acute asthma is more than two log orders lower than normal but normalises with corticosteroid therapy [144]. Similar findings have been reported in COPD and bronchiectasis [145].

EBC can thus be exploited to assess a spectrum of potential biomarkers, generating a fingerprint characteristic of the disease both in stable state and during an acute exacerbation. By assessing the nature of oxidative stress in this manner, the most appropriate therapy can be selected and the response to treatment monitored [133].

1.6.4 Exhaled breath condensate and bronchiectasis

Most inflammatory markers identified in EBC have been described in CF with limited data in non-CF bronchiectasis. H_2O_2 is the only well described marker in bronchiectasis. In the respiratory system H_2O_2 is released both from inflammatory and structural cells. These include neutrophils, eosinophils, macrophages and epithelial cells. EBC H_2O_2 concentrations are elevated in bronchiectasis with a significant inverse correlation between lung function and EBC H_2O_2 levels [146]. No other marker has been clearly identified in EBC of patients with bronchiectasis alone.

1.7 The microbiology of bronchiectasis

1.7.1 Background

The airways of patients in non-cystic fibrosis bronchiectasis become chronically infected with bacteria. This leads to an intense inflammatory response within the bronchi not necessarily with a systemic inflammatory response. This reaction is exaggerated in patients colonised by microorganisms with potential pathogenicity (PPM), the most common being *Haemophilus influenzae*, *Staphylococcus aureus, Streptococcus pneumoniae and Pseudomonas aeruginosa* [4].

Bronchiectasis in the pre-antibiotic era predominantly affected young patients with a high mortality rate [40, 147]. The introduction of antibiotics and immunisation has led to a dramatic improvement in the outcome [8, 40]. However, bronchiectasis remains

prevalent and exacerbations continue to contribute to significant morbidity and poorer HR-QoL [83, 102].

The identification of a respiratory exacerbation in bronchiectasis is complex [8]. Sputum production is continuous and purulence may be common. Systemic symptoms are uncommon. Given that persistent bacterial infection of airways is responsible for progression of disease, it would be logical to identify markers of inflammation and endpoints for therapeutic trials from sputum. To this end either looking for a new pathogen or increase in the numbers of the existing predominant pathogen seems reasonable.

1.7.2 Potentially pathogenic microorganisms in bronchiectasis (PPM)

Effort has always been concentrated on isolating a PPM in sputum. The isolated pathogens include *Haemophilus influenzae, Pseudomonas aeruginosa and Streptococcus pneumoniae* [5, 18, 127].

Haemophilus influenzae is the most commonly isolated organism in stable cohorts [17]. The incidence can vary from 22% up to 47% in a group of patients [17, 46]. *Pseudomonas aeruginosa* is the other commonly isolated pathogen in most series. It is considered a marker of more severe disease and is associated with compromised HR-QoL [62]. These patients also have poor lung function and extensive disease on HRCT [17]. The incidence varies between 12- 27% in some reports [17, 46, 71]. *Moraxella catarrhalis, Streptococcus* pneumoniae and *Staphylococcus aureus* are more infrequent. Commensals are noted most frequently. Absence of pathogens is reported in lower numbers in some series [21%] and up to 60% by others [17, 71]. This

could indicate a fault in detection or perhaps early infection when host defence balance is adequate.

Both *Pseudomonas aeruginosa* and *Haemophilus influenzae* are capable of forming biofilms [17]. The timing of acquisition of *Pseudomonas aeruginosa* is unknown [148]. As in CF early treatment may delay persistent infection. Infection with *Staphylococcus aureus* in patients with bronchiectasis is more frequently associated with ABPA and atypical variants of CF and is a useful marker of these conditions [149].

Anaerobic bacteria are not detected by routine aerobic culture methods. It is possible that these reside within the airway mucous. These may be present in healthy individuals however they are present in larger numbers in lungs of patients with cystic fibrosis. It is possible that they may be contributing to infection and inflammation in non-CF bronchiectasis [150]. Viral infection has yet to be formally studied in bronchiectasis. When neutrophils of patients with bronchiectasis are infected in vitro with Influenza A, there is a reduction in lysozyme release and bactericidal activity. This effect may contribute to increased bacterial load and to acute exacerbations [151]. Microbial clearance, it would follow, may be crucial in therapeutic trials involving antibiotics for acute conditions.

1.7.3 Chronic bacterial colonisation of the lower respiratory tract in bronchiectasis

Microbial clearance may be achieved easily for acute infections. However airway colonisation and biofilms growth in a susceptible airway environment remains a problem and eradication is not always possible.

Identifying the colonising PPM is equally difficult. There has been no standard definition for chronic colonisation. It is unclear if frequency of isolation or duration of isolation should guide this definition. The time duration may vary from 3months to 2 years. Patients are considered colonised if a bacterium is cultured for more than 2 years [34] , or have more than three positive cultures of the same organism at intervals of ≥6 weeks [61] or have more than three isolates of *a PPM* from separate samples over a period of 3 months [145].

We have adhered to a previously used definition of more than three positive cultures within the previous year [3].

Evidence in Cystic fibrosis is more extensive. The first definition for chronic *Pseudomonas aeruginosa* infection was introduced in 1974 and was based on monthly microbiological examination of sputum. Chronic infection was defined as a continuous presence of *Pseudomonas aeruginosa* in the sputum for 6 months [152]. The European consensus definition for chronic *Pseudomonas aeruginosa* infection that is commonly used is at least three positive cultures over >6months with at least 1-month interval between samples [153]. The Leeds criteria in Cystic fibrosis defines chronic infection as >50% of sputum positive cultures with *Pseudomonas aeruginosa* over a 12 month period. While the study suggests that a sample be taken every 3 months, a minimum sample number is not specified [154]. This has been further validated along with anti-*Pseudomonas antibody levels* in a combined paediatric and adult cystic fibrosis cohort. [155]. In cystic fibrosis, it is thought that chronic infection with *Pseudomonas aeruginosa* is preceded by intermittent infection and early detection and intensive

treatment can delay chronic infection [155-157]. Extrapolating data to the non–cystic fibrosis population would have to be done with caution.

In bronchiectasis, once colonised, fifty per cent of patients will retain the same organism at 5 year follow up review. And this group of subjects will have a higher number of exacerbations [17].

1.7.4 Markers of infection in sputa.

Quantitative bacterial load has been previously used as marker of therapeutic efficacy. In a study to assess efficacy of inhaled versus intravenous gentamycin, a 6-10 fold reduction in colony counts has been reported [158]. Longitudinal quantitative microbial data is absent in bronchiectasis. It is unknown if quantitative data correlates to other proven parameters such as HR-QoL markers and spirometric indices. It is time consuming and remains a research tool at the current time.

More useful are markers that involve patients' cooperation and can be measured in the home environment. Sputum volume and appearance are such tools. Sputum production, although common, remains variable in volume. While some patients are daily producers of copious amounts others less so and some only during an infection. It would be an easy tool for bedside testing. Doctor patient correlation has already been established [159].

1.7.5 Non Tuberculous mycobacterial analysis in bronchiectasis

Non-tuberculous mycobacterial (NTM) are ubiquitous environmental organisms that exist in pre-existing lung damage. NTM are inhaled as aerosol droplets and a single positive isolate could indicate recent exposure rather than active infection. However these patients warrant regular follow up. The age group of patients with multiple isolates is 7 years [mean age 62.2 years v 55.1 years] more than those with single isolates [160]. It is possible that the time to active disease from first exposure is a few years.

The prevalence of NTM is estimated to be about 2% in bronchiectasis [17]. A female predominance is noted. Single isolates are frequently *Mycobacterium avium-intracellulare* (MAC). If patients have multiple isolates they are more likely to be smear positive on first sample. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are frequently co-cultured [160].

Patients with bronchiectasis and non-tuberculous mycobacterial disease have a higher prevalence of coexisting *Aspergillus*-realed lung disease than patients with bronchiectasis and without non-tuberculous mycobacteria. Recurrent antibiotic use prior to diagnosis may drive culture findings to fungal isolates, or a common host defence abnormality may be implicated. It may be that lung disease with NTM is destructive and this provides a favourable environment for *Aspergillus fumigatus* [161].

Distinguishing between lung colonisation and disease can be difficult [160]. Pulmonary mycobacterium avium (MAC) complex is recognised as presenting in two distinct ways. It can present as a fibro-cavitatory infection complicating pre-existing lung disease or as an infection in apparently immune-competent individuals with no pre-existing lung disease that then causes nodular bronchiectasis [4]. There is a debate as to whether MAC infection is a cause or simply a complication of bronchiectasis. Patients with bronchiectasis and NTM infection require long term follow up and regular assessment of sputum cultures in order to determine the optimum time to intervene as long term disease progression is likely [4].

It is important to screen all patients with bronchiectasis at referral for NTM infection and thereafter if there is an unexplained deterioration unresponsive to usual therapy [4].

1.7.6 Anti-pseudomonal antibodies in bronchiectasis

Specific serum antibodies could be helpful in defining the status of bacterial infection as well as the response to early treatment in patients. It may also confirm protection following vaccination. However antibodies induced by disease do not seem to offer protection in Cystic fibrosis [12]. The antibody response against *Pseudomonas aeruginosa* in CF is a marker of chronicity of infection and of inflammation and tissue damage. Some patients with non-cystic fibrosis bronchiectasis when infected with mucoid strains of *Pseudomonas aeruginosa* have high levels of anti-pseudomonal antibodies. It is not known if information from CF can be extrapolated to non-CF bronchiectasis [162].

Immunoglobulin G (IgG) antibodies to *Pseudomonas aeruginosa* surface antigens in serum can be estimated by enzyme-linked immunosorbent assay (ELISA). Antibodies to *Pseudomonas aeruginosa* can be directed against alkaline protease (AP), elastase (ELA), exotoxin A (ExoA) or whole cell [153, 163] .The secretory IgA is the initial humoral response to infection in the lungs. While antibodies to IgG are commonly measured, it is possible that at the onset of infection an increase in specific serum IgA antibodies may occur before an increase in serum IgG antibodies [164]. High titres of serum IgG antibodies are associated with a poor clinical state, while low titres are associated with a better clinical state in both chronic and intermittently infected patients with CF [165]. Information on antipseudomonal antibody levels in patients with non-CF bronchiectasis remains limited.

1.8 Cytokines and the lung in bronchiectasis

1.8.1 Cytokines in bronchiectasis

Three distinct pathogenic elements, namely infection, inflammation and enzymatic actions, interact with each other and have been implicated in the pathophysiology of bronchiectasis [166].

When exposed to bacterial endotoxin, bronchial epithelial cells release inflammatory mediators [166]. Some of these mediators are pro-inflammatory and others anti-inflammatory. Among the pro-inflammatory mediators involved, IL-8, IL-1β, and

TNF-α play a role favouring the trafficking of activated neutrophils through the bronchial wall into the bronchial lumen. The anti-inflammatory mediators IL-6 and IL-10 act as a counterpart of pro-inflammatory mediators by promoting the synthesis of natural antagonists IL-1β and TNF-α [127]. Intense neutrophil infiltration into the tracheo-bronchial tree occurs as a result, which further aggravates the release of inflammatory mediators [166].

Evidence now suggests that in bronchiectasis, airway inflammatory response triggered by bacterial stimulation is excessive in relation to the bacterial burden indicating a deregulated cytokine network. It continues to reverberate even after the infection is controlled. The altered homeostasis of airway inflammatory response to bacterial infection in the dynamic process of host–pathogen interaction dictates the clinical manifestations of the lung disease [166, 167].

Finally neutrophil toxic products impair the structure and functioning of the airway mucosa by digesting airway elastin, basement membrane collagen and proteoglycan, contributing in this way to the progression of the disease.

1.8.2 Lower airway inflammation and bronchiectasis

Sputum colour when graded visually relates to the activity of the underlying markers of bronchial inflammation confirming that increased purulence is a result of increased inflammation. Visual measurements of sputum colour strongly correlate with myeloperoxidase, interleukin 8, leukocyte elastase (both activity and total quantity),

and sputum volume [168]. In steady state bronchiectasis, sputum neutrophil elastase levels correlate with the per centage of neutrophils, pro-inflammatory cytokines (IL-8 and TNF-α) and 24-h sputum volume that is a marker of disease activity [166, 169]. The sputum elastase level correlates with sputum production, lung function, and airway cytokine expression in bronchiectasis [170].

There are several chemotactic factors present in secretions and the chemotactic activity of purulent secretions is higher than that of mucoid ones. This is largely related to an increase in IL-8 levels, whereas the contribution of LTB4 remains relatively stable. This observation has important implications in deciding future therapeutic strategies since continued neutrophil recruitment is thought to be important to the pathogenesis and progression of chronic bronchial disease. Removal or reduction of the chemotactic drive may present an attractive therapeutic strategy. However, neutrophil chemotaxis is clearly required during episodes of acute infective exacerbations of bronchial disease. Prevention of this normal response may therefore prove harmful. For this reason measures aimed at removing the IL-8 drive may prove counterproductive, whereas an approach in abrogating the LTB4 drive may be more successful [171].

Neutrophilic infiltration into bronchiectatic airways is also mediated by mediators such as host complement factor 5a (C5a), leukotriene B_4 (LTB_4), IL-8 and IL1, tumour necrosis factor –α and leukotriene (LT) B_4 [6, 172]. These cause release of toxic products such as neutrophil elastase (NE), metalloproteases and reactive oxygen species. Because of the large number of neutrophils present, lung defences are overwhelmed [2, 172, 173]. Patients with more advanced disease have more inflammation within the airway. Levels of TNF-α , IL-1B and IL-10 are higher in

broncho-alveolar lavage fluid of these patients [127]. Activated neutrophils do not differentiate between bacteria and bystander lung tissue. This inflammatory shift is persistent post treatment. Modulation with therapy will need formal study [71].

While individual cytokines have different actions, there may well be an overlap in function. LTB4 promotes neutrophil migration and degranulation. IL-1β mediates airway inflammation and fibrosis; TNF-α interacts synergistically with IL-1 in prostaglandin induction; and IL-8 is one of the most potent chemo-attractants which also degranulates neutrophils in bronchiectatic airways [71]. TNF-α and IL-1 have been shown to induce the breakdown of tight junctions in the blood/brain barrier in vivo [174]. A combination of anti-TNF and anti-IL-1 antibodies completely neutralized cell separation in the vascular endothelium that is induced by *Streptococcus pneumoniae* [175].

A non-clearing adaptive immune response to chronic infection of the lower respiratory tract in bronchiectasis subjects may contributes to the airway inflammatory process. Subjects with bronchiectasis and recurrent infections with non-typeable *Haemophilus influenzae* present a type 2 T-helper cell (Th2) predominant response with production of IL-4 and IL-10. Conversely, cytokine pattern in control subjects was consistent with a Th1 response. Non-typeable *Haemophilus influenzae* also form adherent biofilms on the surface of airways epithelium. The epithelium in turn responds through increased secretion of several innate and adaptive immune factors that mediate airway inflammation *H. influenzae* stimulates respiratory epithelial production of macrophage inflammatory proteins, IL-8 and TNF-α both *in vitro* and *in vivo* [166, 176].

Airway inflammation not related to colonization may be present in the early stages of the disease. However subjects with pathogenic organisms colonizing the airways have a more intense neutrophilic inflammatory reaction than non-colonized patients. Also markers of inflammation increase progressively with the increase in the bacterial load. Although it is speculated that the *Pseudomonas* spp. may cause a more intense bronchial inflammation, no differences in the inflammatory parameters according to the type of bacteria isolated has been noted [127].

Exacerbations are associated with further elevated inflammation in the form of augmented cytokine expression, cellular infiltrate, adhesion molecule expression, loss of lung function and symptomatic deterioration [177].

1.8.3 Systemic evaluation of airways inflammation

Cytokine concentrations of IL-lα, TNF-α and IL-8 are normally below levels of detection in plasma [178]. The relatively low levels of plasma cytokines compared with broncho-alveolar lavage fluid and the poor correlations between them suggest that the inflammatory process in the airways is mostly compartmentalized. A complex interaction between cytokines and their natural antagonists in the local milieu or the relatively low intensity of the bronchial inflammatory process may explain this local effect. The systemic reflection of local inflammatory response is thought to be closely related to the severity of the disease, being more intense in cases of severe pneumonia and ARDS [127].

In stable state chronic obstructive pulmonary disease (COPD) there is no evidence of correlation of inflammation in the systemic and lower airway compartments suggesting that the two compartments may be modulated separately [179]. However in exacerbations of (COPD), the systemic inflammatory response is proportional to that occurring in the lower airways and greater in the presence of a bacterial pathogen. In particular serum IL-6 is correlated significantly to sputum IL-8 [179].

Independent functioning of the two compartments is not fully explained. Perhaps the local inflammation does not spill over or perhaps counter-regulatory mechanisms are in action and need further study.

1.8.4 Cytokines as a marker of inflammation in therapeutic trials

There is no gold standard for measuring disease activity in bronchiectasis. The efficacy of inhaled corticosteroids has been studied using sputum output of leucocytes, IL-1β, IL-8 and LTB4 [93]. The first and only systematic evaluation of sputum pro-inflammatory mediator profiles after fluticasone therapy showed a dramatic reduction in concentrations of IL-1, IL-8, and LTB4 activities in the bronchiectatic airways [71].

There are only a few longitudinal studies on sputum pro-inflammatory mediator profiles in bronchiectasis. Most studies have evaluated these mediators in stable state disease. Investigating these biomarkers has the potential to yield information about underlying mechanisms of disease and aid development of therapeutic strategies [71, 81].

Chapter 2

The COBEX study

Characterisation of exacerbations in bronchiectasis

2.1 Introduction

Acute exacerbations of bronchiectasis are recognised by a change in symptoms with patients complaining of an increase in cough, sputum volume and sputum colour with an associated increase in malaise. In contrast to exacerbations of COPD, treatment of acute exacerbations of bronchiectasis has not been well studied and hard end points of treatment success have not been established. In particular in contrast to asthma and COPD there is no convincing literature to suggest that change in FEV_1 provides a good marker of treatment success in acute exacerbations of bronchiectasis. It therefore remains unclear as to how we could measure treatment success when planning future interventional studies in acute exacerbations of bronchiectasis. Furthermore microbiological endpoints are not well validated in this disease. As infection is chronic, as in Cystic Fibrosis, then eradication of bacteria is a less likely endpoint and reduction of bacterial numbers is a more likely scenario. However, there are no published studies of sufficient size which address this issue in acute exacerbations of bronchiectasis. Measurement of inflammatory markers provides a useful parameter of resolution of acute inflammation associated with an exacerbation but requires repeated blood sampling. The introduction of analysis of breath condensates provides an alternative approach which could be as efficacious as blood

markers but has yet to be investigated in this context. From a patient perspective a non-invasive marker of the need to start treatment for an exacerbation and time to complete treatment would be welcome. Finally it is recognised that in the absence of change in lung function, antibiotic courses are stopped when the patient feels better. Whilst previous studies of the St George's questionnaire in bronchiectasis have assessed stable disease there is a need to further evaluate quality of life measures in response to treatment of an acute exacerbation.

2.2 Definition

COBEX is an acronym for 'Characterisation of bronchiectatic exacerbations'. We chose this for ease of use in day to day functioning of the study. While it does not do justice to the complete title, the purpose of convenience was served.

2.3 Objectives

We set out to study acute exacerbations in Non-CF Bronchiectasis with a view to defining clear end points towards which future interventions can be directed. To this end we intended to use all currently available clinical, biochemical, spirometric and microbiological parameters. Some parameters are easily available in most clinical settings while others are experimental. We have studied patients in stable state (not having had an infection in the preceding 4 weeks) and followed these patients through an exacerbation.

The patient is at the centre of this study. Patients present with symptoms and measuring this would be paramount to any clinical study. Objectively assessing these symptoms will allow wide use with no economic burden. Any disease ultimately

compromises quality of life. Some symptoms however severe will be tolerated; others may be comparatively minor but less well tolerated as quality of life may be compromised. It follows that a measure of disease must include Health related quality of life (HR-QoL). We have employed two commonly used questionnaires to measure the HR-QoL –The St Georges' respiratory questionnaire and the Euroqol.

Copious and purulent sputum production has remained the primary complaint of this disease. Analysing sputa is thought to hold the secret to the persistent infection. Information sought from sputa is many fold. Microbiological load and quality is widely studied [5, 17, 80, 127]. More recently serum inflammatory markers including cytokines have yielded useful information in other lung diseases such as chronic obstructive pulmonary disease [180]. Correlation between microbiological load and inflammatory marker has been sought to explain the pathological process in detail. We have attempted to measure qualitative and quantitative microbiology. We have also analysed both serum and sputum for inflammatory mediators that are commonly described.

Colonization with *Pseudomonas aeruginosa* is a poor prognostic factor in bronchiectasis [62]. Anti-pseudomonal antibodies are meant to be protective against infection. However a malfunction renders these ineffective and may in part be responsible for the persistence of infection. Raised levels are thought to precede colonisation. We have attempted to measure these levels in our study.

Serum inflammatory markers range from regularly used indices such as ESR and CRP to more specific mediators of inflammation such as the cytokines. These have been reported to be raised in bronchiectasis. We have measured ESR, CRP and seven other inflammatory markers: Interferon γ (IFNγ), Tumour necrosis factor (TNF α),

Interleukin 6 (IL-6), Interleukin 8 (IL-8), Interleukin 1β, Interleukin 10 (IL-10) and Interleukin 17 (IL-17) in this study.

Forced expiratory volume in the first second (FEV_1) remains a standard measure by which all lung disease is judged. It may not always provide useful information but will help assess patient's functional level. Forced manoeuvres are difficult for patients with lung disease. Newer methods such as Impulse Oscillometry (IOS) are less cumbersome and may provide the answer in the future. We have used both spirometry and IOS to identify useful markers in bronchiectasis.

Finally, in an attempt to measure lower airway acidity we have used the exhaled breath condensate to predict the changes of an exacerbation. We have compared exhaled breath pH of healthy volunteers to patients with bronchiectasis to set a bench mark.

2.4 Study Design

The study is designed as a longitudinal prospective cohort study. We have followed patients for a period of two years and prospectively studied all reported exacerbations.

2.5 Ethical approval

The initial Ethical approval for this study was granted by the Cambridge 1 Research Ethics Committee on the 1st of August 2006. (MREC ref 06/Q0104/33). An amendment to the protocol was made on the 6th of February 2008 and ethical approval was sought again. A valid notice of approval of a substantial amendment was thereafter issued. Appendix i & ii.

2.6 Funding

We are grateful to the Evelyn Trust (PO Box 27, Hitchin, Hertfordshire, SG4 7ZQ) for their generosity in funding the first year of this study. The second year was funded by the Department of Research and Development, Papworth Hospital, Cambridge CB23RE.

2.7 Time frame

The total study period of two years commenced on the 1st of September 2006 and ended on the 31st of August 2008. Ethical approval was granted in the same year. The first patient was recruited in February 2007. The last patient was recruited in June 2008. The data was analysed thereafter.

2.8 Subjects

Subjects for this study were recruited from the Lung Defence Clinic at Papworth Hospital, Cambridge. The Papworth Lung Defence Clinic has defined a gold standard for characterisation of patients with Non CF bronchiectasis and already has a well-defined cohort of patients [18]. The clinic currently follows over 500 patients with well characterised bronchiectasis. All patients had a diagnosis of bronchiectasis confirmed by previous HRCT and clinical features. All patients entered into this study are white Caucasian.

Fifty-eight patients were recruited. Twenty-two of these patients suffered an exacerbation and were studied through this period.

2.9 Patient criteria

The criteria set out for patient participation is listed below. This was strictly adhered to

2.9.1 Inclusion criteria

- Patients attending the Lung Defence Clinic with well characterised bronchiectasis [18]
- Age 18 and upwards.
- Patient able to cooperate for a period of 12 months.
- Patients were daily sputum producers.
- Fourteen healthy volunteers were recruited to study exhaled breath condensate.

2.9.2 Exclusion criteria

- Patients with Cystic Fibrosis.
- Patients on immunoglobulin therapy
- Patients on treatment for non-tuberculous mycobacterial lung disease.

2.10 Protocol

All subjects were studied in stable state not having suffered an exacerbation in the preceding 4 weeks (Baseline/visit 0). They were asked to contact the Research clinic in case they suffered an acute exacerbation. They attended the clinic again at the onset of an exacerbation prior to commencement of antibiotic treatment. Follow-up visits were

arranged for Day 7, Day 14 and day 42 thereafter. Antibiotic treatment was prescribed based on previous microbiological status. All patients were treated for a period of 14 days with either oral or a combination of oral, nebulised and intravenous antibiotics.

Clinical, biochemical, microbiological, HR-QoL and lung function parameters was measured in all patients entered into the COBEX study and is listed in Table 2.1. All clinical, biochemical and microbiological parameters were measured at each visit. HR-QoL questionnaires are designed to be measures at an interval of either a minimum of 2 (Euroqol) or 4 weeks apart (SGRQ). Hence HR-QoL was measured at baseline, Day 1 and Day 42 for all patients. In addition patients were asked to complete a Euroqol questionnaire at Day 14.

The Clinical research form used – Appendix iii

Table 2.1: Summary of the visit schedule for COBEX study.
Clinical, biochemical, microbiological, HR-QoL and lung function parameters measured in all patients entered into the COBEX study. All clinical, biochemical and microbiological parameters were measured at each visit. Patients completed the Euroqol at baseline, Day 14 and day 42. In addition patients were asked to complete a Euroqol questionnaire at Day 14.

	VISIT 0 **baseline**	**VISIT 1** **Day 1 of acute exacerbation**	**VISIT 2** **Day 7 of acute exacerbation**	**VISIT 3** **Day 14 after onset of acute exacerbation**	**VISIT 4** **Day 42 after onset of acute exacerbation**
Clinicians assessment	X	X	X	X	X
Euroqol	X	X		X	X
St Georges questionnaire	X	X			X
Sputum 24 hour volume	X	X	X	X	X
Qualitative Microbiology	X	X	X	X	X
Inflammatory Markers: Cytokines	X	X	X	X	X
Exhaled Breath Condensate (pH analysis)	X	X	X	X	X
WBC, CRP, ESR	X	X	X	X	X
Spirometry	X	X	X	X	X
Impulse Oscillometry	X	X	X	X	X

Chapter 3

Results

Characteristics of patients in the COBEX study

3.1 Study Subjects

One hundred and sixty-five patients were approached with a view to recruiting them to the COBEX study. Information on the study was given to all patients. Sixty five patients consented to participating in the study. Six of these patients changed their minds prior to commencement of the study. One patient died during the trial of unrelated causes. Fifty-eight patients entered the study and were screened for the baseline visit.

Twenty-seven patients suffered an exacerbation during the period of the study. Five of these patients were unable to attend for a visit at the start of an exacerbation. Three patients felt their symptoms worsen at the beginning of a weekend and commenced their antibiotics as advised. Two patients were unable to attend for other reasons.

Twenty-two patients were screened during an exacerbation of bronchiectasis. Twenty patients finished all visits. Two patients deteriorated before the final visit. Data from these patients is included in all initial visits.

A total of 144 patient visits were recorded between February of 2007 and August of 2008. Only three patients needed to be admitted to hospital for inpatient care. While

two patients had prolonged hospital visits, one patient was admitted for 14 days and attended for the last visit as an out-patient.

The mean age (SD) of our cohort was 64years (11.4). Most patients were female (34/58, 58%).

The use of antibiotics was variable among the patients studied. The mean number of courses of antibiotics used was 3 (range 0 – 12). Intravenous antibiotics were less commonly used as an out-patient and were needed by only one patient.

Eighteen patients (31%) were been admitted to hospital in the preceding one year for treatment of bronchiectasis. Three patients had been admitted more than once. Only one patient needed care on the Intensive therapy unit.

3.2 BMI

The mean (SD) Body Mass Index (BMI) for this group of patients was 25(4). The mean (SD) weight for the men was 80(11) kilograms and for the women 68(22) kilograms.

3.3 Smoking History

Thirty patients had never previously smoked tobacco. Twenty-six patients had previously smoked. Only 2 patients were current smokers. These patients were not studied during an exacerbation as they met the exclusion criteria for exhaled breath condensate collection. The mean use of tobacco for all patients was 21 pack years (Range 5-180). Subject characteristics are summarised in Table 3.1

Table 3.1: Characteristics of patients and healthy volunteers entered into the COBEX study.

	Full cohort n=58	Exacerbators n=22	Healthy volunteers n=14
Mean age (+/-SD)	64years (11.4)	63years (8.5)	65years (5.9)
Sex (M: F)	24:34	5:17	7:7
Smoking history			
Ex-smokers	26	11	9
Non-smokers	31	11	4
Current smoker	1		1
Mean FEV_1 (±SD)	1.9L (0.71)	1.9L (0.61)	2.3L (0.82)
Mean FEV_1 % predicted (±SD)	75 %(25)	81 %(28.2)	100 %(27.1)

3.4 Past medical history

In addition to bronchiectasis, twenty four patients (41%) had asthma, 23(40%) suffered with Gastro-oesophageal reflux disease, 6(10%) had Chronic obstructive pulmonary disease and 8(14%) had Ischaemic heart disease.

3.5 Diagnosis of bronchiectasis

The median age at diagnosis in our cohort was 42 years (range 1-79 years n=55, no data on 3 patients). The duration of disease as identified by patients was (median, IQR) 39 (12.5 - 59) years. The time of potential primary insult was before 35 years of age in 40 % of patients.

3.6 Aetiology of bronchiectasis

This cohort of patients has well defined bronchiectasis as previously described [18]. Aetiology for the bronchiectasis was known in over 70% of patients (41/58). Bronchiectasis as a consequence of childhood infection was the commonest in the group of patients studied. All known causes are listed in Table 3.2.

Aetiology of bronchiectasis in the cohort that was followed through an exacerbation of bronchiectasis is listed below Table 3.3.

Table 3.2: Aetiology of bronchiectasis in all subjects recruited to the COBEX study.

Aetiology	**n=58**
Post pneumonic	3 (5.2%)
Aspiration	2(3.4%)
Ciliary dysfunction	1(1.7%)
Rheumatoid arthritis	3(5.2%)
ABPA	2(3.4%)
Antibody deficiency with normal immunoglobulins	7(12%)
Childhood Infections	23(39.6%)
Idiopathic	22(37.4%)

Table 3.3: Aetiology of bronchiectasis in the cohort that was followed through an acute exacerbation.

Aetiology	**n=22**
Post pneumonic	2(9%)
Aspiration	1(4.5%)
Rheumatoid arthritis	1(4.5%)
Antibody deficiency with normal immunoglobulins	4(18%)
Childhood Infections	7(31%)
Idiopathic	7(31%)

3.7 Antibiotic therapy in bronchiectasis

3.7.1 Prophylactic oral antibiotics

The Lung Defence clinic at Papworth practises a policy of prescribing oral antibiotics as prophylaxis to patients who have previously required frequent courses of treatment. This is thought to reduce the requirement of antibiotic use annually and also reduce the bacterial load. Most patients do so with little or no side effects and are compliant.

Thirty seven (64%) of the patients recruited to the COBEX study were taking oral antibiotics regularly in the absence of an exacerbation. Six (10%) Amoxicillin; 11(19%) Doxycycline; 14(24%) Azithromycin; 1(2%) Erythromycin; 5 (8%) Trimethoprim.

In the group of patients followed through an exacerbation, 2(9%) were taking Amoxicillin, 4(18%) Doxycycline; 6(27%) Azithromycin and 2 (9%) were on Trimethoprim prophylactically.

3.7.2 Prophylactic nebulised antibiotics

Fourteen patients were using nebulised antibiotics prophylactically. Nine (15%) Colomycin; 3(5%) Gentamycin; 1(2%) Tobramycin; and 1(2%) Meropenem.

In the group of patients followed through an exacerbation, 3(5%) patients were Colomycin nebuliser and one patient (2%) on Gentamycin nebuliser prophylactically.

Eight patients were on more than one antibiotic prophylactically. One patient who had end stage bronchiectasis was on four antibiotics at the same time.

3.8 Domiciliary oxygen

Only two patients had domiciliary oxygen. One was on Long term oxygen therapy and another patient was on short burst oxygen therapy.

No patient in this cohort was on domiciliary ventilatory support (Non-invasive ventilation).

3.9 Mucolytic agents

Eleven patients (19%) were prescribed a mucolytic agent.

3.10 Vaccination history

This patient group was well immunized. Fifty four (93%) patients had been immunised with the annual Influenza vaccine and 46(79%) had been immunised with the Pneumococcal vaccination.

3.11 Chest Physiotherapy

Patients in this cohort were well educated in the importance of regular chest physiotherapy techniques. Forty four (76%) followed some form of physiotherapy on a regular basis, 42 (72%) patients did so several times a month. Nineteen patients (33%) used more than one form of physiotherapy. The methods of physiotherapy used are listed in Table 3.4. Frequency of physiotherapy employed by our patients is listed in Table 3.5.

There was no significant difference in the number of exacerbation suffered between the group that did physiotherapy several times a month and those that did so less frequently (p=0.55).Mechanical aids were not frequently used in our cohort of patients.

Table 3.4: Various methods of physiotherapy employed by subjects within the COBEX study.

Method of Physiotherapy used	Number of patients
Active cycle of Breathing Technique (ACBT)	30
Postural Drainage	26
Flutter Valve	3
Acapella	2
Autogenic Drainage	0
General Exercise	2
Percussion & Vibration	5

Table 3.5: Frequency of various techniques in physiotherapy employed by subjects within the COBEX study.

Frequency of use of physiotherapy	Number of patients
Never	14
Once every other month	1
Once a month	1
Several times a month	2
Once a week	8
Several times a week	5
Daily	27

3.12 Use of Inhaled corticosteroid therapy

Forty eight (83%) patients were prescribed and were using inhaled corticosteroids at the time of the study. The average dose of inhaled corticosteroid was the equivalent of 800mcgs beclamethasone. This was not correlated to the number of exacerbations ($r = -0.10$) or the 24hour sputum volume ($r = 0.02$).

3.13 Discussion

Our cohort of patients is comparable to other series in age and sex match. Mean age in other series has been reported as 57.2 [5], 63 [61], 57 [127] and 56 years [109]. Younger patients are rare. In one series only 6% of patients were younger than 30 years [5]. Although some studies have reported a wider age range [19-92 years] [109].

Female predominance is reported in most studies. Our group is therefore representative. Female population within other studies have been reported as 64% [109], or up to 68% [5, 61] .

The Mean (SD) Body Mass Index (BMI) for this group of patients was 25 kg/m^2, indicating a healthy cohort. This also points to a bias in the study as patients who were able to attend the out patients when unwell were more likely to consent to the study. However this is comparable to other groups [21, 22]. Underweight subjects (lower BMI) have a higher mortality rate. Mortality is thought to be higher when the BMI is less than 20 kg/m^2 [21]. Women are also known to have a higher BMI [21].

A formal diagnosis of bronchiectasis can often be delayed. However patients often recall the duration of disease based on symptoms such as chronic production of sputum. Most patients are able to identify the time of onset of symptoms. Patients also identify a definite time at which potential pulmonary insult or injury has occurred. This

varies between 14 years to 20 years of age [5, 127]. There is sometimes a lag between insult and the onset of symptoms and has been reported as 13.6 years in one series [5]. The mean duration of suffering with the disease was 37 years (range 4-85 years). This is well comparable to other reports.

A majority of patients with bronchiectasis are non-smokers. Even those who have previously smoked tend to discontinue due to chest discomfort. In a Texan series 55% of patients were lifetime non-smokers [5], whilst in an Australian cohort 82% of patients were non-smokers [17]. Mean pack years has been reported to be between 8.9-29.8 [5, 21]. Our patients are comparable to other published series with 53% being non-smokers.

Aetiologically, between 30-80% of patients are reported to have idiopathic bronchiectasis [5, 71]. The proportion of patients with idiopathic bronchiectasis is much smaller in our cohort compared to other groups. Our cohort of patients is well characterised and have been studied in some detail [18]. Proportion of patients with other causative factors of bronchiectasis is comparable to our group. In a large series from Texas, 35% had post pneumonic, 20% post childhood infections, 10% post granulomatous disease, 4% genetic disease causing bronchiectasis (α1-antitrypsin disease, immotile cilia syndrome and cystic fibrosis) [5].

The average number of exacerbations in a year seems to vary. While some suggest that patients may suffer an exacerbation only every eight months[27], others think it more frequent, between 2-4 times a year [71, 181]. Our group identifies with the latter two studies and our patients suffered a mean of three exacerbations the preceding year.

Patients with antibiotic resistance seem to suffer significantly more number of exacerbations in a year than those with sensitive organisms [17]. Use of antibiotic

prophylaxis may alter the number of exacerbations that patients suffer in a year. In one study the average number of exacerbation per year significantly reduced from 10 to 5 per year when these patients were treated with long term Azithromycin (mean duration of 9.1 months) [61]. Admissions to Hospital are rare, as most patients are treated with oral antibiotics. Patients needing intravenous antibiotics may be admitted to hospital at some centres. At the Lung Defence Clinic at Papworth, patients are trained to self-administer intravenous antibiotics. This reduces the rate of hospital admissions. Two of our patients were admitted to hospital. Both had other comorbidities such as chronic obstructive airways disease and needed advanced nursing and medical care. Rates of hospitalisation have been variously reported as 0.6-1.6 per year [181].

Inhaled corticosteroids have been shown to have a clear benefit in asthma. However in bronchiectasis evidence for use remains limited. When fluticasone is administered at a dose of 500mcgs twice daily, 24 hour sputum volume is improved but it has no influence on the number of exacerbations. This may suggest that exacerbations are infective while improvement from ICS is inflammatory [71]. We were unable to correlate the dose of inhaled corticosteroids in our study with either the frequency of exacerbations or the 24 hour sputum volume.

The benefit of mucolytic therapy in bronchiectasis is doubtful [8]. Recombinant deoxyribonuclease I administered in conjunction with standard therapies is effective in treatment of patients with cystic fibrosis. However this effect is not observed in non CF bronchiectasis [27]. A 15 day trial of erdosteine (a mucoactive thiol derivative) along with chest physiotherapy has been shown to improve the FEV1 by 200mls in a

randomised controlled trial. However this was in a small group of 30 patients [182]. Inhaled mannitol increases mucus clearance in patients with bronchiectasis by an unclear mechanism. The effect of mannitol on lung function, health status and sputum properties was investigated. Mannitol significantly improved the health status over 12 days and this improvement was maintained for 6-10 days after cessation of treatment. In addition, mannitol reduced the tenacity, increased the hydration of mucus acutely and improved cough clearability in patients with bronchiectasis [183]. A trial of mucolytic is often offered to our patients to see if it helps ameliorate symptoms. Nebulised hypertonic saline can be used safely and effectively as an adjunct to physiotherapy in selected patients. A long-term prospective trial is now indicated to determine its effectiveness on long-term infection rate, quality of life and lung function [184].

We think there may be a bias in recruitment of subjects to the study. Patients who lived within a convenient travel distance to the Hospital tended to consent to the study. Patients living further away were less keen and admitted to being unable to travel all the way when ill. The use of prophylactic antibiotics compared to other cohorts may also introduce a bias within this study. Otherwise our cohort is fairy representative and comparable to groups in other reports in terms of a demographic match.

Chapter 4

Analysis of clinical features in bronchiectasis

Symptoms and signs of disease in stable state and in acute exacerbations from the COBEX study

4.1 Introduction

The Clinical features of bronchiectasis have been described in Chapter 1. It was our aim to establish using the COBEX study cohort whether we could track symptom scores and show differences at an exacerbation with improvement on treatment without the use of diary cards. We employed Visual Analogue scores and the Modified Borg's breathlessness score. We also wished to characterise changes in clinical signs i.e. body temperature, respiratory rate, oxygen saturations and chest auscultatory findings during the course of an exacerbation.

4.1.1 Visual analogue Score

Pain, as a symptom, is often scored on a scale of 1 to 10. Medication is often ordered and administered on the basis of the patient's subjective pain response. A similar self-rating scale could be used for patients with varying levels of symptoms in patients with bronchiectasis.

A Visual Analogue Scale is a measurement instrument that tries to define a characteristic or attitude that is believed to range across a continuum of values that cannot easily be directly measured [142]. From the patient's perspective this spectrum

appears continuous and their symptoms do not take discrete jumps, as a categorization of none, mild, moderate and severe would suggest. The VAS captures this idea of an underlying continuum. Operationally a VAS is usually a horizontal line, 100 mm in length, anchored by word descriptors at each end, as illustrated in Appendix iii. The patient marks on the line the point that they feel represents their perception of their current state. The VAS score is determined by measuring in millimetres/centimetres from the left hand end of the line to the point that the patient marks.

As such an assessment is highly subjective, these scales are of most value when looking at change within individuals, and are of less value for comparing across a group of individuals at one time point. Therefore the VAS is useful in trying to produce interval data out of subjective values that are at best ordinal [185].

Visual analogue scores have been used to assess dyspnoea in bronchiectasis. In a study to looking at the beneficial effects of acupressure in bronchiectasis, the authors used a modified VAS score to measure improvement in dyspnoea [186]. A change in modified VAS has also been used to measure change in "chest unpleasantness due to secretions" after airway clearance techniques [187].

4.1.2 Borg's Breathlessness score

The sensation of breathlessness is a sensory experience that is perceived, interpreted, and rated by the individual [188]. An objective assessment of breathlessness includes clinical examination: evidence of cyanosis, respiratory rate, ability to speak in full sentences. Simple bedside tests used in respiratory diseases include measuring a peak flow, trans-cutaneous oxygen saturation measurement (SaO_2) and blood gas analysis.

The Modified Borg breathlessness (MBS) score is a simple questionnaire that is a valid and reliable assessment tool. Patients were asked to rate their breathlessness by indicating a score between 0 (no breathlessness at all) and 10 (maximal breathlessness). Patients with a Borg score of zero were considered to be free of breathlessness. Subjects with any other Borg score were taken to have breathlessness [189, 190]. Table 4.1 is the modified Borg's breathlessness score used in the COBEX study.

Objective measurement of dyspnoea using the MBS has been described in other respiratory conditions. In a group of patients with asthma the MBS was related to changes in airflow obstruction [191]. In COPD, a strong relationship between MBS and respiratory effort with exercise has been described [192] and an association with a 6 minute treadmill walk test has been reported [193]. In a study using normal volunteers with induced dyspnoea, it was found that subjects could distinguish between different sensations of breathlessness and that the term breathlessness encompassed multiple sensations. It was found that subjects could distinguish between levels of breathlessness using the MBS [52].

Table 4.1: The modified Borg's breathlessness Score (MBS).Using the following score of 0-10, breathlessness is assessed. Zero is nothing at all and 10 is maximal breathlessness.

SCALE	SEVERITY
0	No breathlessness at all
0.5	Very very slight (Just Noticeable)
1	Very slight
2	Slight breathlessness
3	Moderate
4	Somewhat severe
5	Severe breathlessness
6	
7	Very severe breathlessness
8	
9	Very very severe (almost maximum)
10	Maximum

Note: The word "breathlessness" was added in our version of the scale for clarification

4.2 Aims

Symptoms in bronchiectasis are known to deteriorate during an exacerbation. This has not been quantified previously. We have attempted to quantify symptoms at baseline (in stable state) and during an exacerbation. We wished to measure change in symptoms with a view to defining clear end points towards which future interventions can be directed.

A clinician's assessment was also carried out. The aim was to measure clinical parameters during the course of an exacerbation of bronchiectasis.

The clinician's assessment included out-patient objective measurements of

- Blood pressure – Systolic and diastolic
- Respiratory rate
- Oxygen saturations as measured by standard digital pulse oximetry
- Body temperature measured in degrees centigrade
- Examination of the hand for finger clubbing
- Auscultation of the chest

4.3 Methods

As described in Chapter 2, section 2.11 patients were recruited from the Lung Defence Clinic at Papworth Hospital, Cambridge. All patients had well characterised Non CF Bronchiectasis[1]. All patients were screened at Baseline (No exacerbation in the preceding 4 weeks), Day 1 of an exacerbation (not having commenced antibiotics), Day 7 during treatment with antibiotics, Day 14 completion of treatment with antibiotics and Day 42 on recovery from exacerbation.

4.3.1 Study Protocol

4.3.1.1 Visual analogue scale

Patients attended the Research Clinic and a ten point visual analogue scale was completed at each visit by all subjects. The following symptoms were assessed using a ten point visual analogue scale:

- Cough
- Breathlessness,
- Chest pain
- Chest discomfort
- Volume of sputum
- Colour of sputum
- Fatigue

The VAS is included in the Clinical Record form in Appendix iii.

4.3.1.2 Modified Borg's breathlessness score.

All patients completed a Modified Borg's breathlessness score (MBS) at each visit.

The MBS is included in the Clinical record form in Appendix iii.

4.3.1.3 Clinical examination

Blood pressure was measured using an electronic device. Oxygen saturations were recorded using digital pulse-oximetry. Body temperature was measured using a sublingual probe. The clinician then conducted a full respiratory physical examination.

4.4 Statistics

VAS scores are described in absolute values with inter quartile ranges.

MBS scores are described as absolute values with standard deviation.

All correlations are made with linear regression using the Pearson correlation coefficient (r). A global test for repeated measures (ANNOVA) was used to measure change over time. Change was considered significant if $p \leq 0.05$

4.5 Results

4.5.1 Visual analogue scale

4.5.1.1 Stable state bronchiectasis

Fifty-five patients completed the Visual analogue scale questionnaires at baseline. Patients scored fatigue maximally over cough and sputum production. Values from the VAS questionnaires completed in stable state are listed in Table 4.2.

4.5.1.2 Acute exacerbation of bronchiectasis.

Twenty two patients were followed through an exacerbation having been studied at baseline. Nineteen of these patients completed all four visits. There was an increase in the perception of all measured symptoms at Day 1 of exacerbation. This trend was reversed by Day 14 and there was no difference between Baseline and Day 42 recovery from exacerbation. Values from VAS questionnaires completed during visits made at the time of an exacerbation are listed in Table 4.3. Graphical representation of each of the symptoms recorded on the visual analogue score at different times during the exacerbation are shown in figures 4.2 – 4.8

Table 4.2: Median and inter quartile range visual analogue scale scores in centimetres for patients in steady state bronchiectasis (not having had an exacerbation in the preceding four weeks).

	Baseline	Inter quartile range
Breathlessness	2.70	1.2-4.1
Chest pain	0.97	0.15-0.95
Chest tightness	1.66	0.4-2.4
Colour of sputum	2.43	1-3.4
Volume of sputum	2.67	0.95-4.65
Cough	2.95	1.1-4.75
Fatigue	3.86	1.45-5.4

Table 4.3: Median and inter quartile range visual analogue scale scores in centimetres for patients during an acute exacerbation of bronchiectasis.

	Baseline	Day 1	Day 14	Day 42	p value
Breathlessness	2.20 (1.2-3.1)	5.55 (3.5-7.4)	1.95 (1.5-4.65)	1.6 (0.45-3.2)	p<0.001
Chest pain	0.25 (0.1-0.8)	1.75 (0.2-2.9)	0.9 (0.2-1.4)	0.6 (0.2-1.2)	p=0.012
Chest tightness	2.1 (1.3-4.7)	4.8 (2.2-6.9)	1.35 (0.9-2.95)	0.9 (0.2-2.55)	p<0.001

Colour of sputum	1.9 (0.7-2.5)	5.9 (3.1-7.8)	2.0 (0.7-3.4)	1.7 (0.3-4.3)	p<0.001
Volume of sputum	1.5 (0.3-2.9)	4.0 (2.4-6.6)	1.8 (0.8-4.8)	1.1 (0.5-2.8)	p<0.001
Cough	2.1 (1.3-4.7)	6.3 (5.0-7.8)	4.2 (2.0-5.2)	1.4 (0.8-4.4)	p<0.001
Fatigue	3.5 (1.2-5.9)	7.1 (5.7-8.2)	3.1 (1.3-5.7)	1.7 (0.6-3.0)	p<0.001

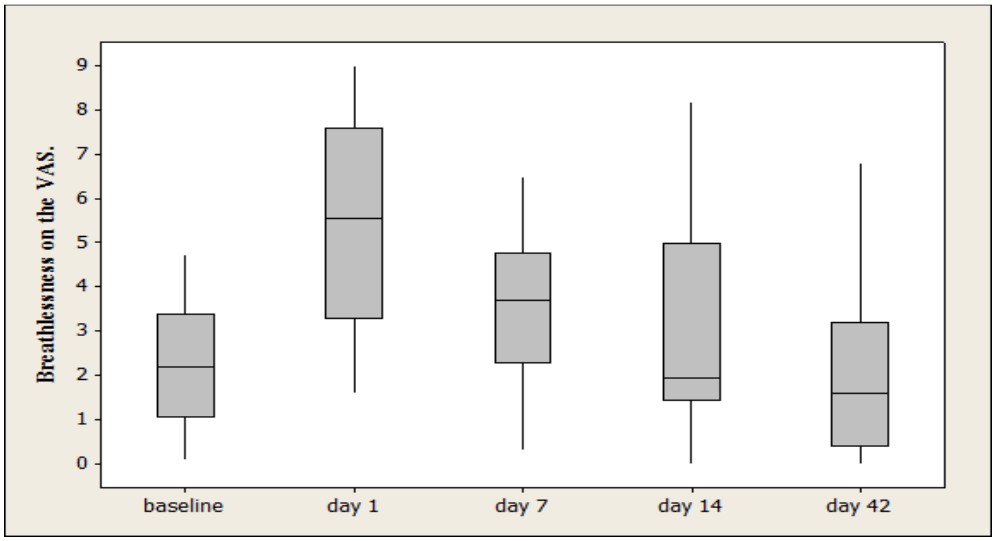

Figure 4.1: Box plot showing the median and inter quartile range in centimetres for breathlessness as scored on the visual analogue score. The x-axis depicts the time of visit - baseline (steady state with no exacerbation in the preceding 4 weeks), Day 1 onset of exacerbation, Day 7 on treatment for exacerbation, day 14 completion of antibiotic treatment and Day 42 recovery from exacerbation. The y-axis depicts visual

analogue score in centimetres. All extreme observations are marked with an *. The change was significant over the course of the exacerbation. p<0.001

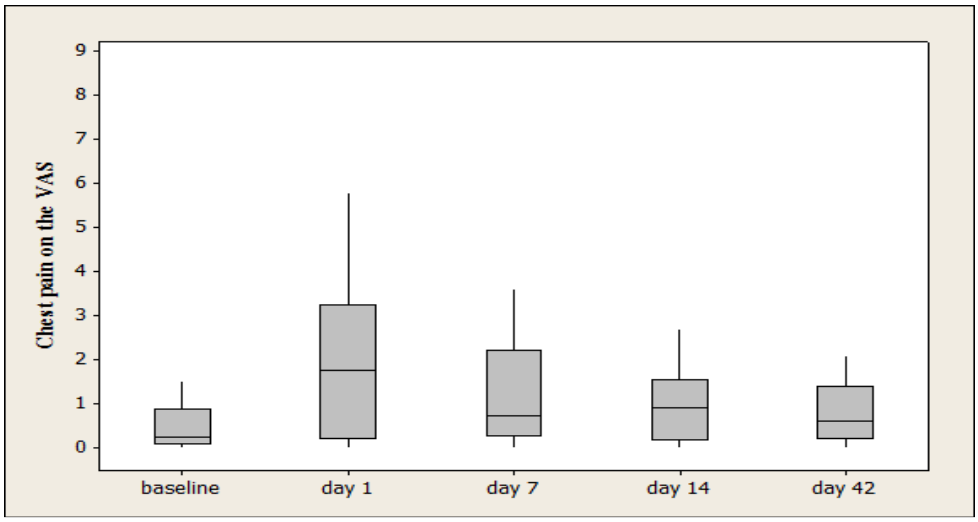

Figure 4.2: Box plot showing the median and inter quartile range in centimetres for chest pain as scored on the visual analogue score. The x-axis depicts the time of visit - baseline (steady state with no exacerbation in the preceding 4 weeks), Day 1 onset of exacerbation, Day 7 on treatment for exacerbation, day 14 completion of antibiotic treatment and Day 42 recovery from exacerbation. The y-axis depicts visual analogue score in centimetres. All extreme observations are marked with an *. The change was significant over the course of the exacerbation. p=0.012

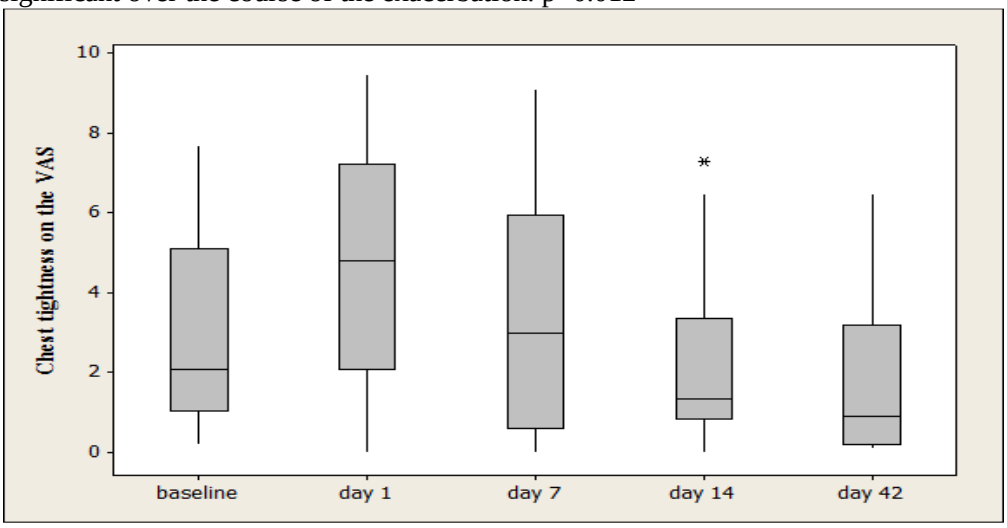

Figure 4.3: Box plot showing the median and inter quartile range in centimetres for chest tightness as scored on the visual analogue score. The x-axis depicts the time of visit - baseline (steady state with no exacerbation in the preceding 4 weeks), Day 1 onset of exacerbation, Day 7 on treatment for exacerbation, day 14 completion of antibiotic treatment and Day 42 recovery from exacerbation. The y-axis depicts visual analogue score in centimetres. All extreme observations are marked with an *. The change was significant over the course of the exacerbation. p<0.001

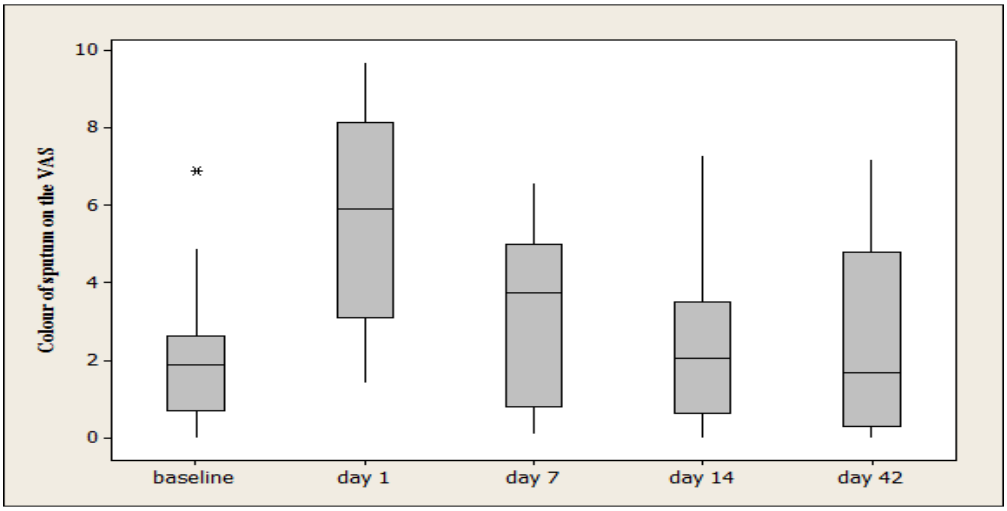

Figure 4.4: Box plot showing the median and inter quartile range in centimetres for colour of sputum as scored on the visual analogue score. The x-axis depicts the time of visit - baseline (steady state with no exacerbation in the preceding 4 weeks), Day 1 onset of exacerbation, Day 7 on treatment for exacerbation, day 14 completion of antibiotic treatment and Day 42 recovery from exacerbation. The y-axis depicts visual analogue score in centimetres. All extreme observations are marked with an *. The change was significant over the course of the exacerbation. $p<0.001$

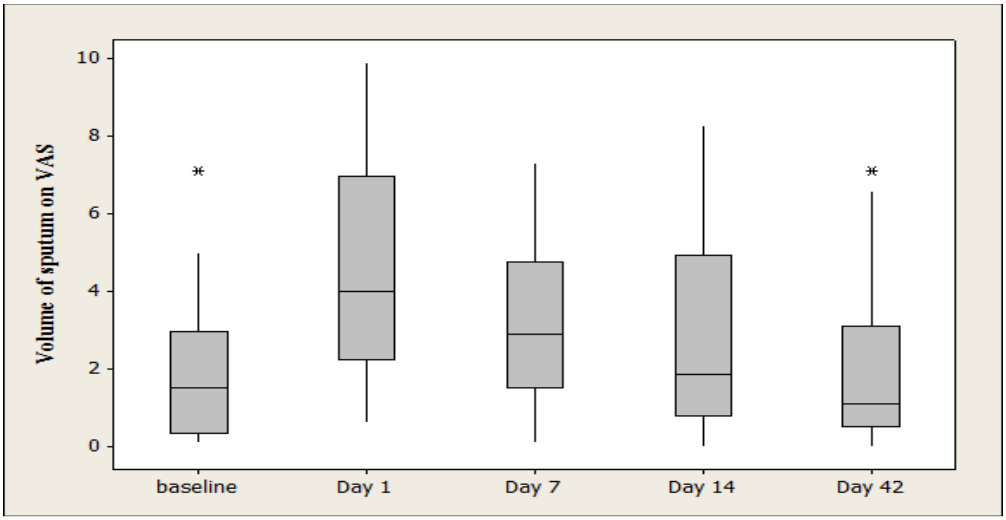

Figure 4.5: Box plot showing the median and inter quartile range in centimetres for volume of sputum as scored on the visual analogue score. The x-axis depicts the time of visit - baseline (steady state with no exacerbation in the preceding 4 weeks), Day 1 onset of exacerbation, Day 7 on treatment for exacerbation, day 14 completion of antibiotic treatment and Day 42 recovery from exacerbation. The y-axis depicts visual

analogue score in centimetres. All extreme observations are marked with an *. The change was significant over the course of the exacerbation. p<0.001

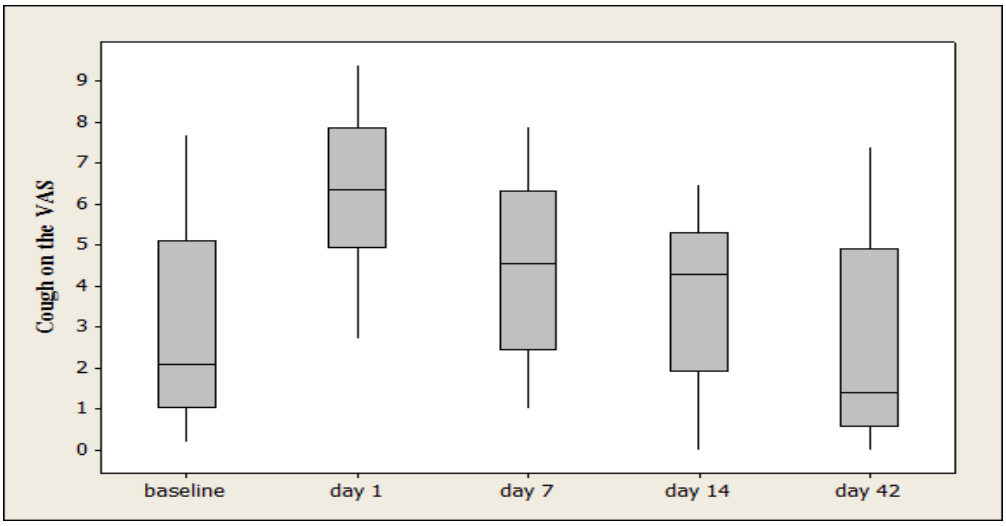

Figure 4.6: Box plot showing the median and inter quartile range in centimetres for cough as scored on the visual analogue score. The x-axis depicts the time of visit - baseline (steady state with no exacerbation in the preceding 4 weeks), Day 1 onset of exacerbation, Day 7 on treatment for exacerbation, day 14 completion of antibiotic treatment and Day 42 recovery from exacerbation. The y-axis depicts visual analogue score in centimetres. All extreme observations are marked with an *. The change was significant over the course of the exacerbation. p<0.001

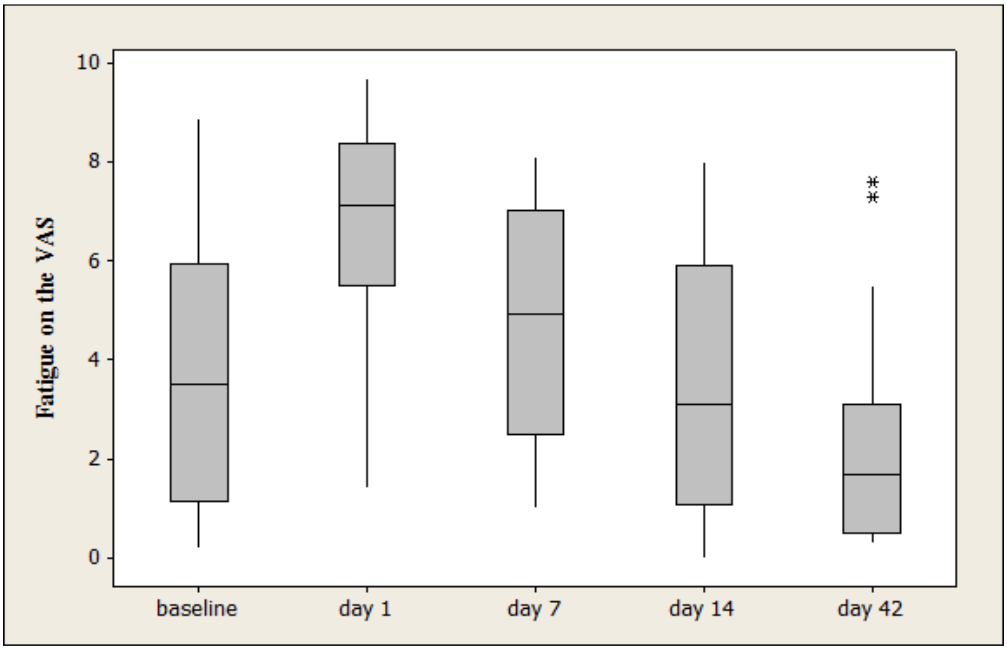

Figure 4.7: Box plot showing the median and inter quartile range in centimetres for fatigue as scored on the visual analogue score. The x-axis depicts the time of visit - baseline (steady state with no exacerbation in the preceding 4 weeks), Day 1 onset of exacerbation, Day 7 on treatment for exacerbation, day 14 completion of antibiotic treatment and Day 42 recovery from exacerbation. The y-axis depicts visual analogue score in centimetres. All extreme observations are marked with an *. The change was significant over the course of the exacerbation. $p<0.001$

4.5.2 Modified Borg's Breathlessness score

4.5.2.1 Stable state bronchiectasis

Fifty eight patients were studied in stable state (not having had an exacerbation in the preceding 4 weeks).

The mean (SD) MBS score 3.32(1.6).

The MBS significantly correlated with the trans-cutaneous oxygen saturations (SaO2). (r=0.160, p=0.000)

The MBS did not significantly correlate with the partial pressure of oxygen while inspiring room air -PaO_2 (r=-0.118, p=0.44, n=45).

The MBS was significantly correlated with FEV_1%predicted (r= -0.334, p=0.012, n=56).

The MBS was significantly correlated with the breathlessness score on the Visual analogue scale (r=0.669, p=0.000, n=55).

4.5.2.2 Acute exacerbation of bronchiectasis

The MBS significantly increased from baseline to day 1(p=0.000). The increase persisted despite 7 days of treatment with antibiotics (p=0.012). This trend was reversed only by day 14 on completion of treatment (p=0.425). There was no difference in the MBS between baseline and day 42 (p=0.209)

Overall there was a significant variation in the MBS over the course of the exacerbation (p=0.000). Figure 4.9

The mean (SD) values of the MBS during the course of an exacerbation are listed in Table 4.4.

Table 4.4: Mean (SD) values of Modified Borg's score at different time points during the exacerbation

Day	Modified Borg's breathlessness score Mean value	Standard Deviation	Number of patients
Baseline	3.30	1.4	22
Day 1	5.95	2.3	22
Day 7	4.85	2.4	22
Day 14	3.55	1.3	22
Day 42	3.60	1.5	20

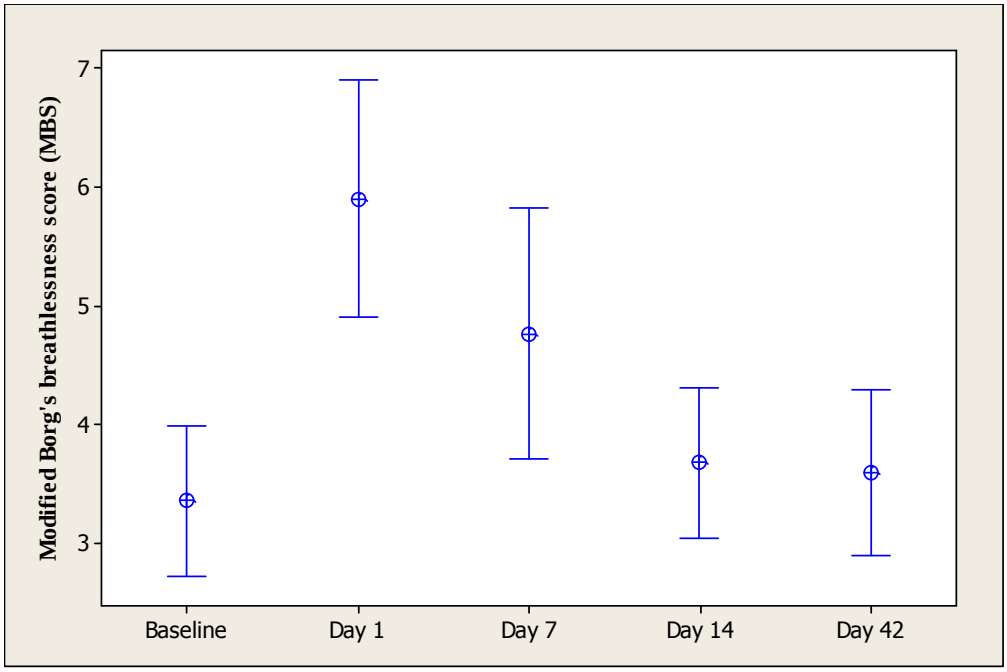

Figure 4.8: Interval plot showing the mean and 95% confidence interval Modified Borg's breathlessness score. The x-axis depicts the time of visit - baseline (steady state with no exacerbation in the preceding 4 weeks), Day 1 onset of exacerbation, Day 7 on treatment for exacerbation, Day 14 completion of antibiotic treatment and Day 42 recovery from exacerbation. The y-axis depicts score as listed on the modified Borg's questionnaire. The change was significant over the course of the exacerbation. p=0.000

4.5.3 Clinical examination

4.5.3.1 Blood pressure

The mean (SD) systolic blood pressure was 137(19) mm Hg and mean diastolic blood pressure was 77(13) mm Hg. This was within the normal range for age and sex of this cohort. (n=57).

The systolic blood pressure demonstrated a fall of 4mmHg between stable state and Day 1. However this was not statistically significant, p=0.120. There was no significant change in the systolic blood pressure during the course of the exacerbation, p=0.849. Figure 4.10 demonstrates the mean and 95% confidence interval change in systolic blood pressure at various visits during the acute exacerbation.

The diastolic blood pressure demonstrated a fall of 4mmHg between stable state and Day 1. However this was not statistically significant, p=0.234. There was no significant change in the diastolic blood pressure during the course of the exacerbation, p=0.785. Figure 4.11 demonstrates the mean and confidence interval change in diastolic blood pressure at various visits during the acute exacerbation.

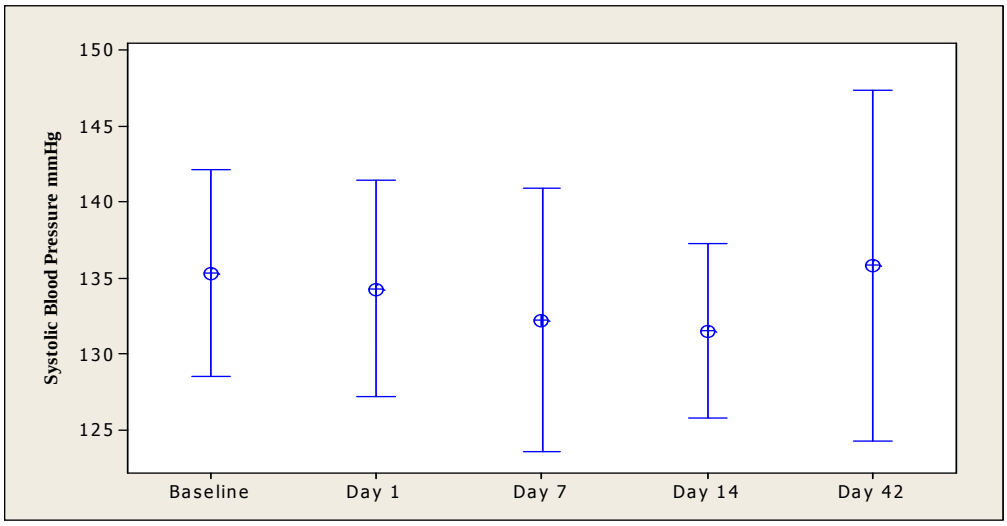

Figure 4.9: Interval plot showing the mean and 95% confidence interval systolic blood pressure. The x-axis depicts the time of visit - baseline (steady state with no exacerbation in the preceding 4 weeks), Day 1 onset of exacerbation, Day 7 on treatment for exacerbation, day 14 completion of antibiotic treatment and Day 42 recovery from exacerbation. The y-axis depicts the blood pressure in mm Hg. The change was not statistically significant over the course of the exacerbation. p=0.849

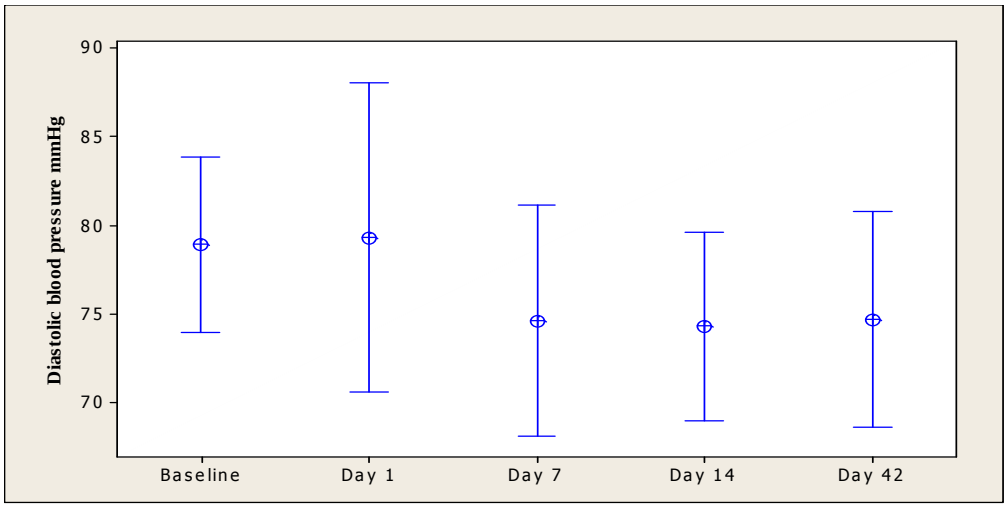

Figure 4.10: Interval plot showing the mean and 95% confidence interval diastolic blood pressure. The x-axis depicts the time of visit - baseline (steady state with no exacerbation in the preceding 4 weeks), Day 1 onset of exacerbation, Day 7 on treatment for exacerbation, day 14 completion of antibiotic treatment and Day 42 recovery from exacerbation. The x-axis depicts the blood pressure in mm Hg. The change was not statistically significant over the course of the exacerbation. p=0.785

4.5.3.2 Oxygen saturations as measured by digital pulse oximetry

The mean (SD) oxygen saturations were 96 (2.2) % on room air (Range 90 – 100%). The mean oxygen saturations as measured by pulse oximetry fell by 1.2% between stable state and Day 1[(p=0.483).There was no significant change in the oxygen saturations measured at room air during the course of the exacerbation of bronchiectasis. (p=0.559). Figure 4.12 demonstrates mean and 95% confidence interval oxygen saturations during the course of an exacerbation.

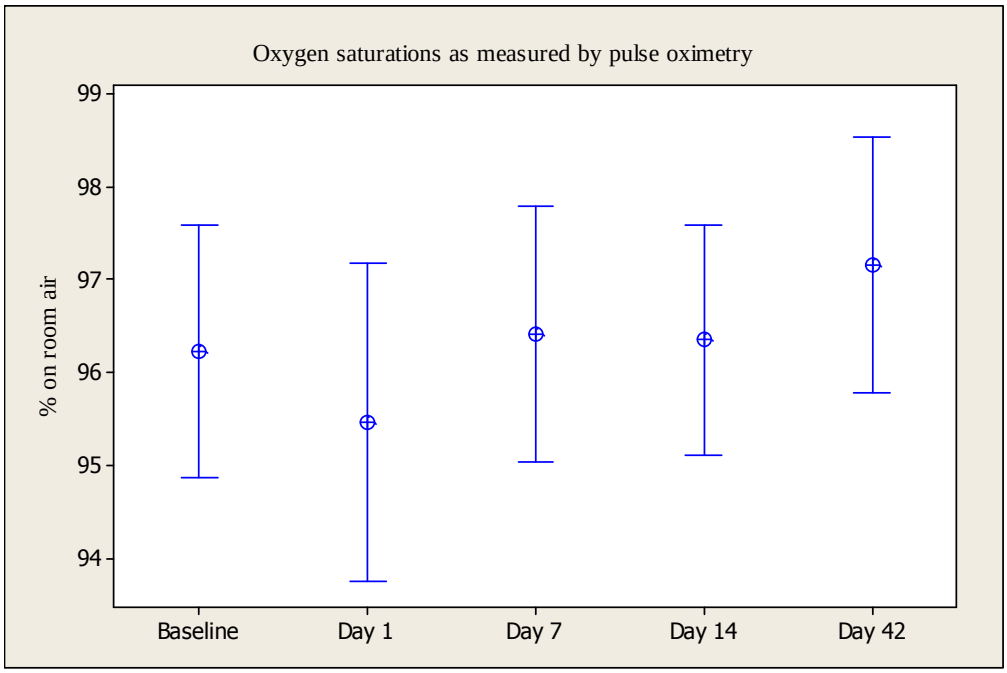

Figure 4.11: Interval plot showing the mean and 95% confidence interval oxygen saturations at room air. The x-axis depicts the time of visit - baseline (steady state with no exacerbation in the preceding 4 weeks), Day 1 onset of exacerbation, Day 7 on treatment for exacerbation, day 14 completion of antibiotic treatment and Day 42 recovery from exacerbation. The y-axis depicts the per centage saturation. The change was not statistically significant over the course of the exacerbation. p=0.559

4.5.3.3 Body temperature as measured by the sublingual probe.

The temperature was less than 38 degrees centigrade in all our patients at baseline (n=58).Only one patient demonstrated a temperature of greater than 38 degrees centigrade at Day 1 of the exacerbation.

4.5.3.4 General clinical examination and auscultatory findings

Only 10% of patients had finger clubbing (6/55). On auscultation of the chest, in steady state, seventeen patients were found to have normal breath sounds. Eleven patients had unilateral crackles, 28 had bilateral crackles and 2 patients had high pitched inspiratory squeaks. There was no significant change in the auscultatory findings during the course of the exacerbations. Table 4.5 lists the auscultatory findings in steady state bronchiectasis.

Table 4.5: Auscultatory findings in patients of the COBEX study in steady state bronchiectasis.

Number of patients	Wheeze	Unilateral crackles	Bilateral crackles	High pitched inspiratory squeaks
Baseline	0	4	7	1
Day 1	2	5	7	1
Day 7	3	4	5	1
Day 14	0	6	0	3
Day 42	0	0	4	0

4.6 Discussion

We have attempted to quantify subjective symptoms in bronchiectasis with a view to measure change. Most symptoms in bronchiectasis vary at the time of an exacerbation. The Visual analogue scores have demonstrated the validity of our definition of an exacerbation.

Our patients gave fatigue a very high score both in steady state and during an exacerbation on the visual analogue scale. This would clearly affect quality of life and is essential that it be addressed when treating an exacerbation. Fatigue can be a presenting feature in bronchiectasis [194] and can be out of proportion to the severity of disease [50]. We have demonstrated a clear measurable change in this symptom on a simple VAS questionnaire.

Breathlessness was the second most severe symptom recorded by our patients. The mean score for breathlessness at baseline as recorded on the VAS- 2.7 cm is comparable to other reports. Mean (SD) score for perception of breathlessness, measured on a visual analogue scale, to assess the usefulness of gravity-assisted drainage positions with a head-down tilt in clearing sputum was 2.3 (1.6) and this improved to 3.3 (2.0) cm [195].

We found a significant deterioration in breathlessness at the onset of an exacerbation as measured on the VAS. This trend was reversed on treatment with antibiotics by day 14 and back to baseline at this time and on recovery from the exacerbation.

Breathlessness as measured on the MBS also increased from baseline to onset of exacerbation. This improved on treatment with antibiotics by Day 14. Other workers have used a change in Borg's score in bronchiectasis to demonstrate improvement in breathlessness in relation to airway clearance techniques and histamine challenges. However they report a change in score rather than absolute values [196, 197]

In our study the visual analogue score strongly correlates to the MBS. This has previously been shown in healthy young volunteers with dyspnoea during exercise [198]. The MBS was also significantly correlated to trans-cutaneous saturations (SaO_2), FEV1 per centage predicted, and the partial pressure of oxygen on room air as measured with a capillary blood gas analyser with samples taken from an ear lobe (PaO2).

Surprisingly, cough featured third in severity in stable state and second at the onset of an exacerbation in our patients. Other reports have placed it as the primary and most important symptom. A recent study has identified different phenotypes of patients with bronchiectasis in relation to the onset of a productive cough and this may explain some of the differences in cohorts [199]. We demonstrated a significant increase in cough as measured by the VAS that improved and reverted to baseline on treatment and on recovery from the exacerbation respectively. The score on VAS for cough in our study in stable state (2.95) is comparable to other published reports. In a study looking at the improvement in symptoms after bronchopulmonary hygiene physical therapy in stable state patients, there were significant improvements in cough symptoms (mean cough VAS before 43.3 vs. after 27.5mm) [105].

The volume and colour of sputum increased significantly at the onset of the exacerbation and reverted to baseline after treatment by Day 14. Chest discomfort and

pain were less important to our patients. However we recorded a significant increase in these symptoms too.

We found no significant abnormalities when patients were clinically examined. Pulse rate, body temperature, systolic and diastolic blood pressure remained unchanged during the course of the exacerbation.

We acknowledge that the small number of patients included in our study is a drawback. Also the patient cohort was clinically less 'sick' and possibly had milder disease.

The visual analogue score for various symptoms as described and the Modified Borg's score are both very effective tools in measuring change and assessing outcomes in the management of bronchiectasis. They could certainly be used as endpoints when future therapeutic interventions are studied. Measurement of blood pressure, body temperature and oxygen saturations are unlikely to be of any benefit in future studies directed at measuring efficacy of any intervention in non cystic fibrosis bronchiectasis.

An exacerbation can be detected on patient history of cough, increased volume of sputum, change in colour of sputum, chest pain and discomfort, breathlessness and fatigue. For future longitudinal studies patients could complete visual analogue scores online without attending the out-patient clinic.

Chapter 5

Quality of Life in stable bronchiectasis and during an acute exacerbation from the COBEX study

5.1 Background

Direct measurement of the impact of disease on patients' lives is necessary to assess whether interventions are of benefit [100]. Health-related quality of life (HR-QoL) is impaired in stable state bronchiectasis and deteriorates during an acute exacerbation [83, 102]. Frequent exacerbations cause a vicious cycle of bronchial wall destruction and further bacterial colonisation [6]. HR-QOL is a relevant and quantifiable outcome of care and is an important marker for evaluating existing and new therapies in bronchiectasis.

Most aspects of bronchiectasis impact on the quality of life. Appropriate management of symptoms on identifying the underlying aetiology has a positive impact on the quality of life in these patients. Daily sputum production, chronic cough, upper airway symptoms and abnormal tiredness are all associated with a reduction in health-related quality of life (HR-QoL) in bronchiectasis [46, 62, 102, 104, 105]. Some studies suggest that the number of exacerbations in the preceding year is an important contributor to HR-QoL [46, 53]. Appropriate treatment with antibiotics improves inflammation and thus cough, wheeze and sputum volume. A significant improvement in HR-QoL between onset of exacerbation and completion of treatment has been previously reported [83].

5.2 Measuring Health-related quality of life in bronchiectasis

Instruments used for the assessment of HR-QoL in bronchiectasis.

Various instruments have been used in assessment of the quality of life in bronchiectasis. The St. George Respiratory Questionnaire (SGRQ) has been used in more than eight studies of stable state bronchiectasis [102, 183, 200-205]. Other instruments include the SF-36 [102], The Chronic respiratory disease questionnaire [83] and nonspecific patient reported quality of life measures [206].

5.2.1 St George's Respiratory Questionnaire

The original St. George Respiratory Questionnaire (SGRQ) was developed in 1990 by Jones et al to quantify the impact of disease on the health and well-being of patients with COPD [207]. Since then, the SGRQ has been translated into many languages and has been validated for use in different ethnic groups. To date, several adaptation and validation studies have been published in English and non-English populations. These studies have performed on Swedish, Spanish, Japanese, and American-English and Chinese speaking populations, who have independently validated the reliability and sensitivity of the translated versions. While other quality-of-life instruments are available, the SGRQ is the only validated instrument in the assessment of patients with bronchiectasis [207-210]

The SGRQ is a self-administrated health-related QoL measure containing 50 items and 76 weighted responses divided into three components: symptoms, activity, and impacts. The subscales include symptoms (8 items), activity (16 items) and impacts

(26 items).The symptoms subscale refers to the frequency and severity of cough, wheezing, expectoration, or exacerbation. The activity subscale in turn refers to the limitations in patient activity due to dyspnoea, and involves 16 dichotomous response items. The impact subscale summarizes the alterations in the psychological, occupational, and social spheres, based on the way in which the patient perceives his or her disease. It consists of 26 items addressed by eight questions. The total questionnaire score and score corresponding to each of the three subscales are calculated as a function of the item scores, based on a range from 0 to 100 points. A higher SGRQ score, either the total score or individual component score, represents a poorer quality of life [207-209]. A 4 unit difference in the total SGRQ score has been established as a clinically significant score [208]. The questionnaire has been designed for self-administration in 10 min. The recall period of the SGRQ symptoms component needs to be shortened to make more appropriate for the use during an acute exacerbation [211].

5.2.2 Euroqol

The Euroqol 5 dimension (EQ-5D) is a generic instrument for the measurement of HR-QoL. The EQ-5D includes single item measures of: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each item is coded using 3-levels (1 = no problems; 2 = some problems; 3 = severe problems). The instrument includes a global rating of current health using a visual analogue scale [142] ranging from 0 (worst imaginable) to 100 (best imaginable).

EQ-5D is designed for self-completion by subjects within a study. It is cognitively simple, taking only a few minutes to complete. Instructions to subjects are included in the questionnaire.

5.2.3 Assessment of HR-QoL during acute exacerbations in bronchiectasis.

Only two studies have so far assessed the quality of life during an acute exacerbation of bronchiectasis [80, 83]. Murray et al used the SGRQ, which is validated in bronchiectasis. The questionnaire was adapted for the end of exacerbation assessment to ask about symptoms in the preceding week. In this prospective cohort study patients were asked to complete the SGRQ at the start of an exacerbation and one week following antibiotic treatment completion. There was a significant improvement in all individual domain scores of the SGRQ following completion of antibiotics. Eighty-nine per cent of patients showed an improvement of more than 4 units in all domains [80].

Courtney et al have used the chronic respiratory disease questionnaire (CRDQ) in their study of acute bronchiectatic exacerbations. In this prospective cohort study patients completed the questionnaire at the onset of an exacerbation, on completion of antibiotics at day 14 and four weeks after the completion of antibiotics. A significant improvement was noted in three of the four domains (dyspnoea, emotional and mastery) of the CRDQ. All the improvement was seen by Day 14 and there was no further change in 4 weeks [83].

5.3 Aims

The aim of this study was to define the change in HR-QOL during the course of an acute exacerbation of bronchiectasis. Describing a significant change will help in using HR-QOL as an endpoint to study therapeutic interventions.

5.4 Methods

In this study two questionnaires have been to assess HR-QoL - The SGRQ and the Euroqol.

The SGRQ was completed by all patients at Day 0 (baseline, in stable state), at Day 1(onset of acute exacerbation and prior to commencement of antibiotics) and Day 42 (on recovery from acute exacerbation).

The Euroqol was completed by all patients at Day 0 (baseline, in stable state), Day 1(onset of acute exacerbation and prior to commencement of antibiotics), Day 14 (on completion of antibiotics) and Day 42 (on recovery from acute exacerbation).

The schedule of questionnaires employed in the study is listed in table 5.1. The questionnaires themselves are appended for information. St Georges respiratory questionnaire employed in this study can be found in the Appendix 2 and the EuroQol questionnaire employed in this study can be found in the Appendix 3.

Table 5.1: Schedule of the two HR-QoL questionnaires employed in the COBEX study. The columns (Day 0, Day 1, Day 14 and Day 42) indicate the visit in stable state and during an exacerbation of bronchiectasis.

	Day 0	Day 1	Day 14	Day 42
Euroqol	√	√	√	√
St George Respiratory Questionnaire	√	√		√

5.5 Statistics

Data analysis was performed using statistical software (SPSS version 14). Analysis of variance was used to compare the questionnaire scores between the four groups and the Fisher test for pair wise comparisons. A 4 point change in the SGRQ was considered a clinically significant improvement. Values are expressed as Mean±SD. Differences were considered significant at p-values less than 0.05.

5.6 Results

5.6.1 St Georges Respiratory Questionnaire

Stable state bronchiectasis

Quality of life scores in the 3 domains and total score at baseline (in stable state) are listed in Table 5.2. The mean score was higher in the symptoms domain and was 65.04. The activities score was less affected in stable state (49.66) than symptoms followed by the impacts score (30.19).

Table 5.2: Mean, minimum and maximum score in the different domains of the St Georges Respiratory Questionnaire during stable bronchiectasis in the study cohort

	Minimum	Maximum	Mean	Std. Deviation
Symptoms score	31.47	95.49	65.04	16.40
Activities score	41.00	93.86	49.66	25.10
Impact score	23.93	57.13	30.19	14.80
Total score	12.90	72.01	42.62	16.34

Acute exacerbation of bronchiectasis

Thirteen patients completed a questionnaire at baseline, Day 1 and Day 42. In the symptom domain, there was no significant change during the course of the exacerbation, p= 0.269. There was no significant deterioration between stable state and onset of the exacerbation, p= 0.160. Table 5.3 lists the mean [SD] values of symptom sub-scores at baseline (Day 0), onset of exacerbation - Day 1 and completion of exacerbation - Day 42. Figure 5.1 is a box plot depicting median values of symptom scores at baseline, Day 1 or onset of exacerbation and Day 42 on completion of the exacerbation.

Table 5.3: Mean (SD) symptom sub-scores on the St Georges respiratory questionnaire during an acute exacerbation of bronchiectasis

	Mean score	Standard deviation	N
Day 0 Baseline, Stable state	66.57	13.30	13
Day 1 Onset of exacerbation Prior to commencement of antibiotics	59.38	21.80	13
Day 42 On recovery from acute exacerbation	60.70	18.50	13

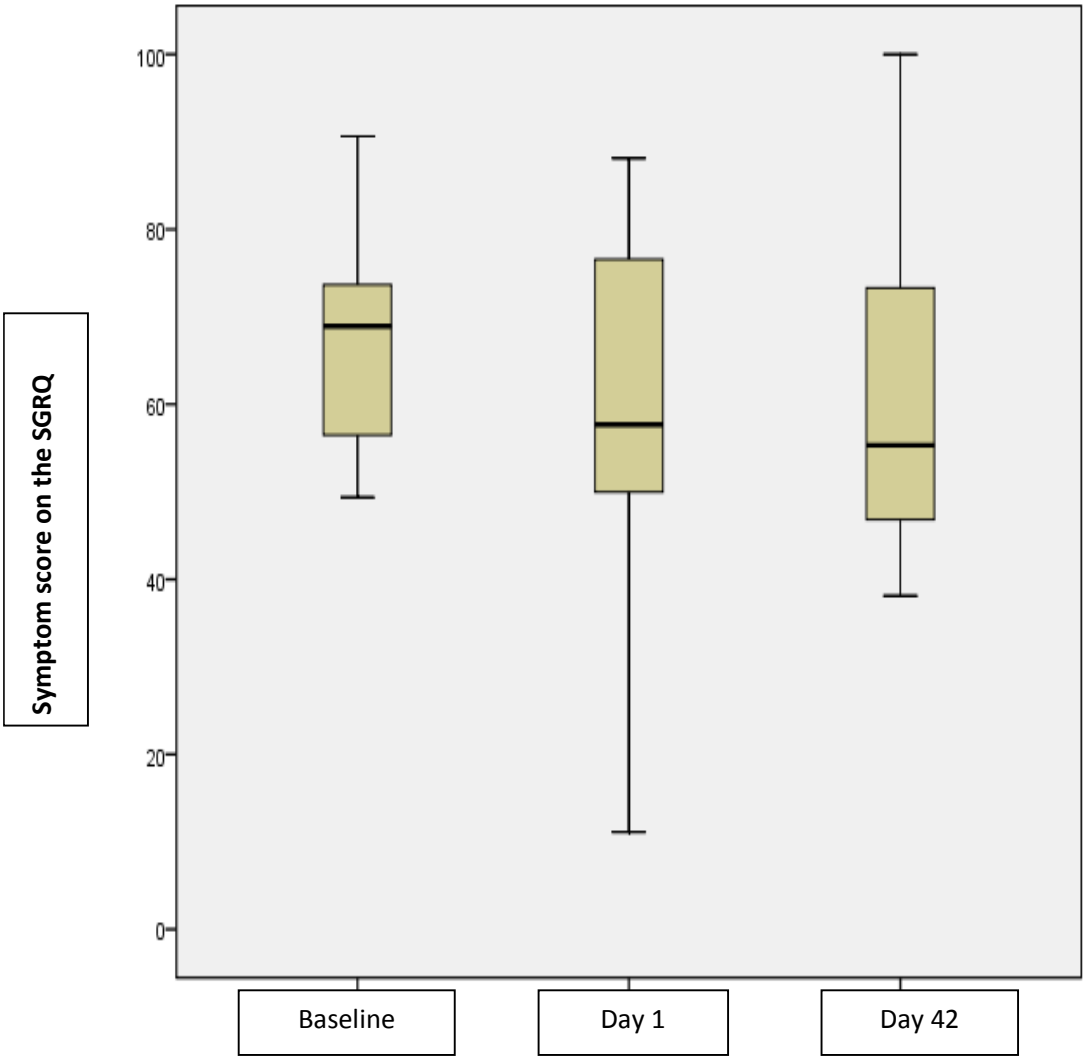

Figure 5.1: Median and SEM values of the symptom sub-score on the St George's Respiratory Questionnaire at baseline (stable state), onset of (Day 1) and 4 weeks later (Day 42), in patients with an acute exacerbation of bronchiectasis. The x-axis lists the time point during the exacerbation and the y-axis is the symptom score on the SGRQ. No significant difference was detected between stable state and onset of exacerbation p=0.160, or over the course of the exacerbation p = 0.269.

In the Activities domain, although there was an increase in the score between baseline and onset of exacerbation suggesting deterioration, however this was not statistically significant, p=0.122. The change during the course of the exacerbation was not statistically significant either, p=0.296. Table 5.4 lists the mean [SD] values of activities score at baseline (Day 0), onset of exacerbation - Day 1 and completion of exacerbation - Day 42. Figure 5.2 is a box plot depicting median values of activities sub-score at baseline, Day 1 or onset of exacerbation and Day 42 on completion of the exacerbation.

Table 5.4: Mean activity sub-scores on the St George's respiratory questionnaire during an acute exacerbation of bronchiectasis

	Mean	Std. Deviation	N
Day 0 Baseline, Stable state	57.17	17.35	8
Day 1 Onset of exacerbation Prior to commencement of antibiotics	59.15	25.79	8
Day 42 On recovery from acute exacerbation	50.84	21.61	8

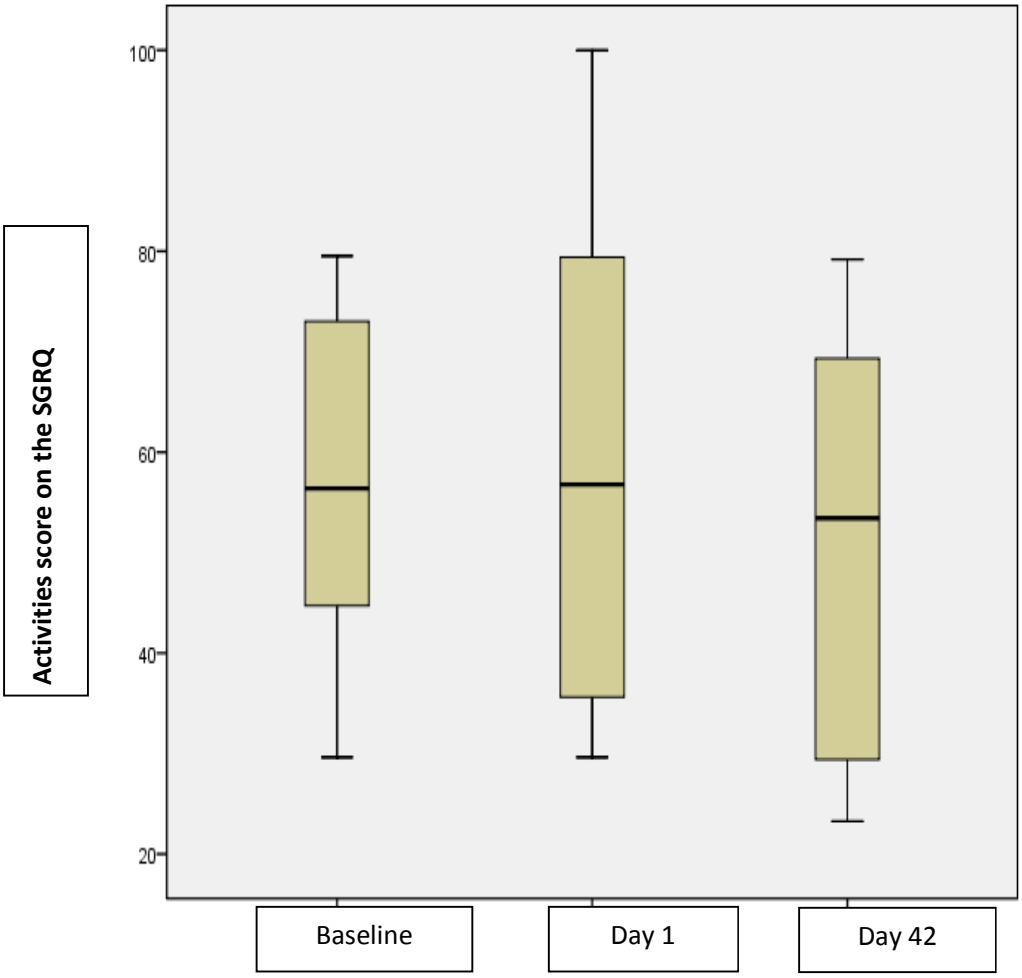

Figure 5.2: Box plot depicting median and SEM values of the activities sub-score on the St George's Respiratory Questionnaire at baseline (stable state), onset of exacerbation-Day 1, and 4 weeks later -Day 42, in patients with an acute exacerbation of bronchiectasis. The x-axis lists the time point during the exacerbation and the y-axis is the activities score on the SGRQ. No significant difference was detected between stable state and onset of exacerbation p = 0.122, or over the course of the exacerbation, p=0.296.

In the Impacts domain, again deterioration in scores was noted at the onset of an exacerbation. However this was not statistically significant p=0.983. There was no significant change during the course of the exacerbation either, p=0.160. However there was a significant improvement in scores between onset of exacerbation ie Day 1 and recovery from exacerbation – Day 42, p=0.044. Table 5.5 lists the mean [SD] values of the impact scores at baseline (Day 0), onset of exacerbation - Day 1 and completion of exacerbation - Day 42. Figure 5.3 is a box plot depicting median values of the impact sub-scores at baseline, Day 1 or onset of exacerbation and Day 42 on completion of the exacerbation.

Table 5.5: Mean (SD) impacts score on the St George's respiratory questionnaire during an acute exacerbation of bronchiectasis.

	Mean	Std. Deviation	N
Day 0 Baseline, Stable state	23.23	15.67	6
Day 1 Onset of exacerbation Prior to commencement of antibiotics	36.86	17.22	6
Day 42 On recovery from acute exacerbation	23.12	17.39	6

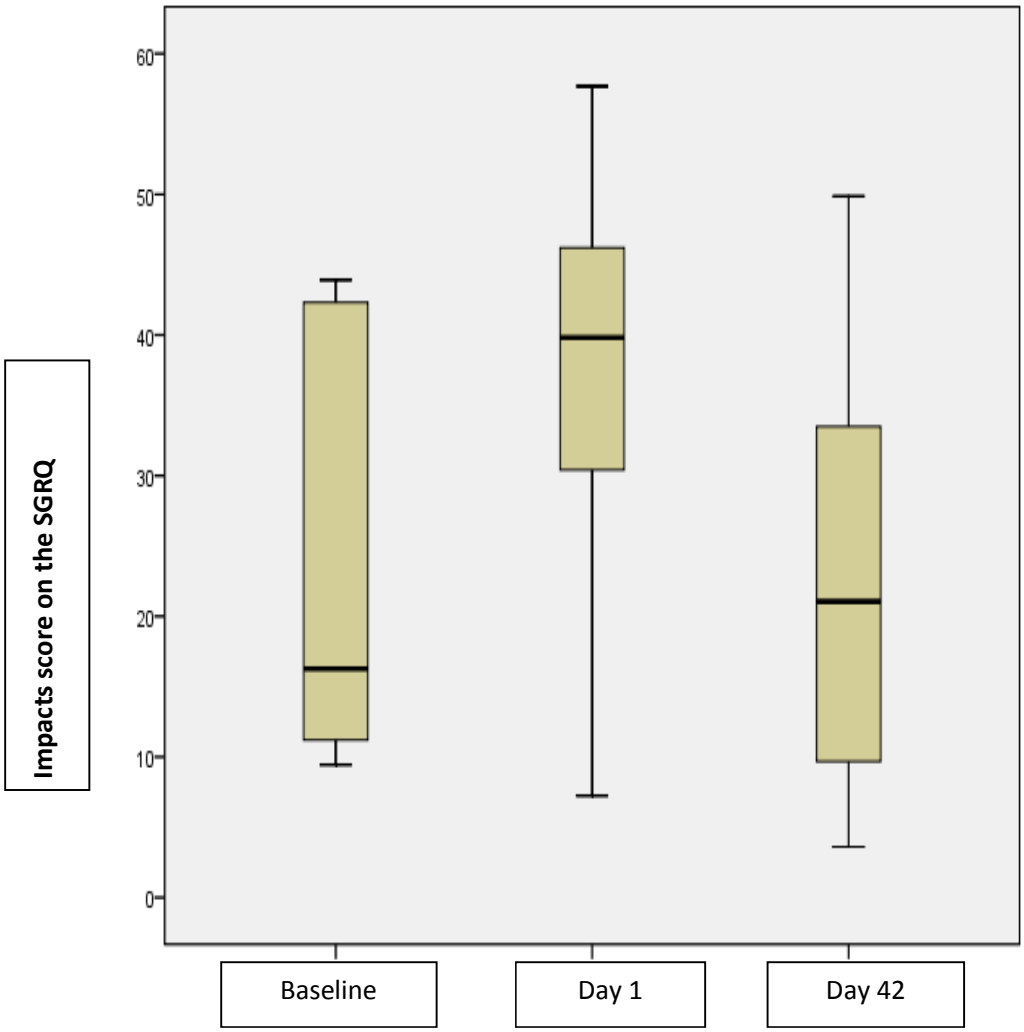

Figure 5.3: Box plot depicting the median and SEM values of the impacts sub-score on the St George's Respiratory Questionnaire at baseline (stable state), onset of (Day 1) and 4 weeks later (Day 42), in patients with an acute exacerbation of bronchiectasis. The x-axis lists the time point during the exacerbation and the y-axis is the impacts score on the SGRQ. No significant difference was detected between stable state and onset of exacerbation $p = 0.983$, or over the course of the exacerbation, $p=0.160$.

Only three patients completed the questionnaires at all periods appropriately to be able to analyse data for total scores. There was no significant change in the total scores during the course of the exacerbation. p=0.769. Table 5.6 lists the mean [SD] values of the total scores at baseline (Day 0), onset of exacerbation - Day 1 and completion of exacerbation - Day 42. Figure 5.4 is a box plot depicting median [95%CI] values of the total scores at baseline, Day 1 or onset of exacerbation and Day 42 on completion of the exacerbation.

Table 5.6: Mean (SD) total scores on the St George's respiratory questionnaire during an acute exacerbation of bronchiectasis

	Mean	Std. Deviation
Day 0 Baseline, Stable state	35.89	8.2
Day 1 Onset of exacerbation Prior to commencement of antibiotics	35.72	16.8
Day 42 On recovery from acute exacerbation	31.80	12.9

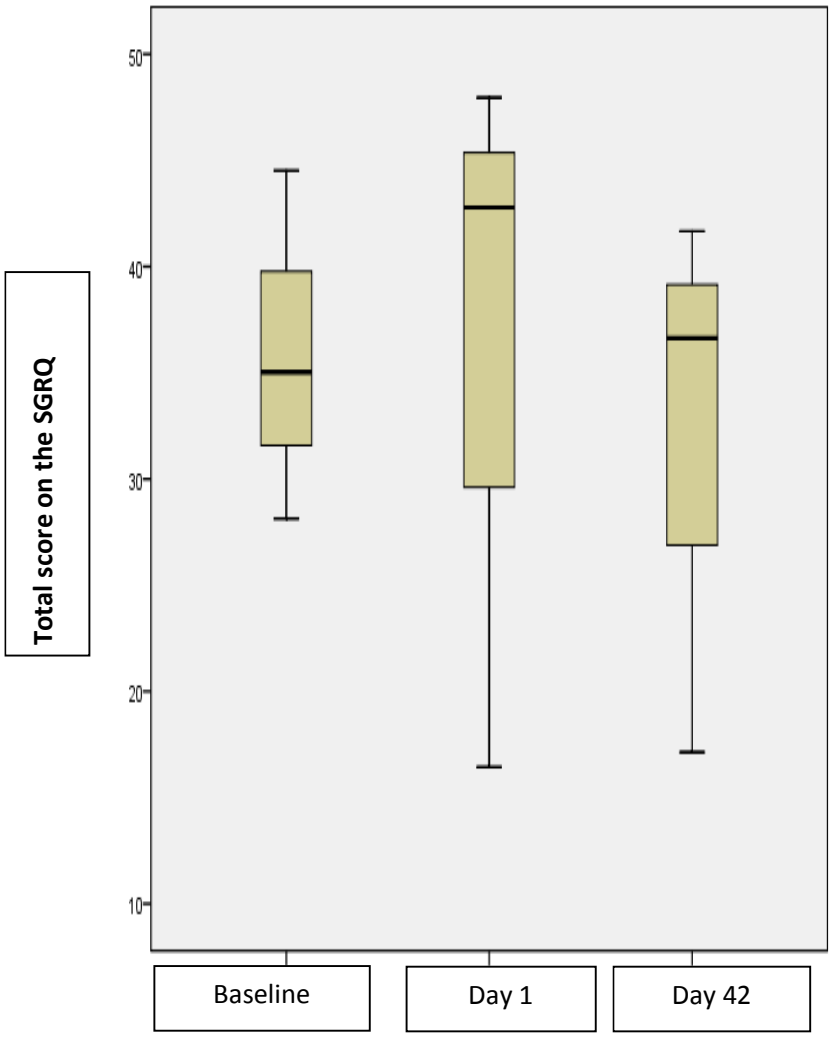

Figure 5.4: Box plot depicting median and SEM values of the total scores on the St George's Respiratory Questionnaire at baseline (stable state), onset of (Day 1) and 4 weeks later (Day 42), in patients with an acute exacerbation of bronchiectasis. The x-axis lists the time point during the exacerbation and the y-axis is the total score on the SGRQ. No significant difference was detected over the course of the exacerbation, $p = 0.769$.

5.6.2 Euroqol

There was a significant change in the quality of life as measured by the Euroqol questionnaire over the course of an exacerbation, (p=0.000). There was a significant decrease in the quality of life as measured by the Euroqol questionnaire between baseline (stable state) and the onset of an exacerbation, Day 1- p=0.003. However this change was reversed by Day 14 at the end of treatment with antibiotics. p=0.800. There was no difference in the quality of life between Day 0 (baseline) and Day 42 (recovery from exacerbation), p=0.797. Table 5.7 lists the mean (SD) Euroqol scores at baseline (Day 0), Day 1-onset of exacerbation, Day 14- completion of treatment and Day 42-recovery from exacerbation. Figure 5.5 is a box plot depicting median and IQR for Euroqol scores at baseline, Day 1, Day 14 and Day 42.

Table 5.7: Euroqol score in stable state and during an acute exacerbation of non-CF bronchiectasis

	Mean score	Standard deviation	p value
Day 0 Baseline, Stable state	0.84	0.16	p= 0.000
Day 1 Onset of exacerbation Prior to commencement of antibiotics	0.67	0.27	
Day 14 On completion of antibiotics	0.86	0.18	
Day 42 On recovery from acute exacerbation	0.80	0.21	

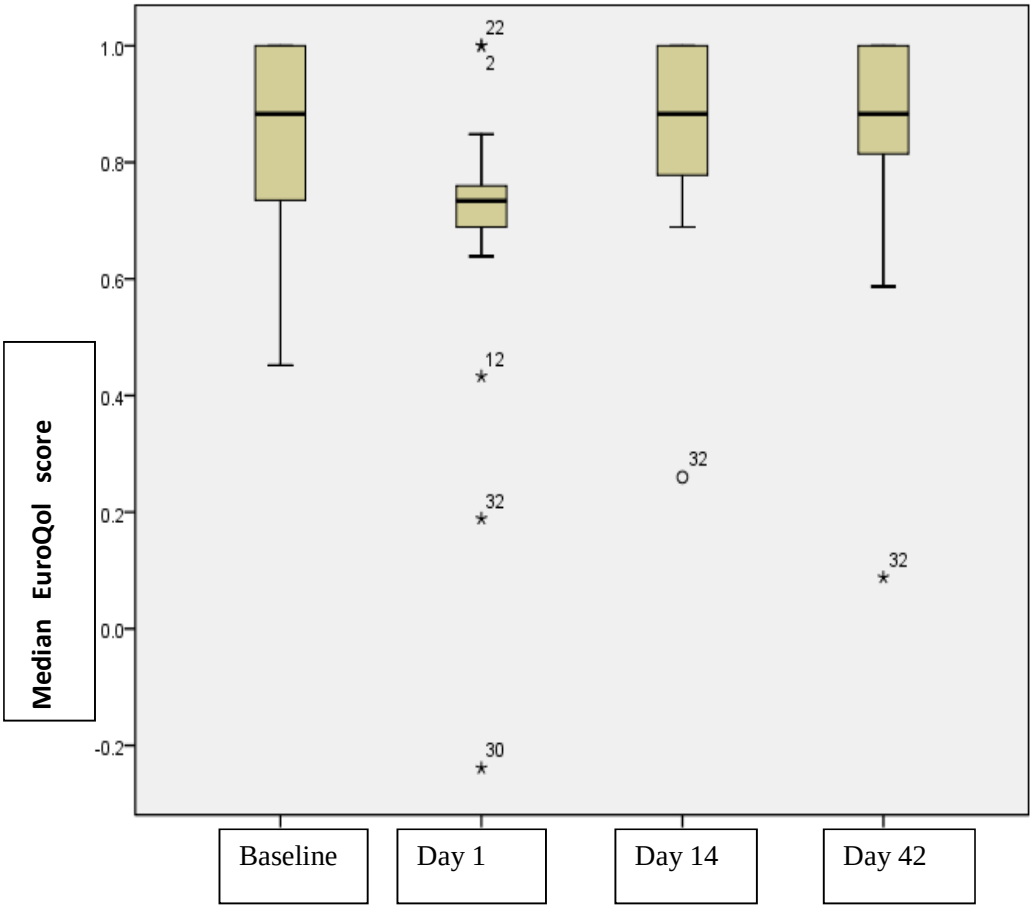

Figure 5.5: Median and SEM values of the total score on the Euroqol at baseline (stable state), onset of exacerbation (Day 1), at the end of treatment (Day 14), and 4 weeks after the onset of an acute exacerbation (Day 42), in patients with bronchiectasis. The x-axis lists the time point during the exacerbation and the y-axis is the median score on the Euroqol questionnaire. Extreme outliers are depicted by an asterix. The numbers accompanying these are patient identification numbers from the study. There was a significant change in quality of life as assessed by the Euroqol questionnaire over the course of an exacerbation, p=0.000.

Quality of life at baseline as measured by Euroqol at baseline (stable state) was significantly correlated to the number of exacerbations the patients has suffered in the preceding 1 year, (r= -0.27, p= 0.04). Figure 5.6 is a scatter plot showing the significant relationship between Euroqol scores at baseline and the number of exacerbations suffered by the patient in the preceding year.

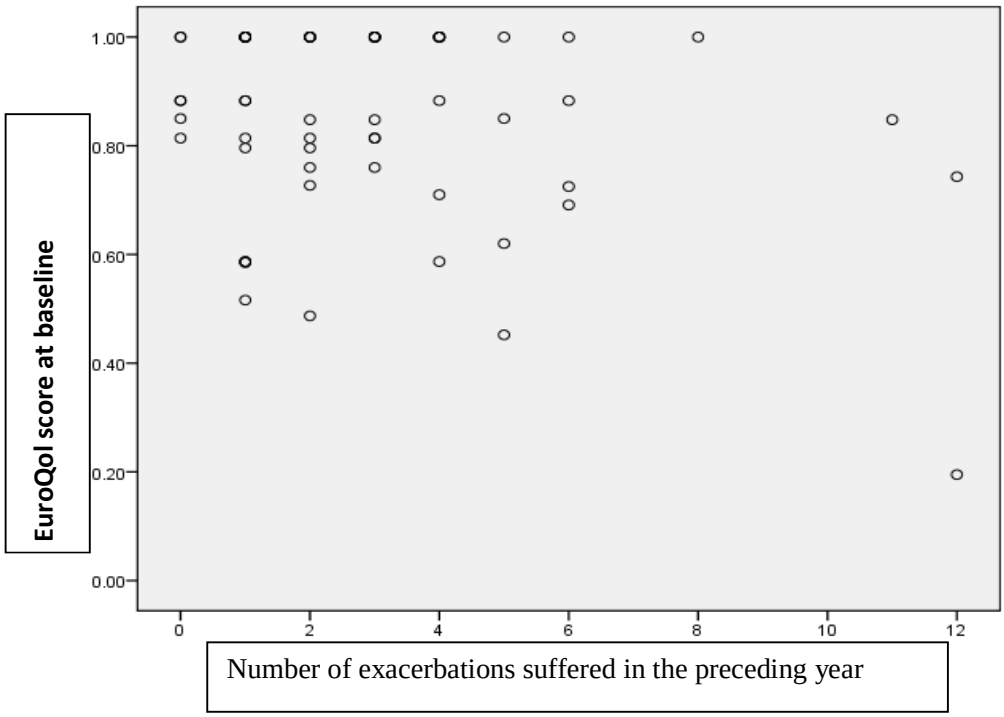

Figure 5.6: Scatter plot of relationship between Euroqol score at baseline and number of exacerbations suffered by a patient in the preceding year. The variables on the x-axis are the number of exacerbations suffered by the patient while the variables on the y-axis are the EuroQol score for the same patients. Extreme outliers are depicted by an asterix. The numbers accompanying these are patient identification numbers from the study. This was a statistically significant relationship. (r= -0.27, p= 0.04)

In stable state bronchiectasis, the quality of life as measured by the Euroqol was not significantly related to the total dose of inhaled corticosteroids being used by the patients(r= -0.025, p=0.85) Figure 5.7 is a scatter plot showing the relationship between Euroqol scores at baseline and the total daily dose of inhaled corticosteroid for patients with bronchiectasis in stable state.

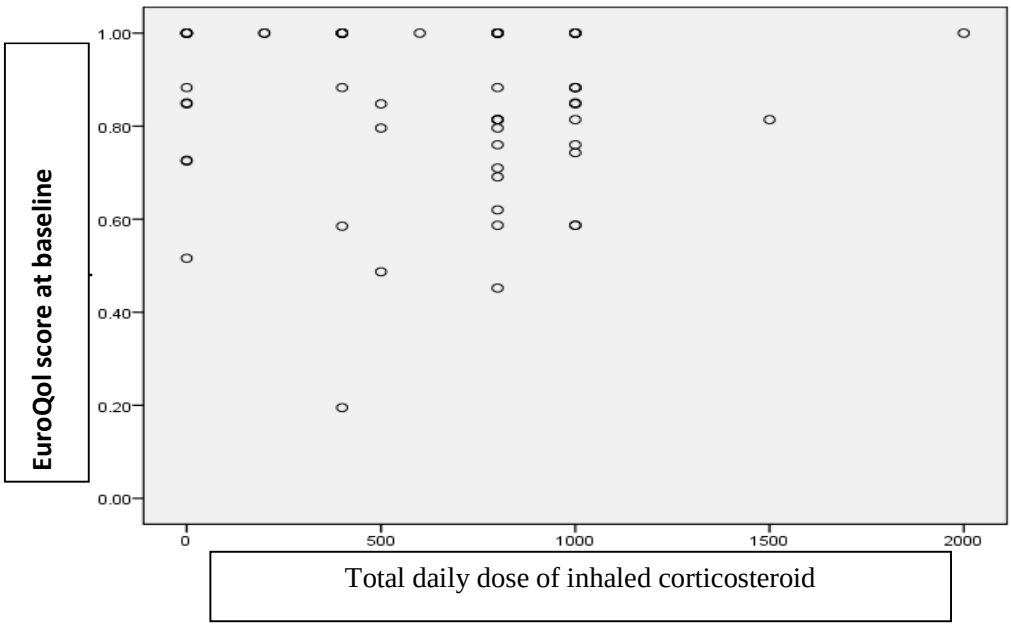

Figure 5.7: Scatter plot of relationship between Euroqol score at baseline and the total daily dose of inhaled corticosteroid used by a patient. The variable on the x-axis is the total daily dose of inhaled corticosteroid used by the patient while the variables on the y-axis are the EuroQol score for the same patients. Extreme outliers are depicted by an asterix. The numbers accompanying these are patient identification numbers from the study. This was not a statistically significant relationship, (r= -0.025, p=0.85).

5.7 Discussion

The aim of this study was to assess HR – QoL in patients experiencing an exacerbation of bronchiectasis with a view to defining end points for future therapeutic interventions. We employed two frequently used questionnaires that have been validated in bronchiectasis- St Georges Respiratory questionnaire and the EuroqoL questionnaire. Patients were studied in stable state prior to the onset of an exacerbation so as establish a baseline to later compare parameters with.

Quality of life scores was assessed in 3 domains (Symptoms, Activity and Impact) by the SGRQ. In 2 of these 3 domains (symptoms and activity) we failed to show a significant change based on the SGRQ scores alone. This was not in keeping with findings in another arm of our study. In particular symptoms as assessed by the Visual analogue scale suggested an initial deterioration at the onset of exacerbation followed by very significant improvement. There was a significant improvement in the impact scores on treatment with antibiotics.

The mean scores on the SGRQ were in our study were (n=22): symptoms score 66±13, activity score 57 ± 17; impact score 23 ± 15 and total score 35 ± 8. These are comparable to other groups of patients who have been studied in stable where the mean scores on the SGRQ were: symptoms score 45.4 ± 18.8, activity score 53.5 ± 23.5, impact score 39.7 ± 19.9 and lower than other reports total score 45.5 ± 17.6 and Symptoms:47±17, Activity:41±17, Impact:55±19 [46, 110, 205].

Local and systemic inflammation improves on treatment with antibiotics but this may not be reflected in HR-QoL parameters as measured by the chronic respiratory disease questionnaire (CRDQ) [83]. Local inflammation may be modified by inhaled corticosteroid. In a randomised study looking at the benefit of inhaled corticosteroid, there were no differences among three treatment groups as regards the total SGRQ score or scores of the three scales. When patients were administered 100mcg of inhaled corticosteroid, a clinically significant improvement (4 points) in total SGRQ score from baseline was noted. 3 months of treatment. This improvement persisted at 6 months [112]. However we were unable to demonstrate any correlation between QoL and the dose of inhaled corticosteroid. Comparing the quality life score in groups of patients according to colonization, patients colonized by *Pseudomonas* have a poorer quality of life on all scores of the SGRQ. Patient groups colonized by other microorganisms also have poor quality of life when compared to those not colonized [109].

The SGRQ in our hands did not prove to be an useful tool to measure change during an exacerbation of bronchiectasis. The small number of patients and the even smaller number of valid SGRQ questionnaires may contribute to this. This is in contrast to other similar studies who have demonstrated a 89% significant improvement in the domains of SGRQ during an exacerbation of bronchiectasis. The nature of our study was such that it involved repeated out-patients clinic visits. We found a bias in our cohort as patients who volunteered, felt they would be able to attend these visits and may have had milder disease.

The Euroqol on the other hand was found to be very useful. We demonstrated a significant change during the course of an exacerbation. The mean score on the Euroqol as measured in our study at baseline was 0.84. Other studies report a slightly lower value of 0.70 in stable state bronchiectasis [212]. This difference may be explained by a difference in the cohort. However the number of patients in both studies is small (less than 20) and results should be viewed with caution [183].

Dyspnoea, FEV1 and daily sputum production are the main variable that best explain HR-QoL [210]. As other workers before us, we found that the number of exacerbations in the preceding year was significantly correlated to the HR-QoL and propose that it is a further determinant. Interventions aimed at reducing these exacerbations will almost certainly improve HR-QoL [213].

Health-related quality of life is a potentially important marker for evaluating existing and new therapies in bronchiectasis. We were able to demonstrate a significant change in the quality of life as measured by the Euroqol questionnaire during the course of an acute exacerbation of bronchiectasis. Quality of life as measured by the SGRQ has proven useful in studies measuring chronic symptoms but this questionnaire did not pick up change from baseline in our hands.

The Euroqol is a valid end point that could be used in the assessment of therapeutic interventions for acute changes in bronchiectasis in the future.

Chapter 6

Lung Function Tests

Spirometry and Impulse Oscillometry in bronchiectasis in the COBEX study.

6.1 Introduction

Spirometry has been considered the mainstay of assessment in bronchiectasis. It is assumed that because an exacerbation represents a flare of airways disease, that spirometry will change at that stage.

Lung function has been well studied in patients with Cystic Fibrosis. Measurement of lung function in these patients is known to reflect both acute and chronic changes [90]. The Forced expiratory volume in one second (FEV1) has been used as a surrogate marker for mortality in Cystic Fibrosis. An acute change in FEV_1 caused by mucous plugging has been clearly demonstrated in exacerbations of CF and provides a short term endpoint [116]. The lung function declines no more than 1% per year in these patients [117]. Chronic colonization with *Pseudomonas aeruginosa* is thought to be an independent factor associated with an accelerated decline of lung function [115]. After antibiotic treatment for an exacerbation, patients infected with Pseudomonas do not show any improvement in FEV1 or FVC, while patients infected with other microorganisms show a significant improvement in both indices [80]. In cystic fibrosis it is recognised that patients with chronic infection with *Pseudomonas aeruginosa* have reduced survival and a more rapid decline in lung function than patients with chronic

infection with *Staphylococcus aureus* [77]. The evidence in non CF bronchiectasis is limited and conflicting. It is possible that Pseudomonas aeruginosa in non-CF bronchiectasis can cause accelerated decline in lung function but this can be modified by the use of nebulised antibiotic therapy [4]. Frequent severe exacerbations and systemic inflammation are also thought to be independent factors associated with an accelerated decline of lung function [115].

Our clinical experience suggested that FEV_1 and FVC did not change despite changes in symptoms. We hypothesised that simple spirometry would not change during the course of an exacerbation. We also sought to investigate IOS – as a tool for longitudinal follow up of bronchiectasis patients.

6.2 Impulse Oscillometry (Forced oscillation technique)

Conventional methods of lung function testing provide measurements obtained during specific respiratory actions of the subject. In contrast, the Impulse Oscillometry technique (IOS) determines breathing mechanics by superimposing small external pressure signals on the spontaneous breathing of the subject. IOS utilises the externally applied pressure signals and their resultant flows to determine lung mechanical parameters. These external forcing signals, may be mono- or multi-frequency, and are applied either continuously or in a time-discrete manner [214] .

These pressure–flow relationships are different to the natural pattern of individual respiratory flows, so that measured IOS results are independent of the underlying respiratory pattern. Therefore, Oscillometry minimises demands on the patient and requires only passive cooperation of the subject.

The impulse oscillation technique allows measurement of up to 10 impedance spectra per second. This allows a useful analysis of intra-breath variation in impedance, comparable to that obtained with mono-frequency applications. However, a disadvantage of such high impulse rates is the inability to record longer respiratory time constants that may be more informative in respiratory abnormalities. Therefore the common application of impulse oscillation utilises recordings of 5 impedance spectra (5 impulses) per second [214].

The resistive component of respiratory impedance, Rrs, includes proximal and distal airways (central and peripheral), lung tissue and chest wall resistance. Normally, central resistance dominates, depending on airway calibre and the surface of the airway walls, while lung tissue and chest wall resistance are usually negligible. Rrs may be considered within normal limits if Rrs at 5 Hz (Rrs5) is within 1.64 standard deviation of the predicted value. Rrs 5 values between 1.64 and 2 SD above predicted may be considered minor, 2+ SD moderate and 4+ SD above predicted severe obstruction [215].

The reactive component of respiratory impedance, Xrs incorporates the mass inertive forces of the moving air column in the conducting airways, expressed in the term inertance (I) and the elastic properties of lung periphery, expressed in the term capacitance (Ca). Respiratory Ca is not identical to compliance. The component of Xrs associated with Ca is defined to be negative in sign. It is most prominent at low frequencies. In contrast, the component of Xrs associated with inertance is always positive in sign and dominates at higher frequencies. Thus, interpretation of Xrs is primarily influenced by the oscillation frequency range under consideration.

Low frequency capacitive Xrs essentially expresses the ability of the respiratory tract to store capacitive energy, mostly in the lung periphery. Xrs5 characterises the lung periphery, but is nonspecific as to the type of limitation. Additional information is needed to differentiate peripheral obstruction from peripheral restriction [214].

The most obvious relationship with other pulmonary function tests concerns the use of spirometry. Spirometry measures maximal forced respiratory efforts, while IOS measures quiet breathing. In bronchiectasis, IOS has not been compared to spirometric techniques as yet.

IOS has been used to monitor response to interventions. IOS has been reported to show greater sensitivity to inhaled corticosteroid or to beta-agonist inhalation in asthma than spirometry. Both inhaled corticosteroids and beta-agonists improve small airways function, and IOS responses manifest prominent changes in indices of peripheral airway obstruction. In contrast, spirometric sensitivity to small airways function is less prominent. Accordingly, it is expected that IOS might provide useful indices of peripheral airway change in response to therapeutic interventions [129, 130].

In patients with chronic obstructive airways disease IOS is able to detect significant change after bronchodilator therapy and FEV_1 is less sensitive [131] . Also IOS measurements, especially indices of peripheral airway function, are significantly correlated with health status and dyspnoea in patients with COPD. IOS indices R5-R20 and X5 are also significantly correlated with the St Georges respiratory questionnaire and the MRC scale. Therefore, in addition to its simplicity and non-invasiveness, IOS may be a useful clinical tool not only for detecting pulmonary functional impairment,

but also to some extent at least estimating the patient's quality of daily life and well-being [132]. Similarly in cystic fibrosis airway resistances can be adequately estimated by forced impulse Oscillometry [216].

IOS remains under investigated in non CF bronchiectasis.

6.3 Aims

1. To measure variability in spirometric indices during the course of an acute exacerbation of bronchiectasis.

   The Functional indices measured are as follows

- Forced expiratory volume in the first second- actual(FEV_1)
- Forced expiratory volume in the first second per centage predicted (FEV_1% predicted)
- Forced Vital Capacity-actual (FVC)
- Forced Vital Capacity %predicted (FVC % predicted)

2. To determine the sensitivity of Impulse Oscillometry (R5, X5) in patients with stable bronchiectasis by comparing it to standard spirometric manoeuvres.

6.4 Methods

6.4.1 Spirometry

Dynamic lung volumes were assessed at all visits. Static lung volumes were performed at Baseline visit only. Total lung capacity and residual volume were measured by helium dilution technique. All manoeuvres were performed after the exhaled breath condensate was collected. All measurements were performed to ARTP/BTS guidelines.

FEV_1 and FVC were expressed as absolute values and as per cent predicted values for the patient's age, sex, and height using European Community of Steel and Coal references values. A maximum of three manoeuvres were performed to obtain a coefficient of variation of < 5% in FEV_1 relative to the best manoeuvre.

6.4.2 IOS

Impulse Oscillometry was performed at Baseline visit only.

IOS measures airway resistance by sending a pulse-shaped sound wave produced by a loudspeaker to the patient's lungs and listening for the reflection of that wave. The overall impedance of the pulse is due to the resistive and viscoelastic forces of the respiratory system. This is reported as R5, R20 (respiratory resistance at 5 and 20Hz, respectively) and X5 (reactance at 5 Hz). The point at which reactance is zero is known as the resonant frequency (RF) and is measured in Hertz.

Subjects were connected to the IOS machine via a mouthpiece with tongue depressor and instructed to breathe quietly at FRC. All measurements were performed using a nose peg with the patient sitting in an upright position and head in a neutral position. Three to five tests were performed each 60 seconds in length. Data with glottis closure,

swallowing or irregular breathing was discarded and the remaining clean data analysed.

IOS indices

Resistance was measured at an impulse frequency of 5Hz (R5). R5 reflects total respiratory resistance i.e. resistance attributable to both central and peripheral airways.

Reactance was measured at an impulse frequency of 5Hz(X5). Since inertance is negligible at lower frequencies X5 is used to measure the elastic properties of the peripheral respiratory system. Reactance comprises reactive elastance and inertance.

6.4.3 Equipment used

The Jaeger Masterscreen PFT and body have been used to measure FEV_1, FVC, and Residual volume and total lung capacity (RV/TLC).

The Jaeger Impulse Oscillometry System (IOS) have been used to measure R5 and X5.

6.4.4 Statistical Analysis

All data were tabulated as the mean and SD for quantitative variables, and as absolute values and per centages for qualitative variables. The normality of all variables was verified. In the absence of a normal distribution, nonparametric tests were used. Global test ANNOVA has been used to measure change over time and paired t-test to make

within group comparisons. Any comparison with a p value < 0.05 was considered to be statistically significant.

6.5 Results

6.5.1 FEV_1 (Forced expiratory volume in first second)

Patients, who had an infective exacerbations, had FEV_1 actual measured at each visit during the study. The mean (± SD) values for FEV_1 at these time points are listed in Table 6.1.

Table 6.1: Mean (SD) FEV_1 at different time points during an exacerbation of bronchiectasis.

FEV_1	Number of patients	Mean (L)	Standard Deviation
Baseline	22	1.91	0.62
Day1	20	1.95	0.62
Day7	22	1.93	0.83
Day14	22	1.97	0.73
Day42	18	2.00	0.67

There was no significant change in FEV_1 measured over the time course of an exacerbation, n=17, p=0.996. Figure 6.1 is an interval plot demonstrating change in the mean FEV_1 during the course of an exacerbation.

Twenty patients had FEV 1 measured at baseline and Day 1 {mean (± SD) 1.91(0.61) v 1.95(0.61)}.There was no change in FEV_1 between stable state and onset of an exacerbation (baseline v Day 1) p= 0.917. There was no difference between FEV_1 in stable state and on recovery from the exacerbation, (n=18, baseline v Day 42) p=0.466.

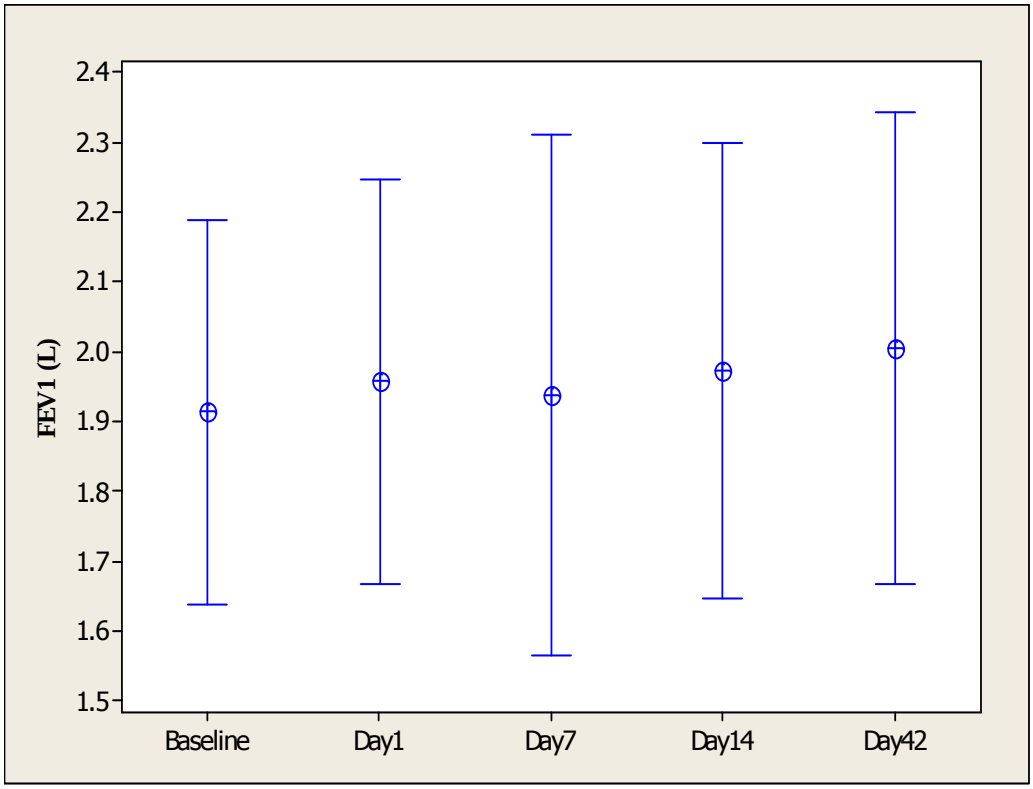

Figure 6.1: Interval Plot of Mean (95% confidence interval) FEV1 at baseline (stable state), onset of exacerbation (Day 1) and 4 weeks later (Day 42), in patients with an acute exacerbation of bronchiectasis. The x-axis lists the time points during the course of an exacerbation. The y axis is the FEV_1 value in litres. No significant difference was detected between stable state and onset of exacerbation, or at different time points during an exacerbation of bronchiectasis. p=0.996

6.5.2 FEV_1 % predicted.

Patients, who had infective exacerbations, had FEV_1 % predicted measured at each visit. The mean (± SD) values for FEV_1 % predicted at these time points are listed in Table 6.2.

Table 6.2: Mean (SD) FEV_1% predicted at different time points during an exacerbation of bronchiectasis.

FEV1 %predicted	Number of patients	Mean	Standard Deviation
Baseline	22	80.32	28.3
Day1	20	84.00	26.7
Day7	21	80.14	31.3
Day14	21	82.14	30.6
Day42	18	83.89	27.6

There was no significant change in FEV 1% predicted measured over the time course of an exacerbation, n=15, p=0.988. Figure 6.2 is an interval plot demonstrating change in the mean FEV_1 % predicted during the course of an exacerbation.

Twenty patients had FEV 1 % predicted measured at baseline and Day 1 [mean (± SD) 83.4(27.3) v 84(26.7)].There was no change in FEV_1 % predicted between stable state and onset of an exacerbation (baseline v Day 1) p= 0.853. There was no difference between FEV_1% predicted between stable state and after the completion of an exacerbation (n=18, baseline v Day 42) p=0.293.

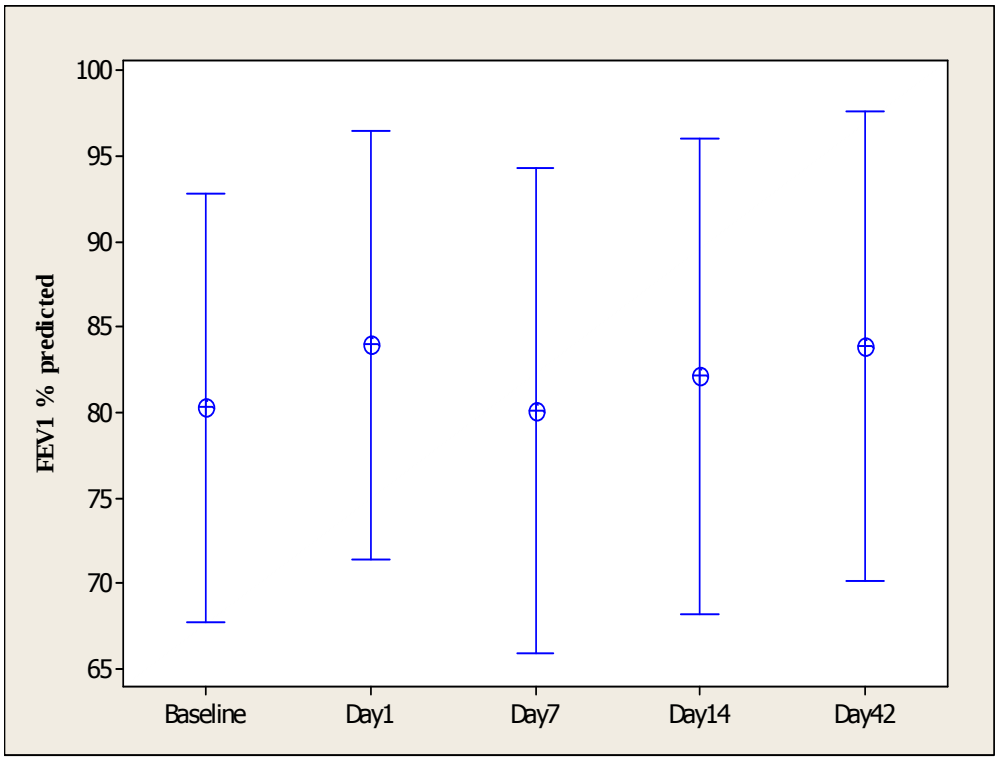

Figure 6.2: Interval Plot of Mean (95% confidence interval) FEV 1% predicted at baseline (stable state), onset of exacerbation (Day 1) and 4 weeks later (Day 42), in patients with an acute exacerbation of bronchiectasis. The x-axis lists the time points during the course of an exacerbation. The y axis is the FEV_1 % predicted value. No significant difference was detected between stable state and onset of exacerbation or at different time points during an exacerbation of bronchiectasis. p=0.988.

6.5.3 FVC Actual

Patients, who had infective exacerbations, had FVC measured at each visit. The mean (± SD) values for FVC at these time points are listed in Table 6.3.

Table 6.3: Mean (SD) FVC at different time points during an exacerbation of bronchiectasis.

FVC actual	Number of patients	Mean (L)	Standard Deviation
Baseline	22	3.07	0.69
Day1	19	2.86	0.77
Day7	22	2.81	0.77
Day14	21	2.96	0.70
Day42	17	3.05	0.76

There was no significant change in FVC actual measured over the time course of an exacerbation, n=14, p=0.749. Figure 6.3 is an interval plot demonstrating change in the mean actual FVC values during the course of an exacerbation.

Nineteen patients had FVC measured at baseline and Day 1 [217].There was a significant fall in the measured FVC actual (L) between stable state and onset of an exacerbation (baseline v Day 1) p= 0.032. This fall in FVC recovered to baseline values only on completion of treatment with antibiotics (Day 14) n=19, p=0.200. There was no difference in the FVC actual measured in stable state and on recovery from an exacerbation (Baseline v Day 42) n=17, p=0.116.

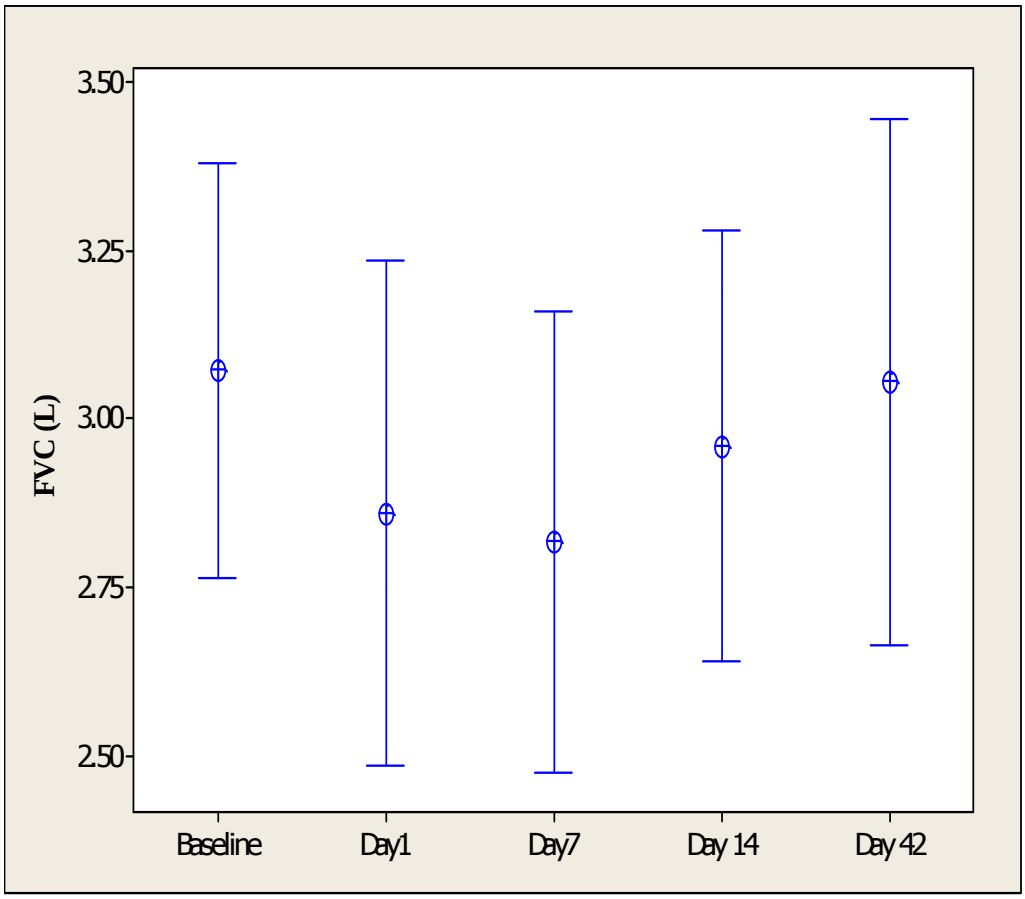

Figure 6.3: Interval Plot of Mean (95% confidence interval) FVC actual at baseline (stable state), onset of exacerbation (Day 1) and 4 weeks later (Day 42), in patients with an acute exacerbation of bronchiectasis. The x-axis lists the time points during the course of an exacerbation. The y axis is the FVC actual value in litres. No significant difference was detected between stable state and onset of exacerbation or at different time points during an exacerbation of bronchiectasis, p=0.749.

6.5.4 FVC % predicted

In sixty three patients, at baseline, the FVC % predicted was measured as Mean (± SD) 98% (22).

Patients, who had infective exacerbations, had FVC % predicted measured at each visit. The mean (± SD) values for FVC% predicted at these time points are listed in Table 6.4.

Table 6.4: Mean (SD) FVC% predicted at different time points during an exacerbation of bronchiectasis.

FVC % predicted	Number of patients	Mean	Standard Deviation
Baseline	22	105.3	23.9
Day1	19	103.1	25.1
Day7	21	99.1	29.0
Day14	20	100.9	22.9
Day42	17	104.6	25.9

There was no significant change in FVC % predicted measured over the time course of an exacerbation, n=17, p=0.933. Figure 6.4 is an interval plot demonstrating change in the mean FVC % predicted values during the course of an exacerbation. Nineteen patients had FVC% predicted measured at baseline and Day 1 - Mean (± SD) [110(21.8) v 103(25.1)].There was no change in FVC % predicted between stable state and onset of an exacerbation (baseline v Day 1) p= 0.077. There was no difference between FVC % predicted in stable state and on recovery from an exacerbation (n=17, baseline v Day 42) p=0.199.

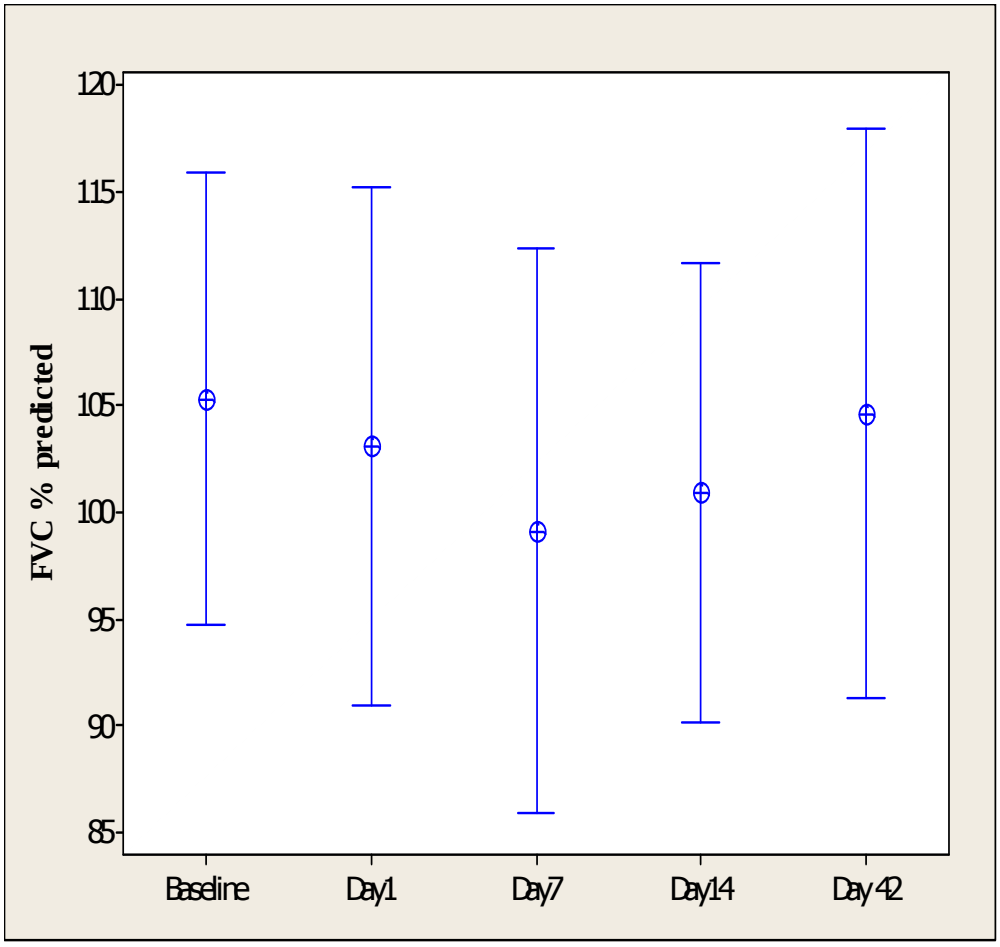

Figure 6.4: Interval Plot of Mean (95% confidence interval) FVC % predicted at baseline (stable state), onset of exacerbation (Day 1) and 4 weeks later (Day 42), in patients with an acute exacerbation of bronchiectasis. The x-axis lists the time points during the course of an exacerbation. The y axis is the FVC % predicted value. No significant difference was detected between stable state and onset of exacerbation or at different time points during an exacerbation of bronchiectasis, p=0.933.

6.5.5 Spirometric indices and chronic colonisation with bacteria.

Chronic bacterial colonisation with *Pseudomonas aeruginosa* was seen in 17 patients. Only three patients were colonised with other organisms [*Haemophilus influenzae* and *Streptococcus pneumoniae*]. There was no difference in the absolute or % predicted values of spirometric indices between groups. Table 6.5 lists the mean (SD) values for all measured spirometric indices.

Table 6.5: Mean (SD) values of spirometric indices in patients chronically colonised with bacteria.

	Number of patients	FEV_1 Absolute Mean (SD)	FEV_1 %predicted Mean (SD)	FVC Absolute Mean (SD)	FVC %predicted Mean (SD)
Colonised With *Pseudomonas aeruginosa*	17	1.67 (0.63)	66 (22)	3.05 (0.24)	96 (21)
Colonised With Other PPM	3	1.86 (0.30)	82 (35)	3.43 (0.45)	116 (24)
p values		0.446	0.532	0.508	0.315

6.6 IOS

IOS indices were significantly correlated to spirometric indices. In stable state bronchiectasis, fifty seven patients completed spirometry and 52 patients had complete data for IOS indices. Although the study planned to measure IOS indices only in stable state bronchiectasis, some measures of IOS indices were taken during an exacerbation in 42 patients –number of patients- Day 1-n=12, Day 7-n=10, Day 14-n=11 and Day 42-9.

Resistance

Resistance increases as FEV_1 decreases (Figure 21). At lower FEV_1 values, R5 shows greater increase per unit decrease in FEV_1. Resistance increases as FEV_1% predicted decreases (Figure 22). R5 is influenced both by the size of the lungs and the degree of airways obstruction. Resistance is significantly correlated (negatively) to FEV_1 in both stable state and during an exacerbation of bronchiectasis (Table 6.5 & 6.6).

Reactance

Reactance increases in magnitude as FEV_1 decreases (Figure 23). At lower FEV_1 values, X5 shows greater increase in magnitude per unit decrease in FEV_1. Reactance increases in magnitude as FEV_1 % predicted decreases (Figure 24), suggesting airways obstruction has a negative impact on lung elastance. X5 is influenced both by the size of the lungs and the degree of airways obstruction. Reactance is significantly correlated (negatively) to FEV_1 in both stable state and during an exacerbation of bronchiectasis (Table 6.6 & 6.7).

Table 6.6: Pearson's correlation between spirometric and IOS indices in stable state bronchiectasis. **Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level.

IOS parameters	FEV_1	FEV_1 %predicted	RV/TLC
R5	-0.550**	-0.283*	0.333**
X5	-0.686**	-0.542**	0.601**

Table 6.7: Pearson's correlation between spirometric and IOS indices during an exacerbation of bronchiectasis. **Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level.

IOS parameters	FEV_1	FEV_1 %predicted
R5	-0.309 *	-0.069
X5	-0.629 **	-0.495*

Figure 6.5 and 6.6 are scatter plots depicting the significant relationship between Resistance at 5Hz (R5) and FEV_1 actual [p=<0.001] and FEV_1 % predicted [p=<0.05] in stable-state bronchiectasis.

Figure 6.7 and 6.8 are scatter plot depicting the significant relationship between Reactance at 5Hz (X5) and FEV1 actual [p=<0.001] and FEV_1 % predicted [p=<0.001] in stable-state bronchiectasis.

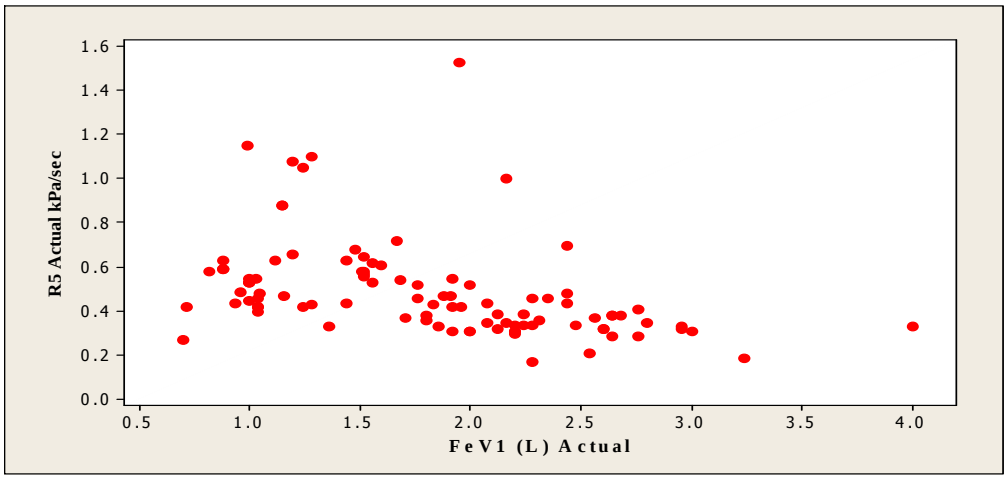

Figure 6.5: Scatter plot depicting significant relationship between Resistance at 5Hz (R5) and FEV_1 actual in stable-state bronchiectasis. The variables on the x-axis are FEV_1 actual values in litres and variables on the y-axis are R5 actual values in kPA/sec. There was a significant correlation between FEV_1 actual and Resistance at 5Hz, p=<0.001.

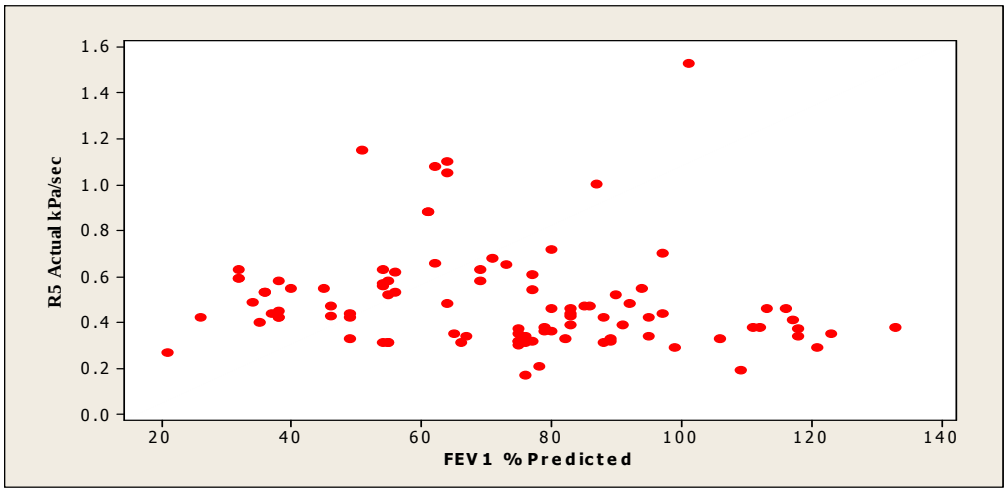

Figure 6.6: Scatter plot depicting significant relationship between Resistance at 5Hz (R5) and FEV_1 %predicted in stable-state bronchiectasis. The variables on the x-axis are FEV_1 %predicted values and variables on the y-axis are R5 actual values in kPA/sec. There was a significant correlation between FEV_1 % predicted and Resistance at 5Hz, (p=<0.05).

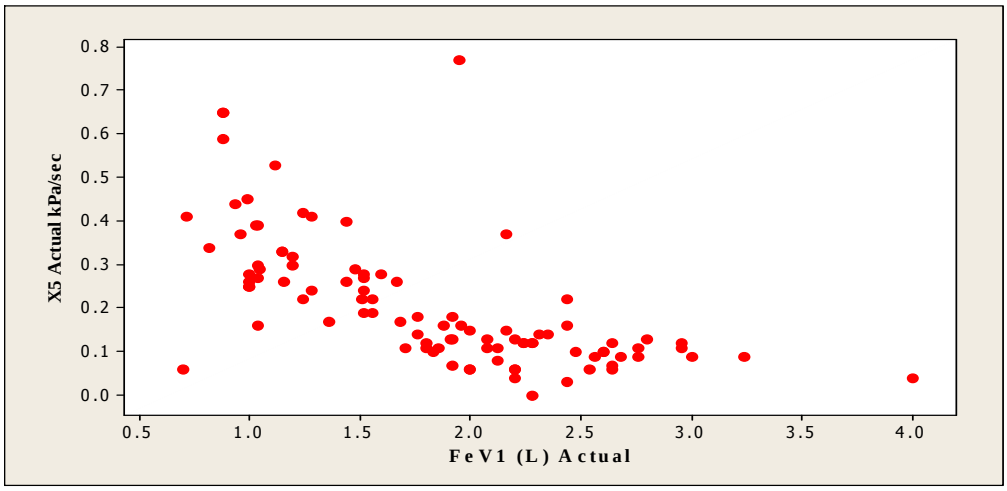

Figure 6.7: Scatter plot depicting significant relationship between Reactance at 5Hz (X5) and FEV_1 actual in stable-state bronchiectasis. The variables on the x-axis are FEV_1 actual values in litres and variables on the y-axis are X5 actual values in kPA/sec. There was a significant correlation between FEV_1 actual values and Reactance at 5Hz, p=<0.001.

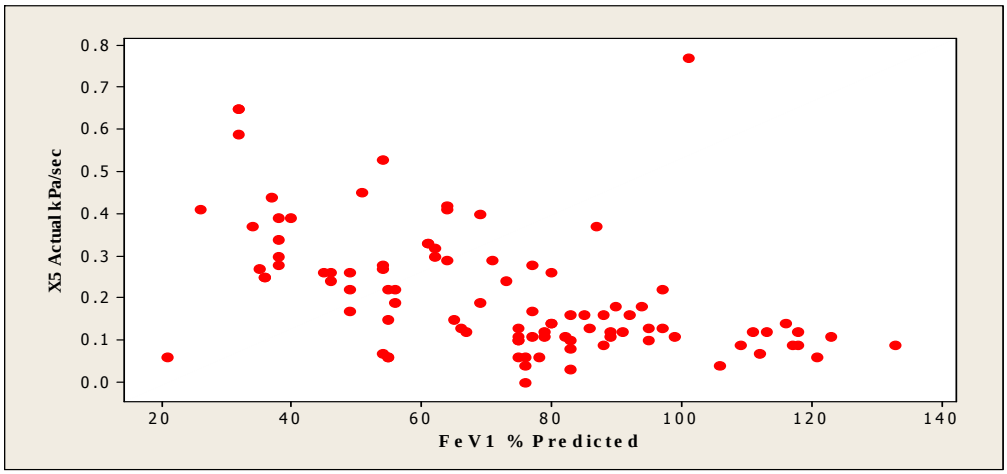

Figure 6.8: Scatter plot depicting significant relationship between Reactance at 5Hz (X5) and FEV_1 %predicted in stable-state bronchiectasis. The variables on the x-axis are FEV_1 %predicted values and variables on the y-axis are X5 actual values in kPA/sec. There was a significant correlation between FEV_1 % predicted and Resistance at 5Hz, Scatter plot depicting significant relationship between Reactance at 5Hz (R5) and FEV_1 %predicted, p=<0.001.

Correlation of gas trapping

A linear relationship exists between R5 and RV/TLC. Total respiratory resistance increases as the degree of gas trapping increases r=0.356, p=0.007. Figure 6.9 demonstrates this linear relationship between R5 and RV/TLC.

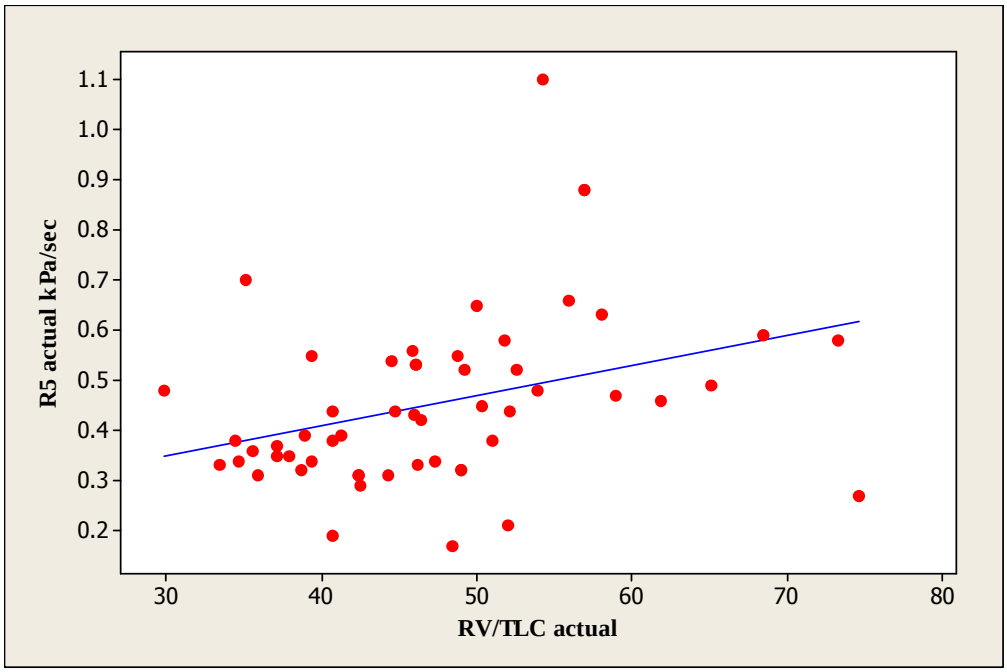

Figure 6.9: Scatter plot depicting the significant linear relationship between Resistance at 5Hz (R5) and gas trapping measured as RV/TLC in stable state bronchiectasis. The x-axis variables are the actual values of RV/TLC, while the y-axis variables are the measures R5 values, r=0.356, p=0.007.

6.7 Discussion

Our aim was to measure change in spirometric indices during exacerbation in bronchiectasis with a view to identifying markers that may help in future therapeutic trials.

In stable state bronchiectasis, the mean FEV_1 in our cohort was 1.9 L [95% predicted]. This indicates a relatively healthier cohort. At the onset of an exacerbation, our cohort had a mean FEV_1 of 1.9 L. Other groups have reported FEV1 actual values between 1.5 and 1.6 L for a similar cohort of patients at the onset of an exacerbation [80, 83]. Similarly the FVC values at the onset of an exacerbation were higher than other studies at 2.86 L. Other reports put this value at 2.35 – 2.45L [80, 83]. We have identified a selection bias in our study. It was felt that subjects only volunteered when they felt they were able to make the many visits that the schedule involved. This may have resulted in a group of patients with milder disease.

Our aim was however to check for change in these measured spirometric values. We were unable to demonstrate any clear change or improvement in the FEV1, FVC. There is no evidence in bronchiectasis to suggest that treatment of acute exacerbations modifies spirometric measures. Pre and post antibiotic spirometry has been convincingly investigated in two studies of adult patients with bronchiectasis. Both studies had a similar cohort of patients to us. Both studies failed to show a statistically significant change in these parameters [80, 83]. It may be assumed that the bronchi in children are possibly more elastic. But a similar paediatric study failed to demonstrate any improvement in spirometry on treatment with antibiotics [218]. These results must however be viewed with caution. As in our study the number of patients in all these other reports were limited to 18 [83], 32 [80] and 30 [218].

Reversibility of FEV_1 has been demonstrated in bronchiectasis. Up to 37% of patients have been noted to a have significant (>20%) bronchodilator induced reversibility in FEV_1 and up to 60% patients can have an improvement in the FEF 25-75 [217]. Peak flow readings at the bedside can increase by 26% after an inhaled bronchodilator. These patients who respond to bronchodilators cannot be identified by clinical or immunological features [219]. An obstructive defect is common in bronchiectasis. A reduced FEV_1/FVC ratio of less than 70% documenting airway obstruction was found in most (54%) patients in a cohort from Texas [5]. The mean FEV_1/FVC ratio in our study was 59%. Other workers report this to vary between 50% [220] and 60% [80].

Forced respiratory manoeuvres remain uncomfortable for our patients. Our aim was to establish a relationship between spirometry and IOS so that we could offer our patients a less distressing procedure that would still help us investigate airways. To this end we were successful. In our cohort, IOS indices significantly correlated to spirometric indices (FEV_1 and FVC). FEV_1 in particular shows a striking relationship. While we primarily studied stable disease, we included some measures from patients with exacerbation. The relationship remained significant. IOS is a useful measurement in patients with stable bronchiectasis. Further study will need to be done to validate its use in acute exacerbations of bronchiectasis.

Bacterial colonisation is thought to affect the FEV1. Patients colonized by *Pseudomonas* have lower values of FEV_1 and FVC than patients not colonized.**,** Comparing patients colonized by *Pseudomonas* against a heterogeneous group of patients colonized by other microorganisms also finds the group colonized with *Pseudomonas* has worse pulmonary function values [109]. The FEV_1 and FVC values were lower in the group colonised with *Pseudomonas aeruginosa*, however this

difference did not reach statistical significance. The number patients in our study were too small for us to interpret this difference accurately.

Patients get breathless during an exacerbation of bronchiectasis; however these clinical symptoms cannot be quantified with spirometric indices. Spirometry is unlikely to be a useful measure as end point in future therapeutic trials. IOS is strongly correlated to spirometry but needs further investigation.

Chapter 7

Exhaled breath condensate in bronchiectasis in the COBEX study.

7.1 Introduction

7.1.1 Exhaled breath condensate

"A stuck tuning slide is one of the hazards of old flutes. The nasty organic compounds in your breath condensate continue working on the metals your slide is made from and effective weld them together". Unknown

Flautists have known for a long time that there are "nasty" compounds in our breath that work on metals. Exhaled breath in the gaseous phase contains volatile substances that we wished to investigate.

7.1.2 pH of exhaled breath condensate

Airway pH homeostasis is maintained by a balance of different buffer systems and the production and release of acids and bases in the airways [142]. This airway pH homeostatic process is not completely understood**.** EBC pH is determined by volatile and non-volatile components [221-226]. In health, exhaled breath condensate pH is slightly alkaline [225]. The pH of the airway has been reported to be in the range of 7–8 [225, 227, 228].

Altering the pH of the airway environment is known to affect airway function. Nebulised citric or acetic acids are used to trigger bronchoconstriction and cough for testing anti-tussive agents. Chlorine gas is thought to cause wheezing and coughing in substantial part because of the rapid formation of hydrochloric and hypochlorous acids upon contact with the airway lining fluid [225, 226].

Neutrophils are an important part of the natural defences against acute bacterial infections. In healthy state there is rapid clearing of airway neutrophils. In bronchiectasis this inflammation persists and causes release of tissue damaging neutrophil products such as neutrophil elastase and myeloperoxidase. Reduced pH values in EBC have been associated with neutrophilic inflammation [229]. Carbon dioxide (CO2) is the major volatile component of EBC. In the aqueous environment CO2 forms H^+ and $HCO3^-$ and profoundly affects the pH of dilute solutions. An acidic pH has been shown to have detrimental effect on the airway defences. Reduction in ciliary beat, enhancement of bacterial binding to mucous, and reduction of bactericidal effect of antibiotics are some of these effects [230]. Mild acidification (below pH 6.5) increases mucous viscosity, converting it from sol to gel and may cause mucous plugging [231, 232] An acidic environment has also shown to increase the adherence of *Streptococcus pneumoniae* [233]. Exposure to acidic solutions causes action potential discharge in Aδ fibres and C fibres of airway afferent nerves in guinea pigs both of which mediate the cough reflex [48].

It has also been suggested that mild airway acidification may be a subtle and innate host defence mechanism, one which takes advantage of the weak endogenous acids to defend the airway against airborne pathogens. Airway acidosis may have antimicrobial effects mediated through protonation reactions involving reactive nitrogen and oxygen species. Nitrite acidification has been proposed as a mammalian host defence

mechanism [234]. The abundant NO_2 of the airway is present as bacteriotoxic HNO_2 in relevant quantities only when the pH is low. *Mycobacterium tuberculosis* produces a gene product specifically protecting against the cidal effects of HNO_2 [235]. Some of the toxicity of HNO_2 occurs because of its reactive decomposition to NO, which is known to inhibit mycobacterial growth [236] for females and males [225].

It is this diversity of EBC itself that has prevented it from achieving clinical applicability yet [142]. None of the biomarkers in EBC has been validated sufficiently for clinical use. Measurement of pH of EBC has been reported in respiratory diseases including asthma, chronic obstructive airway disease, bronchiectasis and cystic fibrosis. There are no markers of dilution either yet.

Age of subject does not seem to affect exhaled breath condensate pH [225]. There is conflicting evidence with regards to smoking and pH of EBC. Some authors claim an effect and others deny it [133, 225]. Collection of EBC can be affected by diurnal variation [133]. Evaluation of the reproducibility of EBC pH measurements from seven consecutive mornings has revealed a mean coefficient of variation of 4.5% (full intra subject range 0.9 to 20%). The mean intraday coefficient of variation in the same cohort has been reported as 3.5% (range 0.6 to 23%) [142].

7.1.3 Collection of exhaled breath condensate

Most lung diseases involve chronic inflammatory changes and oxidative stress. Peripheral blood markers are unlikely to be adequate as the important mediator and cellular responses occur locally in the airways. Means to measure this inflammation in the past has always been invasive and not easily repeatable. Methods to assess this airway inflammation has included the use of bronchoscopy and induced sputum for

analysis. The use of induced sputum may be less invasive but involves inhalation of hypertonic saline that may induce coughing and bronchoconstriction. The technique itself cannot be repeated for 24 hours. A non-invasive technique that is easily repeated, useful in children and possible in patients with severe disease who may be intubated has led to the increase use of EBC to study inflammation in the airways [224]. While it is currently used as a research tool, further studies could make it more useful as a home device to study response to treatment and to monitor improvement.

In healthy subjects, the pH of EBC immediately tested tends to be unstable. To enhance the stability of the readings, de-aeration (gas standardisation) with a CO_2 free gas (such as argon, nitrogen oxygen or another CO_2-free gas) can be performed. During de-aeration, the pH gradually rises to a point when stable reading can be obtained. In healthy subjects, EBC pH after de-aeration has a mean pH of 7.7, with a range of normal considered by the investigators to be 7.4–8.8. These values are obtained from orally collected EBC samples. From intubated subjects without lung disease, the mean pH of de-aerated samples is likewise 7.7 with no difference from matched oral collections [142].

The collection time in our study was 10 minutes. This time period is recommended so that a typical yield of 1–2 mL is obtained. Subjects usually tolerate this period of sampling without fatigue. Direct comparison for pH levels, shows no effect of changes in collection time between 3–20 min on EBC pH in healthy subjects [142, 237].

7.1.4 Markers of inflammation in exhaled breath condensate and bronchiectasis

Condensate measurement reflects different markers and molecules derived from the mouth (oral cavity and oropharynx), tracheobronchial system and alveoli. It is assumed that the airway surface liquid becomes aerosolised during turbulent airflow, so that the contents of the condensate reflects the composition of the airways surface liquid, although large molecules may not aerosolize as well as small soluble molecules [238]. A strong correlation exists between levels of CO2 and O2 in exhaled fluid and exhaled breath suggesting that aerosol particles exhaled in human breath reflect the composition of bronchoalveolar extracellular lining fluid [239].

There are also many potential chemo attractants including Interleukin (IL)-8 and leukotriene (LT) B4 in EBC. The final common pathway is oxidative stress which results in airway damage. This can be quantified using reactive oxygen species (Hydrogen peroxide) or Isoprostanes (IPs) [240]. Most inflammatory markers identified in EBC have been described in CF with limited data in non-CF bronchiectasis. In the respiratory system H_2O_2 may be released both from inflammatory and structural cells. These include neutrophils, eosinophils, macrophages and epithelial cells. EBC H_2O_2 concentrations are elevated in bronchiectasis with a significant inverse correlation between lung function and EBC H_2O_2 levels [146].

We hypothesised that the inflammatory burden increases during an exacerbation and this would lead to a fall in pH of EBC. This could be used to assess the onset of an exacerbation and also outcome of treatment. As the technique of EBC collection is simple it could be modified for monitoring and use in the home environment in patients with bronchiectasis.

7.2 Aims

We hypothesised that

a) Airways in non CF Bronchiectasis would be acidified

b) This may be worsened during an infective exacerbation and

c) This could be used to confirm onset of an exacerbation and outcome on treatment with antibiotic therapy.

We wished to investigate exhaled breath condensate (EBC) pH as a biomarker of infection and or inflammation in these patients

7.3 Methods

7.3.1 Subjects

Fifty eight adult patients with well characterised non CF bronchiectasis [18] (34 women) of mean (SD) age 64(11.4) attending the Lung Defence centre at Papworth Hospital Cambridge were recruited. An exacerbation was defined as a patient having at least two of the following symptoms: increased cough, increased volume of sputum, increased dyspnoea or wheezing and at least one of the following: fever>/= 38^{o}C or malaise. Fourteen healthy volunteers (seven men) of mean (SD) age 63(5.9) acted as controls.

7.3.2 Study protocol

All patients were initially seen when in stable state (no infective exacerbation in the preceding 4 weeks). Twenty two of these patients were followed through an exacerbation as described in Chapter 2. EBC was always obtained prior to spirometry.

Exhaled breath condensate collection was performed using the RTube^{TM} EBC collection system (Respiratory Research, Inc, Charlottesville, Virginia, USA). As per the American Thoracic society/European Respiratory Society task force recommendations [142], subjects were asked to breath at tidal volumes for a period of ten minutes, wearing a nose clip. A typical yield of 1-2mls of condensate was obtained. pH was measured immediately using a portable pH meter (pH boy, Camlab pocket pH meter, UK). The aluminium sleeves were stored in a freezer (– 4 to -17^{o}C). The aluminium plunger never comes into contact with the EBC sample. The collected condensate has been stored at -80 ^{o}c. The plunger was cleaned between patients.

Sputum appearance was recorded as described in Chapter 8. Spirometry was recorded as described in Chapter 6

7.3.3 Statistical analysis

Data are expressed as mean ± Standard deviation (SD). Change over time was measured using the global test ANNOVA. Comparison between groups was made using the unpaired t test. Correlation between groups was determined by nonparametric Spearman correlation analysis. Significance was defined as a value of $p < 0.05$.

7.4 Results

7.4.1 Patient characteristics

Patient characteristics for our cohort, subjects followed through an exacerbation and the healthy volunteers are listed in Tables 7.1. This has previously been described in Chapter 3. It is included here for the ease of reference.

Table 7.1: Patient characteristics for the exhaled breath condensate analysis arm of COBEX study.

	Full cohort n=58	Exacerbators n=22	Healthy volunteers n=14
Mean age (+/-SD)	64years (11.4)	63years (8.5)	65years (5.9)
Sex (M:F)	24:34	5:17	7:7
Smoking history			
Ex-smokers	26	11	9
Non-smokers	31	11	4
Current smoker	1		1
Mean FEV_1(±SD)	1.9L(0.71)	1.9L(0.61)	2.3L(0.82)
Mean FEV_1 % predicted (±SD)	75%(25)	81%(28.2)	100%(27.1)

7.4.2 Stable state bronchiectasis

The pH of EBC was significantly lower in our patient cohort than the healthy control group [Mean (SD) n=58, 6.26(0.41) v n=14, 6.75(0.17); p= 0.000]. Figure 7.1 is an interval plot demonstrating the difference in the mean pH of EBC between patients with bronchiectasis in stable state and healthy volunteers.

When pH of EBC fluid was compared to sputum appearance alone there was a trend with the purulent sputum being more acidic than the mucopurulent and mucoid sputum {n=56, 6.06(0.40), 6.28(0.43), 6.45(0.28)} this was not significant p= 0.180. Figure 7.2 is a box plot demonstrating the differences between pH of EBC at different time points during the course of an exacerbation.

There was no correlation between EBC pH and lung function (% predicted FEV_1) {r= 0.108, p=0.430}, CRP (r=0.11, p=0.434, n=53) or ESR (r=0.052, p= 0.721, n=49).

There was a significant correlation between EBC pH and the total bacterial load (r= -0.471, p=0.03, n=21). Figure 7.3 is a scatter plot demonstrating the significant relationship between pH of EBC and the total bacterial count in sputum.

There was no difference in the EBC pH between patients, in whom *Pseudomonas aeruginosa* was cultured in stable state as compared to those without (13:43), {6.19(0.39) v 6.29(0.43), p=0.421}. There was also no difference in EBC pH between patients in whose sputa a PPM was isolated as compared to those in whom it wasn't isolated {n=27:29 6.29(0.43) v 6.29(0.39), p=0.684}

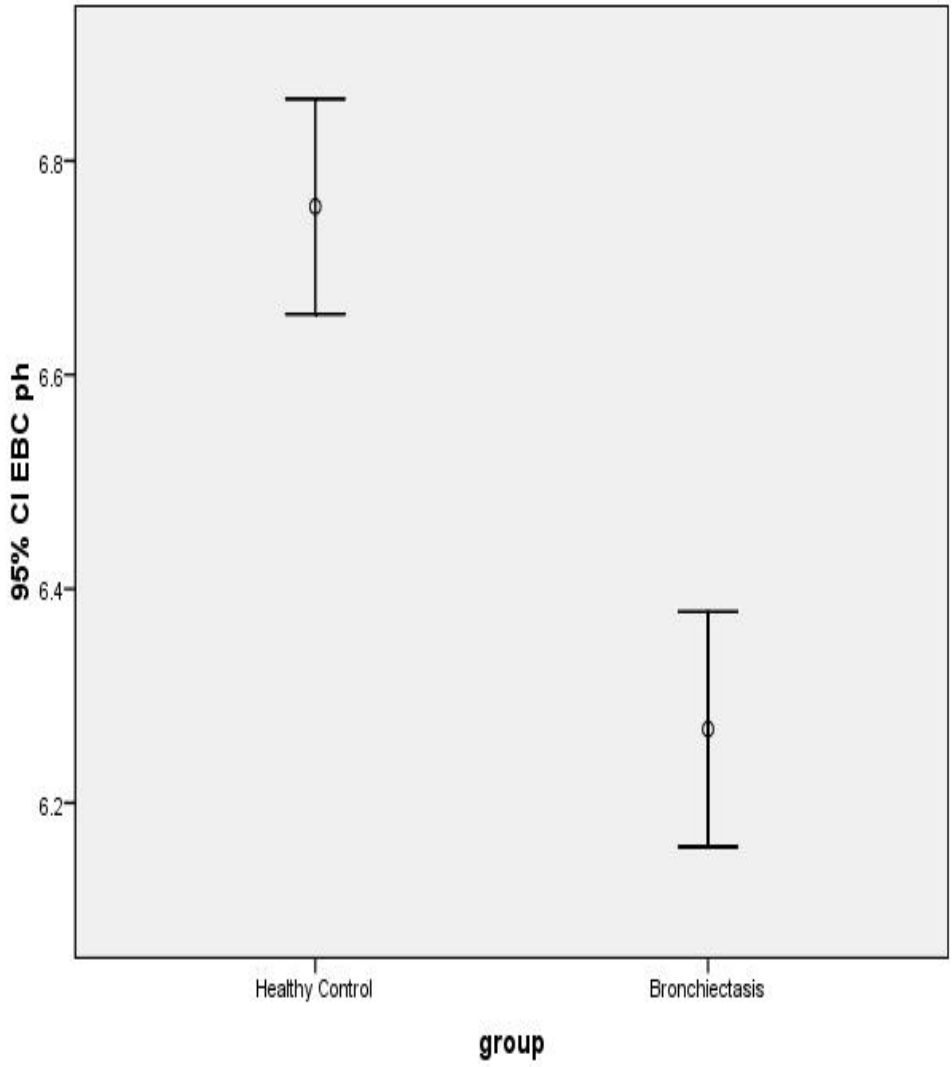

Figure 7.1: Interval plot of Mean (95% confidence interval) EBC pH in stable state bronchiectasis v healthy volunteers. The x-axis lists the group of patients i.e. with Bronchiectasis or healthy volunteers and the y-axis is pH values of exhaled breath condensate. There was a statistical significant difference in the two groups, p= 0.000.

Sputum appearance

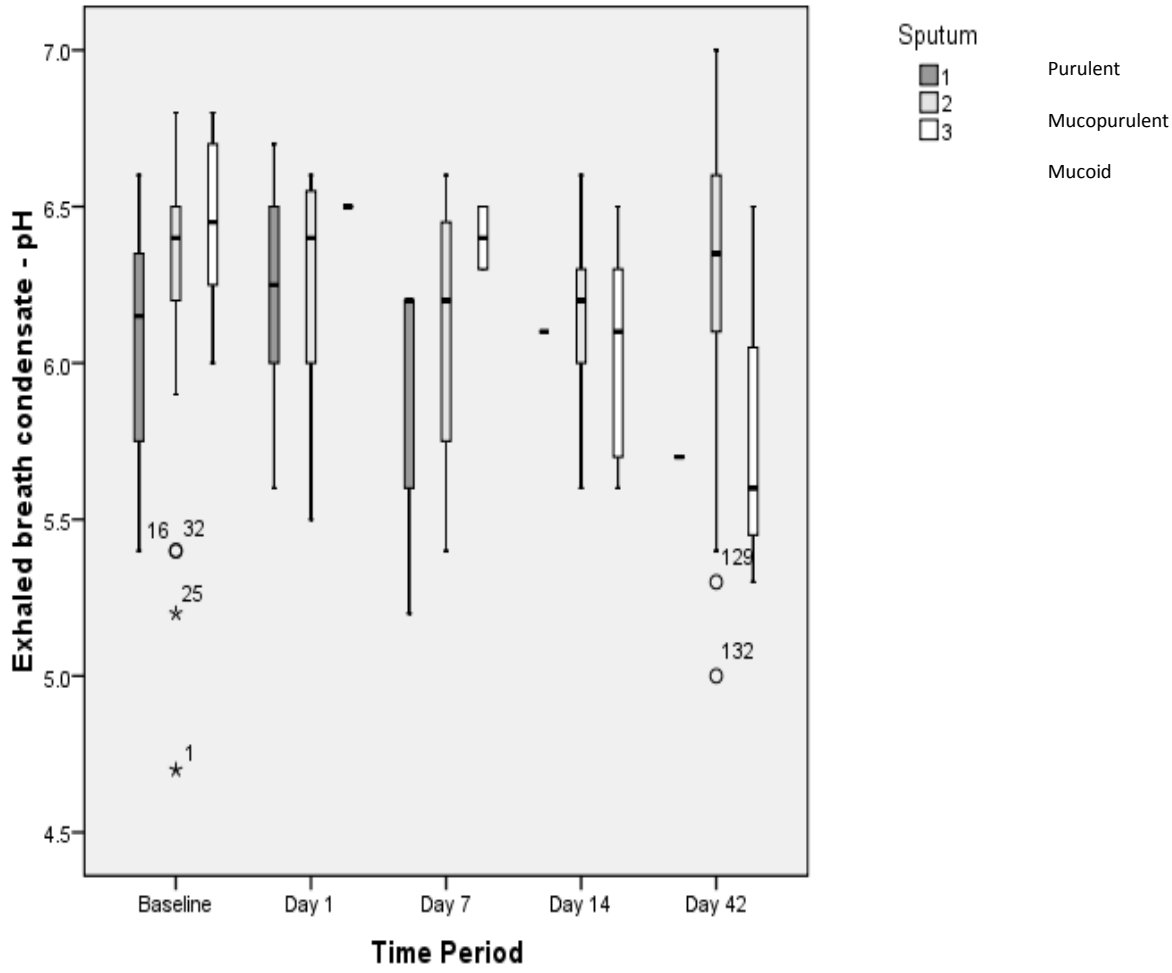

Figure 7.2: Interval plot of EBC pH when compared according to sputum appearance. Sputum was classified on appearance as purulent, mucopurulent, and mucoid. The x-axis lists the time point at which the pH of EBC was measured i.e. baseline or stable state, Day 1, Day 7, Day 14 of the exacerbation, or Day 42 after recovery from exacerbation. Outliers are marked with an o and extreme outliers are marked with an asterisk. Numbers accompanying these are patient identification numbers for the study. There was no significant difference in pH of EBC when compared to appearance alone, p=0.180.

7.4.3 Acute exacerbation of bronchiectasis

The pH of EBC did not significantly change between baseline and Day 1(onset of exacerbation, prior to antibiotic treatment), {n=22, mean (SD) 6.21(0.45) v 6.28(0.35), p=0.595}. The pH of EBC did not alter significantly when followed longitudinally through an exacerbation {Day 14, at the end of treatment, 6.1(0.29)] or Day 42, after recovery from the exacerbation 6.15(0.56)} p=0.525. There was no significant change between EBC pH at baseline and Day 42 (n= 20, p=0.079). Figure 7.4 is an interval plot demonstrating change in mean pH of EBC from baseline, during the course of an exacerbation and on recovery from an exacerbation.

At Day 1, there was no correlation between CRP (r=-0.205, p=0.387, n=20) or ESR (r=0.066, p= 0.802, n=17).There was no significant correlation between EBC pH and lung function (% predicted FEV_1) {r= 0.44, p=0.059, n=19}. At day 7, there was no correlation between EBC pH and lung function (% predicted FEV_1) {r= 0.098, p=0.673, n=21}, CRP (r=0.156, p=0.511, n=20) or ESR (r=-0.242, p= 0.318, n=19). At Day 14, there was no correlation between EBC pH and lung function (% predicted FEV_1) {r= 0.374, p=0.104, n=20}, CRP (r= -0.168, p=0.479, n=20) Figure 16 or ESR (r=0.066, p= 0.807, n=16). At day 42, there was no correlation between EBC pH and lung function (% predicted FEV_1) {r= 0.280, p=0.261, n=18}, CRP (r=-0.084, p=0.749, n=17) or ESR (r=0.026, p= 0.931, n=14). Table 7.2 lists the Pearson's correlation coefficient between EBC pH, ESR, CRP and % predicted FEV_1.

There was no significant correlation between EBC pH and the total bacterial load during and after the exacerbation. {Day 1(r= 0.231, p=0.30, n=21), Day 14(r= 0.160, p=0.318, n=21). Day 42(r= -0.336, p=0.18, n=17)}.

Table 7.2: Correlation between EBC pH and markers of inflammation- ESR and CRP and % predicted FEV_1. Pearson's correlation coefficient 'r' and p values for measured parameters and pH of EBC at baseline and during the course of an exacerbation.

	Baseline	Day 1	Day 7	Day 14	Day 42
EBC pH and % predicted FEV_1	r= 0.108 p=0.430 n=49	r= 0.44 p=0.059 n=19	r= 0.098 p=0.673 n=21	r= 0.374 p=0.104 n=20	r= 0.280 p=0.261 n=18
EBC pH and ESR	r=0.052 p= 0.721 n=49	r=0.066 p= 0.802 n=17	r=-0.242 p= 0.318 n=19	r=0.066 p= 0.807 n=16	r=0.026, p= 0.931 n=14
EBC pH and CRP	r=0.11 p=0.434 n=53	r=-0.205 p=0.387 n=20	r=0.156 p=0.511 n=20	r= -0.168 p=0.479 n=20	r=-0.084 p=0.749 n=17

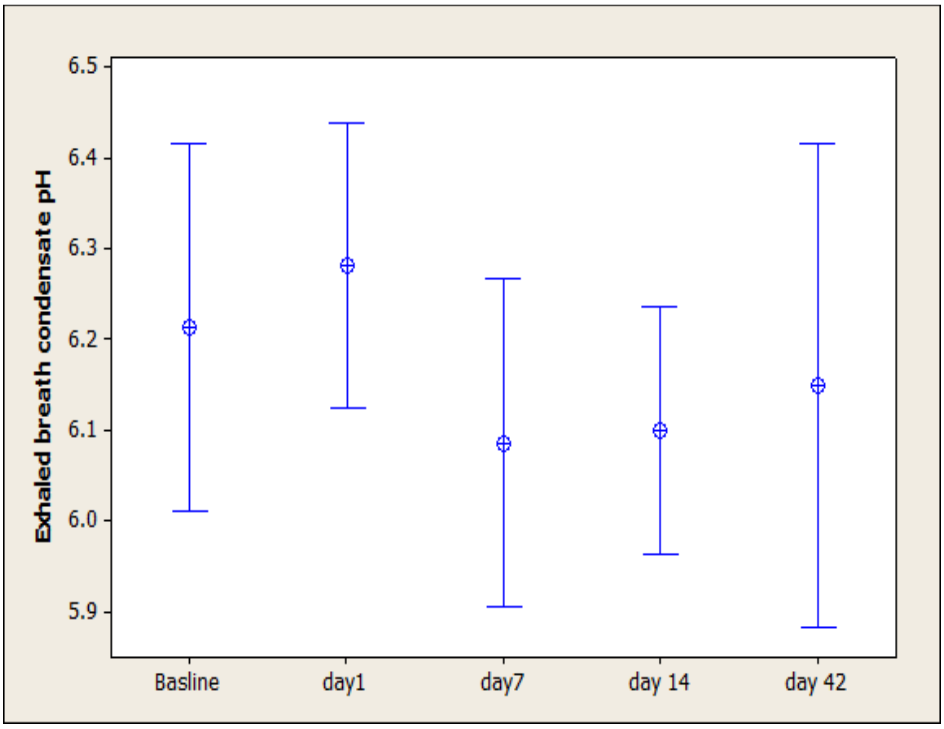

Figure 7.3: Interval plot depicting Mean (95% CI) EBC pH at baseline and during the course of an exacerbation. Variables on the x-axis are the time points during the study when EBC pH was measured i.e. baseline, day 1, day 7, day 14 and day 42. Variables on the y-axis are the EBC pH values. Global test ANNOVA has been used to calculate change over time. There was no significant change in the EBC pH during the course of an exacerbation in the COBEX study, p = 0.525

7.5 Discussion

Exhaled breath condensate pH is considered to reflect the acid-base balance within the airways [224]. The contributors to this pH balance are yet to be explained. The pH is currently considered the most robust variable of EBC [225].

Our study was designed to evaluate EBC pH in stable state bronchiectasis, to compare it to healthy volunteers and study its variation during the course of an exacerbation of Bronchiectasis.

The EBC pH in our cohort was significantly lower than in the healthy volunteer group. This confirms our hypothesis that the pH of EBC in patients with bronchiectasis is more acidic than normal subjects. In bronchiectasis caused by cystic fibrosis [12], the pH is significantly lower (5.67) [241] than if CF is excluded 7.19 (0.08) [145]. The values of pH of EBC in our study lie between published values and may be explained by difference in the methodology. The lower pH values that are observed in bronchiectasis are attributed to the neutrophilic inflammation. This in turn is due to the activity of the enzyme neutrophil myeloperoxidase that catalyzes the reaction between hydrogen peroxide and a chloride to form hypochlorous acid. This acid is highly volatile and might be responsible for the acidification of exhaled breath condensate [145].

The mean (SD) EBC pH {6.75(0.17)} for healthy volunteers in our cohort was lower than some reports and comparable to others. The mean pH in healthy subjects has been reported variously as 8.26 [242], 7.65 [144], 7.57 [145] and 6.15 [241]. This may be explained by the different methodology employed to collect and measure the pH. While the former two workers [144, 242] used frozen and defrosted EBC samples and the latter [145] used neat samples, all three groups performed argon de aeration prior to

measurement of pH. Tate et al measured pH from neat EBC and that may explain a comparable value [241]. We acknowledge that our method of pH measurement does not confirm to the task force recommendations [142].

We expected to find a fall in pH with the onset of an exacerbation however found no significant change. The pH of EBC is almost a log order lower in exacerbations of Cystic Fibrosis [241]. The small size of our cohort may have been inadequate to measure a significant change but a significant change has been detected with as few as eleven patients with CF [241]. Other components that contribute to this pH balance require further study.

Exhaled breath analysis has proven useful in monitoring therapy with corticosteroids in asthma. The breath condensate in acute asthma is acidified and this normalises to control values on treatment [144]. The pH in our cohort however did not rise on treatment with antibiotics and there was no significant difference between baseline and recovery from exacerbation. The eosinophilic inflammation in asthma is treated easily with corticosteroids but the neutrophilic inflammation implicated in bronchiectasis may be multifactorial and not affected by antibiotics alone.

Patients with bronchiectasis that are chronically colonized by *P. aeruginosa* have a significantly lower pH (6.96, 95% CI, 6.88–7.05) [145]. This was not our finding despite having a large number of patients with steady state bronchiectasis. It has also been shown *in vivo* that leukocyte metabolism in the presence of live bacteria could produce a fall in pleural fluid pH. This may contribute to the low pH observed in patients with bronchiectasis chronically colonized by bacteria [145]. However there was no difference in the EBC pH between patients whose sputa isolated Streptococcus pneumoniae and those that did not {6.27(0.71) ± 6.26(0.37), p=0.98}. We do not think

that the colonising pathogen contributes significantly to the EBC pH. We found no correlation between EBC pH and % predicted FEV_1. Other workers have found a significant relationship between the FEV_1 % predicted and pH of EBC but in a smaller cohort of patients [145].

We acknowledge that the number of patients in this study is small. We have not adhered to the task force recommendation on pH measurement methodology either. The pH in our study was measured immediately following collection of EBC. Our aim was to be enable patients to use this equipment at home to confirm the onset of an exacerbation. However as our samples are preserved we hope to reanalyse with deaeration and should be able to then compare results. We also did not measure intra subject variability which has previously been reported as 4.5% [225].

pH of exhaled breath condensate is more acidic in patients with stable bronchiectasis than in healthy subjects. pH of EBC in our limited cohort failed to confirm the onset of an exacerbation or recovery in a significant way. There may be other markers of inflammation or oxidative stress that may be more significant in bronchiectasis and will need to be investigated. New and more sensitive assays will allow detection of these other markers and help produce a specific finger print for bronchiectasis.

Chapter 8

The microbiology of stable state and exacerbations in bronchiectasis –the COBEX study

8.1 Introduction

Microorganisms with potential pathogenicity (PPM) - *Haemophilus influenzae, Staphylococcus aureus, Streptococcus pneumoniae, Moraxella catarrhalis and Pseudomonas aeruginosa* - lead to an intense inflammatory response within the bronchi in patients with bronchiectasis [4, 17]. Exacerbations are common and continue to contribute to significant morbidity and poorer HR-QoL [83, 102]. As there is persistent bacterial infection of airways, it would be logical to identify markers of inflammation and endpoints for therapeutic trials from sputa.

8.1.1 Use of qualitative analysis of sputum as an end point in therapeutic trials in bronchiectasis.

Pathogens isolated in sputa include *Haemophilus influenzae, Pseudomonas aeruginosa Staphylococcus aureus, Moraxella catarrhalis* and *Streptococcus pneumoniae* [5, 18, 127]. *Pseudomonas aeruginosa* is considered a marker of severity and is associated with compromised HR-QoL [62]. Its presence is associated with poor lung function and extensive disease on HRCT [17]. As HR-QoL is compromised to begin with, deterioration may not be easily measured. Microbial clearance in sputa may or

may not always be achieved. Both *Pseudomonas aeruginosa* and *Haemophilus influenzae* are capable of forming biofilms [17]. Eradication therefore can be difficult. However microbial clearance can be achieved in a majority of patients [80].

8.1.2 Quantitative total bacterial counts as markers of infection.

Quantitative bacterial load has been previously used as marker of therapeutic efficacy. In a study to assess efficacy of inhaled versus intravenous gentamicin, a 6-10 fold reduction in colony counts has been reported [158]. Longitudinal quantitative microbial data is absent in bronchiectasis. It is unknown if quantitative data correlates to other proven parameters such as HR-QoL markers and spirometric indices. It is time consuming and remains a research tool at the current time.

More useful are markers that involve patients' cooperation and can be measured in the home environment. Sputum volume and appearance are such tools.

8.1.3 Twenty-four hour sputum volume as a marker of infection

Twenty four hour sputum has been used in studies to assess the outcome of treatment in exacerbations of bronchiectasis. An improvement in sputum volume on treatment with antibiotics, during an exacerbation has been demonstrated. This alone would validate it for future use in therapeutic interventions. However a baseline reference volume from stable state was not included [80]. A standardised approach to sputum volume collection for future studies would also be useful. Most workers have used single day collections. A mean volume from a three day sputum collection may give a more accurate measurement [46, 71]. Presence of a PPM is known to affect this

volume. Patients with *Pseudomonas aeruginosa* produce more sputum than those with other or no pathogens in their sputa. [17]. A potential limitation of 24 hour sputum volume as an outcome measure is the reliance on patient compliance for collection [243]. The volume of sputum produced remains variable. While some patients are daily producers of copious amounts others less so and some only do so during an infection.

8.1.4 Sputum appearance as a marker of infection

Sputum appearance and colour can predict bacterial colonisation accurately [159]. A standardised approach to sputum description would be useful. Currently sputum description can be scored between 0 and 10 as follows: 1-absence of, 2-completely transparent, 3-almost transparent, 4-translucent but colourless, 5-opaque and 6-milky white, 7-grey, 8-pale green, 9-moderately green and 10-dark green sputum or is dependent on the investigator [71]. We have simply classified sputum as mucoid, purulent and mucopurulent.

Sputum appearance would be an easy tool for bedside testing. A significant correlation has already been established between the doctor's and the patient's interpretation of sputum appearance [159].

8.1.5 Non-tuberculous mycobacterial infection in bronchiectasis

It is important to screen all patients with bronchiectasis at referral for NTM infection and thereafter if there is an unexplained deterioration unresponsive to usual therapy [4]. It is also known that patients with bronchiectasis and non-tuberculous

mycobacterial disease have a higher prevalence of coexisting *Aspergillus*-related lung disease. Outcome in such cases is poor and therefore it is important to investigate frequent exacerbators for both not tuberculous mycobacterial disease and *Aspergillus* related lung disease [161].

8.1.6 Obtaining sputum samples for analysis

Finally obtaining the sample of sputum for analysis is crucial. Spontaneously expectorated samples are easy to obtain and cause the patient least trouble. Induced sputum and bronchoscopic lavage may be alternatives in adults who do not expectorate. Oropharyngeal swabs have a high predictive value in the paediatric population with cystic fibrosis [148]. For this study only spontaneously expectorated samples were used.

It has been hypothesised that the inflammation within the bronchi is increased during an exacerbation [31]. We have sought to measure this increase with a view to using it as clinical endpoint in future therapeutic trials.

8.2 Aims

Our aim was to study the bacterial profile of our patients with bronchiectasis in stable state and during the course of an exacerbation.

We wished to investigate the following:

In stable state

- The prevalence of bacterial colonisation in our patients.
- The correlation between serum inflammatory markers and total bacterial count in sputa.
- The relationship between antibiotic prophylaxis and prevalence of PPM in the study cohort.

During an acute exacerbation

- Change in sputum appearance [alone]
- Change in 24 hour sputum weight
- Change in bacterial profile of sputa.
- Change in total bacterial count of sputa

We hoped to identify markers of infection that respond to treatment and could be used as valid end points in future therapeutic trials.

8.3 Materials and methods

We prospectively studied 58 patients with bronchiectasis in a stable clinical state. Fifty five of these patients were able to provide samples of sputa. All patients had well characterised non CF Bronchiectasis.[18] We followed 22 of these patients through an exacerbation. The patients were studied at

Baseline - No exacerbation in the preceding 4 weeks

Day 1 - of an exacerbation (not having commenced antibiotics)

Day 7 - during treatment with antibiotics

Day 14 - on completion of treatment with antibiotics

Day 42 - recovery from exacerbation

8.3.1 Specimens

Fresh sputum samples were collected for analysis at each visit. We aimed to process the sputum within two hours of expectoration. In addition patients were advised to collect all sputum expectorated over a 24 hours period. At Day 1 of their exacerbation they were asked to collect the 24 hour sputum following the study visit. This sample was weighed and the result recorded. The 24 hour sputum sample provided at the screening visit was also used to check for presence of non tuberculous mycobacterium (NTM). For the purpose of this study it has been assumed that sputum weight is equal to volume i.e. 1g=1ml. All samples were collected in sterile containers. Samples at Day 1 were collected prior to antibiotic use.

8.3.2 Sputum analysis

Sputum was classified as purulent, mucopurulent and mucoid based on appearance alone. The specimen was then divided into three parts – Two samples were a hundred microlitre aliquot each (Samples A&B) and the third was a three hundred microlitre aliquot (Sample C). Analysis of sample is described in Appendix iv (Sample B).

8.3.3 Antibiotic treatment advice

Antibiotics chosen to treat an exacerbation were usually based on previous sputum microbiology history. All patients received 14 days of antibiotic treatment. When given intravenously, two agents were used. Oral antibiotics used included Ciprofloxacin, Clarithromycin, Oofloxacin, Trimethoprim, Doxycycline, Moxifloxacin, Flucloxacillin and Septrin. Intravenous antibiotics used were Tobramycin, Meropenem, Ceftazidime and Aztreonam. One patient received nebulised antibiotic used was Tobramycin. If patients were on long term oral Azithromycin or on nebulised Colomycin, they were advised to remain on these antibiotics during the exacerbation

8.3.4 Statistical analysis

Data is expressed as Mean and standard error of mean or Mean and Standard deviation as appropriate. Change over time has been calculated using a global test [Repeated measures ANOVA] . Paired t-test has been used to assess difference between values when required. Chi squared test has been used to analyse differences in tabulated data. All bacterial counts are expressed $\log_{10}$ unless otherwise specified. Results were considered significant when p value was less than 0.05.

8.4 Results

Fifty seven patients were studied in stable state. Twenty two of these patients were followed through an acute exacerbation. Number of samples of sputa analysed varies between different sections described below. The reasons for this is - i) specimens obtained were not always adequate in quantity; ii) patients did not expectorate sputum within two hours of the clinic visit; iii) patients did not attend a visit.

8.4.1 Sputum appearance

In stable state bronchiectasis

Appearance is described in 56 sputum specimens. Most patients had mucopurulent sputum when in stable state 40/56, [72%]. Eight patients [14%] produced mucoid sputa and eight [14%] produced purulent sputa at baseline.

Acute exacerbation of bronchiectasis

In the group of patients followed through an exacerbation, sputum appearance was most commonly mucopurulent at baseline {n =14/21 (1=no sputum)}. There were more patients with purulent sputum at the onset of an exacerbation. (Baseline 3/21, Day 1 10/22) and this change was statistically significant, p=0.046. By Day 7 fewer patients had purulent sputum n=5/22. By day 14 and Day 42, only one patient had purulent sputum. There was significant change in appearance over the course of an exacerbation, p=0.018. Figure 8.1 is a bar chart demonstrating change in frequency of sputum appearance from baseline and during the course of an exacerbation in the study cohort.

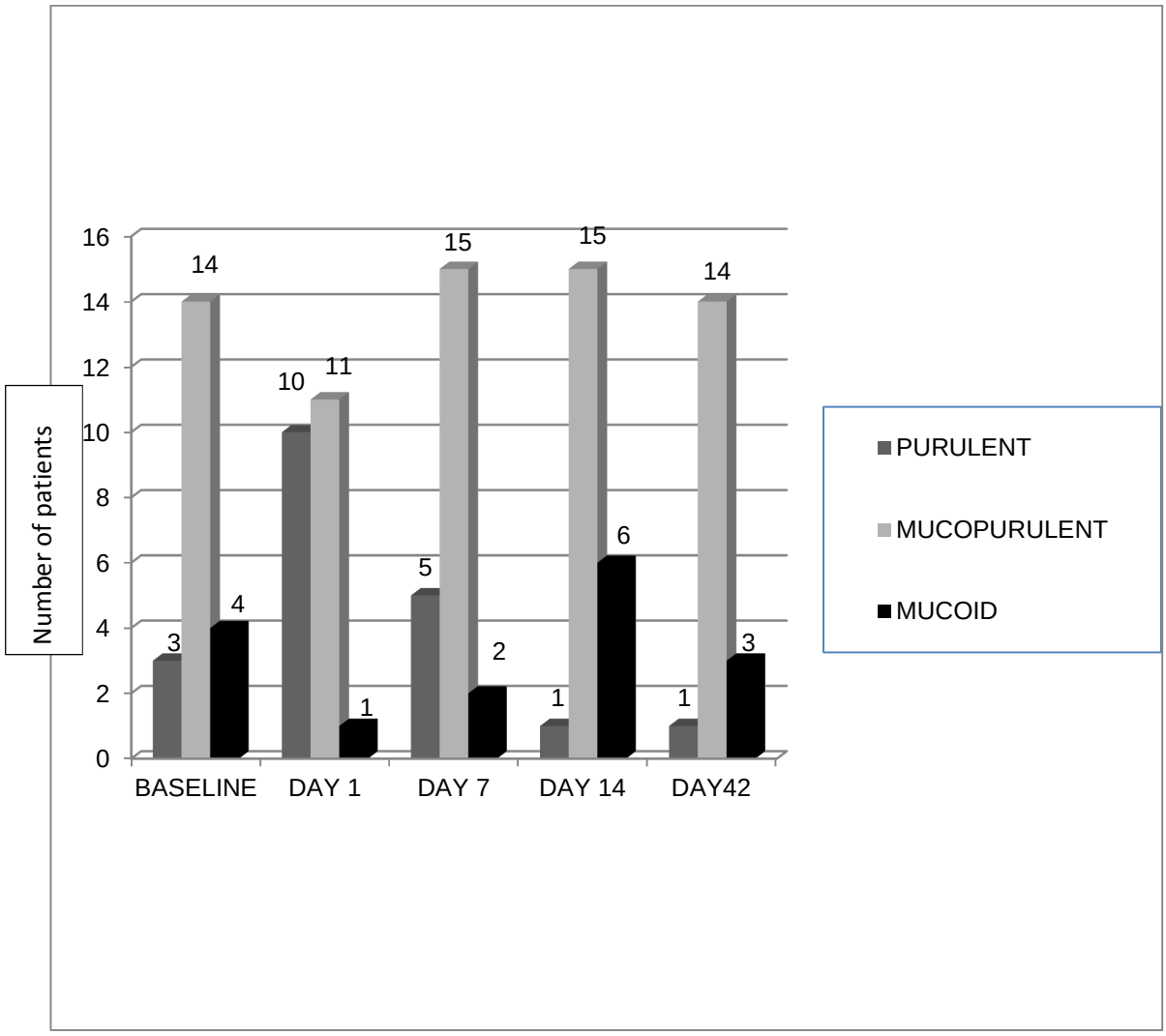

Figure 8.1: Bar chart showing frequency of change in appearance of sputa at each during the study. Variables on the x-axis are the time points in the study when sputum appearance was described i.e. Day 0-baseline, Day 1,7,14 during the course of an exacerbation and Day 42 on recovery from the exacerbation, p=0.018. Baseline v Day 1-p=0.046, Day 1 v Day 7-p=0.270, Day 1 v Day14-p=0.004, Baseline v Day 42-p=0.632. The black bars denote purulent sputum, the grey bars denote mucopurulent sputum and the white bars denote mucoid sputum. There was a significant change in sputum appearance during the course of the exacerbation.

8.4.2 24 hour sputum collection

In stable state bronchiectasis

The mean (SD) of 24 hour sputum weight (g) was 9.89(8.72).

There was no significant difference in sputum weight between patients who isolated *Pseudomonas aeruginosa* in sputa when compared to those that isolated *Haemophilus influenzae or* in whom no pathogen was isolated. {*Pseudomonas aeruginosa* n=10, 12.39(9.86) v *Haemophilus influenzae* n=3, 11.20(9.95) v no pathogen n=21, 9.23(8.61)} p=0.658.

Acute exacerbation of bronchiectasis

The 24 hour sputum weight was measured at baseline and Day 1. There was no significant change, n=12, baseline-9.0 (7.2) v Day 1-11.6 (12.2), p = 0.444.

There was no significant change in 24 hour sputum weight between Day 1 and Day 7 of the exacerbation,, n=14, Day 1-11.5 (11.3) v Day 7-7.8 (6.2) p=0.184.

There was no difference in the 24 hour sputum weight between Day 1 (onset of exacerbation) and Day 14 (completion of treatment), n=16, 10.2 (8.7) v 8.7 (6.4), p = 0.549.

There was no significant change in 24 hour sputum weight during the course of an exacerbation, n=7, p=0.273. Table 8.1 lists the mean (SD) 24 hour sputum weight at baseline and during the course of an exacerbation in the study cohort. Figure 8.2 is an interval plot demonstrating change in the mean 24 hour sputum weight during the course of an exacerbation.

Table 8.1: The mean (SD) 24 hour sputum weight in grams at baseline and during the course of an exacerbation in the study cohort followed through an exacerbation of bronchiectasis.

Visit	Number of patients	24 hour sputum weight Mean ± SD (g)
Baseline	17	9.89 ± 8.72
Day 1	14	11.5 ± 11.38
Day 7	20	8.36 ± 7.25
Day 14	20	7.87 ± 6.27
Day 42	14	7.76 ± 6.13

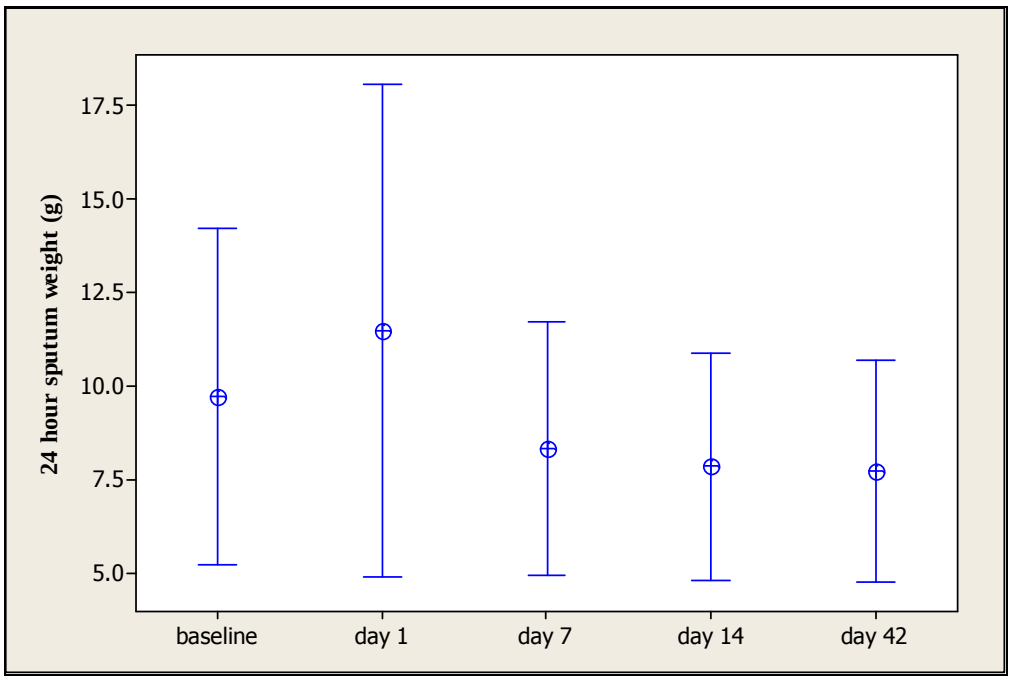

Figure 8.2: Interval plot demonstrating change in 24 hour sputum weight during the course of an exacerbation in the study cohort. The variables on the x-axis are the time points at which 24 hour sputum weight was measured during the exacerbation i.e. baseline, Day 1, Day 7, Day 14 and Day 42. The variable on the y-axis is the mean 24 hour sputum weight in grams.

8.4.3 Qualitative analysis of sputum

In stable state Bronchiectasis

Forty seven per cent (26/55) of patients had PPM above the established threshold ($\geq 10^3$ cfu/ml) in their sputa.

The PPM isolated in sputa were: 13 *Pseudomonas aeruginosa*, 7 *Streptococcus pneumoniae*, 4 *Haemophilus influenzae*, 2 *Staphylococcus aureus*, 1 *Stenotrophomonas maltophilia* and 1 *Achromobacter xylosoxidans.*

More than one PPM was isolated in two patients. No pathogen was isolated in twenty nine patients (53%) at baseline.

Acute exacerbation of bronchiectasis

At baseline, in the group of patients followed through an exacerbation, many patients isolated only mixed upper respiratory tract flora (10/21-48%) in their sputa. Nine patients had a single PPM in their sputum. More than one PPM was isolated in sputum of only one patient.

The PPM isolated were: 5 *Pseudomonas aeruginosa*, 2 *Streptococcus pneumoniae*, 2 *Haemophilus influenzae*, 1 *Staphylococcus aureus. Achromobacter xylosoxidans* was isolated along with *Pseudomonas aeruginosa* in one patient.

At Day 1, the onset of an exacerbation, twelve of the 22 patients (55%) isolated a PPM in their sputa. These were: 5 *Pseudomonas aeruginosa*; 4 *Haemophilus influenzae*; 2 *Streptococcus pneumoniae*; 3 *Staphylococcus aureus*; 1 *Moraxella catarrhalis*;

1 *Achromobacter xylosoxidans.* Four patients (33%) isolated more than one organism in the sputa.

At the onset of an exacerbation, Day 1, similar organisms were cultured to baseline in 15(71%) patients. Of these, 6 isolated a PPM while 9 continued to have only mixed upper respiratory tract flora in their sputa. A different or entirely new PPM was isolated in the sputa of 5 patients. An additional organism to their baseline isolate was found in one patient. Two patients who had mixed upper respiratory tract flora at base line converted to PPM at Day 1. (1 *Streptococcus pneumoniae*; 1 with *Haemophilus influenzae* and S*taphylococcus aureus*).

The total number of patients isolating a PPM in sputum at reduced from 12 at Day 1 to 7 at Day 7. Overall microbial clearance was achieved in 7 patients. Out of five patients who isolated *Pseudomonas aeruginosa* at Day 1, microbial clearance was achieved in two patients. However, interestingly, 2 patients had isolated a new PPM having had only upper respiratory tract flora at Day 1 in their sputa.

At Day 14, seven patients had achieved microbial clearance. However 4 patients had isolated a new PPM in their sputa (1 *Pseudomonas aeruginosa*, 2 *Stenotrophomonas maltophilia*, 1 Coliform organism). Of the 3 patients with *Pseudomonas aeruginosa* at Day 1, only one had achieved microbial clearance.

On recovery from the exacerbation, Day 42, only 14 sputum samples were analysed. Four patients did not attend the final visit (2 still hospitalised, 1 further exacerbation, 1unable to attend). A further four patients were not able to expectorate sputum at the time for analysis. Eight of these patients had returned to the baseline profile of sputum. Of the remaining 6, 2 patients had lost the PPM and isolated only mixed upper respiratory tract flora. The remaining 4 patients had acquired a new PPM (3 patients

isolated *Pseudomonas aeruginosa*, 1 *Stenotrophomonas maltophilia* and one a Coliform species).

Table 8.2 lists the PPM isolated in sputa at baseline and at Day 1 of the exacerbation for the cohort followed through an exacerbation.

Table 8.3 lists the PPM isolated or the lack of it in sputa during the course of the exacerbation at each visit. If no sputum sample was obtained, the numerical 10 has been used and UA has been used if patients were unable to attend. Table 8.4 is the key to Table 8.3.

Table 8.2: Microorganisms isolated in sputa at baseline (steady state) and at onset of symptoms (Day 1) in patients followed through an exacerbation of bronchiectasis.

	Organism isolated in stable state	Organism isolated at Day 1
1	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
2	Mixed upper respiratory tract flora	Mixed upper respiratory tract flora
3	Mixed upper respiratory tract flora	Mixed upper respiratory tract flora
4	Mixed upper respiratory tract flora	Mixed upper respiratory tract flora
5	*Pseudomonas aeruginosa*	*Haemophilus influenzae*
6	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
7	*Pseudomonas aeruginosa, Achromobacter xylosoxidans*	*Pseudomonas aeruginosa, Achromobacter xylosoxidans*
8	Mixed upper respiratory tract flora	Mixed upper respiratory tract flora
9	Mixed upper respiratory tract flora	Mixed upper respiratory tract flora
10	Mixed upper respiratory tract flora	Mixed upper respiratory tract flora
11	Mixed upper respiratory tract flora	Mixed upper respiratory tract flora
12	No sputum	*Pseudomonas aeruginosa*
13	*Streptococcus pneumoniae*	*Streptococcus pneumoniae, Staphylococcus aureus*
14	*Staphylococcus aureus*	*Staphylococcus aureus*
15	Mixed upper respiratory tract flora	*Staphylococcus aureus, Haemophilus influenzae*
16	*Streptococcus pneumoniae*	Mixed upper respiratory tract flora
17	Mixed upper respiratory tract flora	Mixed upper respiratory tract flora
18	Mixed upper respiratory tract flora	*Streptococcus pneumoniae*
19	*Pseudomonas aeruginosa*	*Haemophilus influenzae*
20	Mixed upper respiratory tract flora	Mixed upper respiratory tract flora
21	*Haemophilus influenzae*	*Haemophilus influenzae, Pseudomonas*

		aeruginosa
22	*Haemophilus influenzae*	*Moraxella Catarrhalis*

Table 8.3: Microorganisms isolated during the course of and on recovery from an exacerbation of bronchiectasis.

Baseline	**E1D1**	**D7**	**D14**	**D42**
1b, 1c	1c,1d	1c, 1d	8	1c
8	8	8	5	8
8	8	1a	8	8
8	8	8	8	10
1c	3	8	8	1c
1c, 1a	1c,1a	1a	1c,1a	UA
1a, 1b, 7	1a,7	1a,1b	1a,1b	1a,1b,7
8	8	10	8	8
8	8	8	8	8
8	8	8	8	11
8	8	8	8	10
11	1c	8	8	10
2	2,6	8	5	1c,5
6	6	6	6	11
8	3,6	8	8	11
2	8	8	1c,1d	1a
8	8	9	9	9
8	2	8	2	8
1c	3	8	8	8
8	8	8	8	10
3	3,1a	7	8	1b
3	4	8	8	8

Table 8.4: Key to Table 8.3

1a	*Pseudomonas aeruginosa - mucoid*
1b	*Pseudomonas aeruginosa - dwarf*
1c	*Pseudomonas aeruginosa - rough*
1d	*Pseudomonas aeruginosa - small*
2	*Streptococcus pneumoniae*
3	*Haemophilus influenzae*
4	*Moraxella catarrhalis*
5	*Stenotrophomonas maltophila*
6	*Staphylococcus aureus*
7	*Achromobacter xylosoxidans*
8	Mixed upper respiratory tract flora
9	Coliform *sp.*

10	No Sputum
11	Unable to attend

8.4.4 Antibiotic prophylaxis and microbiological profile of the study cohort

In our stable cohort of fifty seven patients, thirty eight (67%) were on prophylactic oral antibiotics. Fourteen (25%) patients were on aerosolised antibiotics. Eight patients were on more than one antibiotic prophylactically. One patient with end stage bronchiectasis was on four antibiotics at the same time.

In the cohort of 22 patients followed through an exacerbation, 15 (68%) were on prophylactic antibiotics. Being on prophylactic antibiotic did not affect the isolation of a PPM in sputum.

Table 8.5 lists the relationship between isolation of or lack of a PPM in sputum with the use of prophylactic antibiotic.

Table 8.6 lists the antibiotic prophylaxis prescribed to patients and the microbiological profile of the study cohort followed through an exacerbation.

Table 8.5: Relationship between isolation of or lack of a PPM in sputum with the use of prophylactic antibiotic.

	On Prophylactic Antibiotic	Not on prophylactic Antibiotic

PPM in sputa	17	9
No PPM in sputa	21	8

Chi square test χ^2 =0.371, p=0.573

Table 8.6: Antibiotic prophylaxis prescribed to patients and the microbiological profile of the study cohort followed through an exacerbation. The column on the left lists the sputum microbiological profile at baseline and the column on the right lists the profile at onset of an exacerbation. The column in the centre lists the prophylactic antibiotic prescribed to the patient.

Baseline	**Antibiotic Prophylaxis**	**Day 1**
Pseudomonas aeruginosa		*Pseudomonas aeruginosa*
Mixed upper respiratory tract flora	Azithromycin	Mixed upper respiratory tract flora
Mixed upper respiratory tract flora	Doxycycline & aerosolised Colomycin	Mixed upper respiratory tract flora
Mixed upper respiratory tract flora		Mixed upper respiratory tract flora
Pseudomonas aeruginosa		*Haemophilus influenzae*
Pseudomonas aeruginosa	Aerosolised Gentamicin	*Pseudomonas aeruginosa*
Pseudomonas aeruginosa & *Achromobacter xylosoxidans*	Azithromycin	*Pseudomonas aeruginosa* & *Achromobacter xylosoxidans*
Mixed upper respiratory tract flora	Azithromycin & aerosolised Colomycin	Mixed upper respiratory tract flora
Mixed upper respiratory tract flora	Azithromycin	Mixed upper respiratory tract flora
Mixed upper respiratory tract flora	Doxycycline	Mixed upper respiratory tract flora
Mixed upper respiratory tract flora	Trimethoprim	Mixed upper respiratory tract flora
No sputum sample		*Pseudomonas aeruginosa*
Streptococcus pneumoniae		*Streptococcus pneumoniae* & *Staphylococcus aureus*
Staphylococcus aureus	Doxycycline	*Staphylococcus aureus*
Mixed upper respiratory tract flora	Doxycycline	*Haemophilus influenzae* & *Staphylococcus aureus*
Streptococcus pneumoniae		Mixed upper respiratory tract flora
Mixed upper respiratory tract flora	Trimethoprim	Mixed upper respiratory tract flora
Mixed upper respiratory tract flora		*Streptococcus pneumoniae*
Pseudomonas aeruginosa	Amoxicillin	*Haemophilus influenzae*
Mixed upper respiratory tract flora	Azithromycin & aerosolised Colomycin	Mixed upper respiratory tract flora
Haemophilus influenzae	Azithromycin	*Pseudomonas aeruginosa*
Haemophilus influenzae	Amoxicillin	*Moraxella Caterrhalis*

8.4.5 Quantitative data

The mean (SEM) $\log_{10}$ total bacterial count of the PPMs isolated from 26 of 56 patients sampled at steady state (baseline) – not the smaller cohort followed through an exacerbation at was 7.17(0.19), n=26.

Table 8.7 describes the mean (SEM) $\log_{10}$ total bacterial count in sputa on all patients followed through an exacerbation.

Table 8.7: Mean (SEM) $\log_{10}$ total bacterial counts in sputa for all patients followed through an exacerbation of bronchiectasis.

	Baseline	Day 1	Day 7	Day 14	Day 42
Number of patients	21	22	21	22	14
Mean	4.51	5.43	3.13	3.90	4.13
Standard error of mean	0.60	0.68	0.40	0.57	0.64

In thirteen of the 22 patients, samples of sputa were obtained at all visits. When no PPM was isolated in sputum a minimum value of 10^2cfu/ml has been substituted for the purpose of calculation as this was below our leve0ls of detection. There was no significant change in the $\log_{10}$ total bacterial counts during the course of an

exacerbation. Using a Global test (repeated measures ANOVA) the p-value over all time points was 0.132 (n=13). Figure 8.6 is an interval plot demonstrating change in the mean $\log_{10}$ total bacterial counts (PPM) during the course of an exacerbation in the study cohort.

The total bacterial count at baseline was 0.5 units lower on the log scale (95%CI -1.9, 0.3) than at Day 1 (n=21, p=0.167). Counts at Day 1 were 1.4 units higher on the log scale than at Day 7 (95%CI 0.96, 3.9, n=21, p=0.003). Counts at Day 14 were 1.5 units lower on the log scale than at Day 1 (95%CI -0.186, 3.3.24, n=20, p=0.078) There was no significant difference in the bacterial counts between baseline and Day 42 (n=14, p=0.171).

The mean total bacterial count at baseline was poorly correlated with systemic markers of inflammation CRP (r=0.15) and ESR (r=0.041). [Method explained in Chapter 10]

8.4.6 Chronic bacterial colonisation of lower airways in stable state bronchiectasis

In the stable cohort of 57 patients, 20 (35%) patients were colonised by a PPM*:* *Pseudomonas aeruginosa* 17(30%); *Haemophilus influenzae* 2(3%); *Streptococcus pneumoniae* 1(2%)

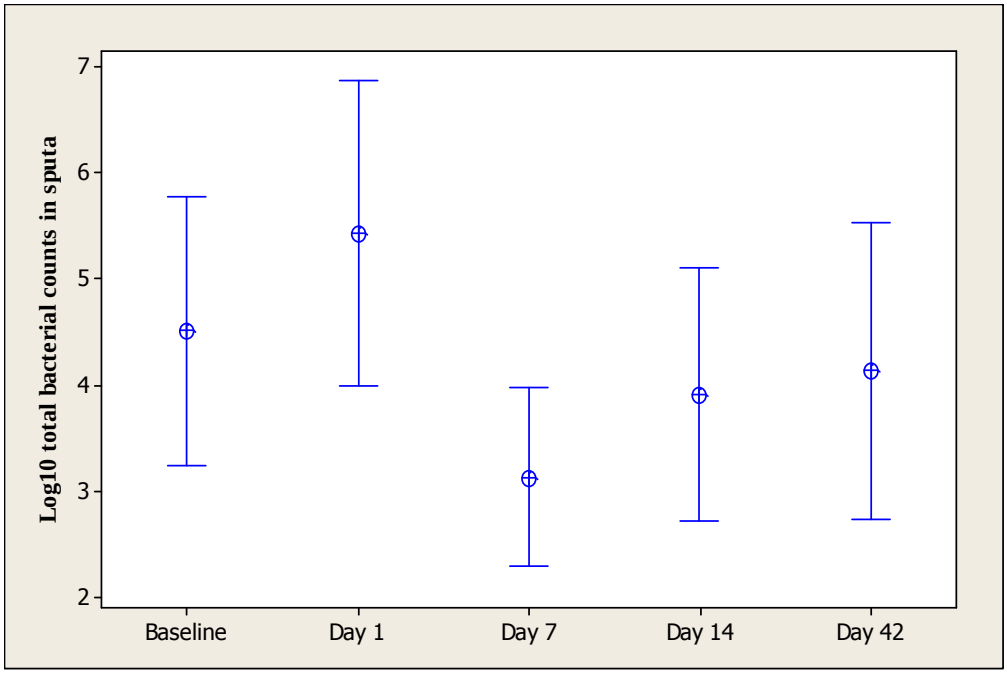

Figure 8.3: Interval plot [95% CI] demonstrating change in the mean bacterial count during the course of an exacerbation of bronchiectasis in the COBEX study. The variables on the x-axis are the time points during the study when total bacterial count in sputa was measured i.e. baseline, Day 1, Day 7, Day 14 and Day 42. The y-axis variables are the mean and 95%CI $\log_{10}$ total bacterial count in sputa. There was no significant change in the total bacterial count during the exacerbation, p=0.132

8.5 Discussion

Clinically stable patients with bronchiectasis have a high prevalence of bronchial colonisation by potentially pathogenic microorganisms [108]. This damages the mucociliary escalator preventing bacterial clearance and allowing the persistence of pro-inflammatory mediators [244]. The prevalence of chronic colonisation in our series was only 35%. Rates of chronic colonisation in our cohort is higher than some reports of 16% [17] but well below most others 48% [46] , and 54% [61, 127]. It is known that when given antibiotics prophylactically this colonisation rate falls. This may explain why the lower rate of colonisation within our cohort [17]. Up to 66% of our cohort was treated with antibiotics prophylactically. This would have been based on previous chronic colonisation data and therefore our current estimate may not be accurate for this population.

Two thirds of our patients were on prophylactic antibiotics and a third of patients isolated no pathogenic microorganism at baseline. We think this to be to the patient's advantage as higher colonisation rates are associated with frequent exacerbations [17]. However statistically we were unable to prove a significant difference in the isolation rates between those on and those without prophylactic antibiotics.

We found no significant relationship between serum inflammatory markers (ESR & CRP) and the total bacterial load at baseline. Patients who are colonised with a PPM are known to have significantly higher levels of neutrophils, elastase, MPO, and IL-8 levels in bronchoalveolar lavage fluid. It is possible that in stable state this inflammation remains local and may only spill over during an exacerbation [127].

Appearance and colour of sputum can predict bacterial colonisation [159]. We were able to validate the utility of sputum based on appearance alone. Most patients have mucopurulent sputa in stable state but this appearance significantly changed to purulence during an exacerbation. In our study, this appearance was described by a single investigator. A doctor patient correlation of describing sputum appearance is proven [159]. Seventy two per cent of our patients had mucopurulent sputum at baseline. Other groups report higher levels of purulence, in some cases up to 57% [71]. Our cohort may have milder disease and antibiotic prophylaxis may be a confounding factor in the outcome.

Eighty per cent of patients are known to have a more than fifty per cent fall in 24 hour sputum volume at the end of an exacerbation [80]. We were unable to confirm this in our study. Our mean daily sputum weight was less than 10gs. Mean daily sputum volume has been variously reported as 10mls [71], 30mls [80], 35mls [17, 27]. There are many confounding factors to this volume collection. As volume varies everyday an average of a 3 day collection may be more accurate. Compliance and sputum swallowing also remains a problem. Collection of this volume from hospital in patients may be easier. Office goers in our study cited an accurate collection as being difficult. Again nocturnal volumes may go unmeasured. We certainly found a trend with increased sputum volume at the onset of the exacerbation and this was reversed by Day 7. Perhaps the small number of patient in ours study may have reduced the power of detecting the significance in this trend. Patients with *Pseudomonas aeruginosa* produce more sputum than those with other or no pathogens in their sputa [17]. While this was true in our cohort the result was not statistically significant.

Microbial clearance as an end point in an exacerbation would seem logical. We noticed an increase in the isolation of a PPM at the onset of an exacerbation. By Day 7 this was

reduced and fell further by Day 14. Qualitative bacterial clearance can be achieved in up to 78%of patients [80]. We achieved microbial clearance in 31% of patients. However we also isolated new PPM in the sputa of some patients indicating an altered microbial balance within the airways. Antibiotic prophylaxis could once again be responsible for the lower isolation rates seen in our study.

As in most series, no pathogens are isolated in a large proportion of patients. Commensals or upper respiratory tract flora are seen in the majority of patient [71, 245]. There may be many reasons for this. The gold standard for collection of specimens remains a bronchoscopic lavage. Induced sputum may also give a better yield. Most studies, like ours, use spontaneously expectorated samples. Our methodology did not allow us to detect a bacterial count of less than 10^2cfu/ml. Isolating a new organism at Day 1 having isolated only mixed upper respiratory tract flora before, could mean that these organisms were already present in undetectable numbers previously (less than 10^2cfu/ml). We assume that a count lower than this is of no clinical significance. Other techniques such as polymerase chain reaction may increase the yield [17].

Pseudomonas aeruginosa is the most commonly isolated pathogen followed by *Haemophilus influenzae* [5, 71]. *Pseudomonas aeruginosa* was the most commonly isolated pathogen in our cohort too. *Streptococcus pneumonia* was however the second most common followed by *Haemophilus influenzae.* Our antibiotic prophylaxis policy may again be responsible for this change in balance. Other antibiotic naive populations report a higher incidence of *Haemophilus influenzae* isolates. The low incidence of S*taphylococcus aureus* in our group is in keeping with other series. [149].

Microbial clearance is certainly possible for most species but may be less likely if patients are infected with mucoid strains of *Pseudomonas aeruginosa* [80].

We found no significant increase in the quantitative bacterial load between baseline and Day 1- onset of the exacerbation. There was a significant fall in total bacterial counts between Day 1 and Day 7 suggesting effectiveness of antimicrobial treatment. There was no significant difference in total bacterial counts during the course of an exacerbation in our cohort.

A slighter lower yield in our study is also caused by our strict requirement of sputum sample to be expectorated within two hours of the analysis during the scheduled visit. All patients were not able to provide this easily. Bacterial density of spontaneous sputum is affected by the time and mode of sample storage. It can be stored for up to 6h following expectoration at 25 degrees C; beyond this it is associated with a significant increase in bacterial load [246].

We find that sputum appearance is the only useful markers of infection that could be used as endpoint in future therapeutic trials. While 24 hour sputum weight, microbial clearance and total bacterial counts show promise, larger studies would be required to confirm our findings.

Chapter 9

Anti-pseudomonal antibodies and bronchiectasis in the COBEX study

9.1 Background

Specific serum antibodies could be helpful in defining the status of bacterial infection as well as the response to early treatment in patients. It may also confirm protection following vaccination. However antibodies induced by disease do not seem to offer protection in Cystic fibrosis [12]. The antibody response against *Pseudomonas aeruginosa* in CF is a marker of chronicity of infection and of inflammation and tissue damage. Some patients with non-cystic fibrosis bronchiectasis when infected with mucoid strains of *Pseudomonas aeruginosa* have high levels of antipseudomonal antibodies. It is not known if information from CF can be extrapolated to non CF bronchiectasis [162].

Immunoglobulin G (IgG) antibodies to *Psa* surface antigens in serum can be estimated by enzyme-linked immunosorbent assay (ELISA). Antibodies to *Psa* can be directed against alkaline protease (AP), elastase (ELA), exotoxin A (ExoA) or whole cell [153, 163]. The secretory IgA is the initial humoral response to infection in the lungs. While antibodies to IgG are commonly measured, it is possible that at the onset of infection an increase in specific serum IgA antibodies may occur before an increase in serum IgG antibodies [164].

In CF, the *Pseudomonas aeruginosa* antibody response is related to the degree of inflammation and lung tissue damage [162]. Therefore it is postulated that it may help

differentiate between chronic and intermittent infect [155]. Specific serum IgG to whole–cell *Pseudomonas aeruginosa* has been measured in CF patients with chronic infection (>50% positive cultures of *Pseudomonas aeruginosa* in previous 12 months), intermittent infection (≤50% positive cultures) and those never infected. Antibody levels are significantly higher in patients with chronic infection and very low in those never infected. A sputum culture positive for *Pseudomonas aeruginosa* with a negative antibody titre suggests early superficial infection rather than advanced chronic infection [153]. Antibody titres measured in patients undergoing antibiotic treatment for eradication of *Pseudomonas aeruginosa* are significantly higher in patients chronically infected compared to those with negative cultures [153]. The duration of infection with *Pseudomonas aeruginosa* and the total number of days patients were treated with intravenous antibiotics also correlates with the antibody levels [155].The *Pseudomonas aeruginosa* antibody level can be followed up to evaluate success of anti-pseudomonas treatment [247].

In CF, longitudinal assessment of antibody titres assessed before and after inhaled antibiotic therapy in patients with first *Pseudomonas aeruginosa* isolation showed a significant decrease in antibody titres against AP and ExoA in patients clearing *Psa* infection, whereas titres increase in patients in whom antibiotic therapy fails to eradicate the organism [153].

Eradication of bacteria is more likely after early antibiotic treatment of pulmonary infection, given the problem of poor antibiotic access from serum to the intrapulmonary cavity, which is compounded by the tissue damage that occurs during infection. Alternatively, a defective IgA response may be present in some patients, which contributes to pulmonary infection. The ratio of IgA and IgG concentrations may provide an indication of the depth of pulmonary infection, and also an insight into

the reasons for the observed differences in prognosis of different patients with *Psa* infection [164].

Variability between patients is considerable, and whether treatment decisions should be based on *Pseudomonas aeruginosa* antibody levels alone remains controversial. While some suggest it may not be ideal [153] others report benefit. Patients with high serum IgG titres against *Psa* when treated with antipseudomonal treatment at intervals of four months until the serum IgG titre return to the control range, show improvement in the frequency of *Psa* isolation when compared with an observation only group [164].

High titres of serum IgG antibodies are associated with a poor clinical state, while low titres are associated with a better clinical state in both chronic and intermittently infected patients with CF [165]. Information on antipseudomonal antibody levels in patients with non-CF bronchiectasis remains limited. No study has previously described change in anti-pseudomonal antibody titres in exacerbations of bronchiectasis.

9.2 Aims

1. To investigate the prevalence of antipseudomonal antibody titres within this study cohort
2. To study the prevalence of antipseudomonal antibody in patients in whom we did not isolate *Pseudomonas aeruginosa* from corresponding sputa sample
3. To study variation in anti-pseudomonal antibodies titres during the course of an exacerbation of bronchiectasis.

9.3 Methods

Ten ml sample of venous blood was collected into a sterile vacutainer, centrifuged and the serum stored at -70^{o}C. Serum samples obtained from patients were sent to the Respiratory and Systemic Infection Laboratory (HPA, Colindale, London NW9 5HT) for analysis of antipseudomonal antibodies. Antibodies to A-band lipopolysaccharide antigen were analysed in this study.

Data was analysed using SPSS statistical software. Data are expressed as mean ± Standard deviation (SD). Comparison between different timepoints was made using the unpaired t test. Correlation between groups was determined by nonparametric Spearman correlation analysis. Significance was defined as a value of $p < 0.05$.

9.4 Results

Thirty one patients had antipseudomonal antibodies measured at baseline. Twenty of these patients had negative titres (below 0.99).Six patients had slightly elevated levels (0.99-1.49), 4 had clearly elevated levels (1.49-1.99) and only one patient has significantly elevated level (>2). Titres of antipseudomonal antibodies at baseline are listed in Table 9.1. Table 9.2 indicates when titres were slightly, clearly or grossly elevated and is a key to Table 9.1.

Seven patients had elevated titres of antipseudomonal antibodies despite not having isolated *Pseudomonas aeruginosa* in corresponding sputum samples. Three of these

seven patients had clearly elevated titres. Two of these patients were previously colonised with *Pseudomonas aeruginosa* and were already on regular antibiotic prophylaxis (oral Azithromycin + oral Doxycycline and nebulised Colomycin). One of these patients isolated *Pseudomonas aeruginosa* in sputum during the course of an exacerbation. Only one patient was neither colonised nor on antibiotic prophylaxis. This patient did not subsequently isolate *Pseudomonas aeruginosa* at baseline and had no further samples of sputum analysed during this study.

Ten patients had anti-pseudomonal antibodies measured at all visits during the course of an exacerbation. Whilst there was a trend with increased titres at the onset of an exacerbation, there was no significant change during the course of the exacerbation, p=0.996. There was no significant change between baseline and Day 1 either p=0.756.

Pseudomonas aeruginosa antibody titres were higher at the onset of exacerbation in our patient cohort however this was not statistically significant (mean baseline - 0.793 v Day 1- 0.873, p=0.672, n=11). The titres fell after 7 & 14 days of treatment with antibiotics but this fall was not statistically significant (mean Day 1- 0.874 v Day 7 – 0.853, p=0.884, n=21). There was no significant change in antibody titres over the course of the exacerbation (p=0.996).

Table 9.3 lists the mean (SD) antibody titres at all visits during the course of an exacerbation in our patient cohort.

Figure 9.1 is an interval plot demonstrating change in the mean anti-pseudomonal antibody titres during the course of an exacerbation.

Table 9.1: Anti-pseudomonal antibody titres in steady state bronchiectasis

Patient number	Anti-pseudomonal antibody titres
1	1.386**
2	0.711
3	0.937
4	1.281**
5	1.762***
6	0.454
7	0.729
8	1.343**
9	1.169**
10	1.641***
11	0.249
12	0.751
13	1.731***
14	1.183**
15	0.599
16	1.704***
17	0.525
18	0.471
19	0.561

Patient number	Anti-pseudomonal antibody titres
20	0.244
21	0.906
22	0.804
23	0.366
24	1.108**
25	0.546
26	2.197*^
27	0.526
28	0.839
29	0.488
30	0.822
31	0.699

Table 9.2: key to Table 9.1

Interpretation	
Negative	0.00 - 0.99
**= Slightly elevated	1.0 - 1.49
*** = Clearly elevated	1.5 - 1.99
*^ = Grossly elevated	2.0 +

Table 9.3: Mean (SD) anti-pseudomonal antibody titres during the course of an exacerbation, n=10.

	Mean	Standard deviation
Baseline	0.811	0.408
Day 1	0.867	0.488
Day 7	0.854	0.453
Day 14	0.811	0.454
Day 42	0.815	0.417

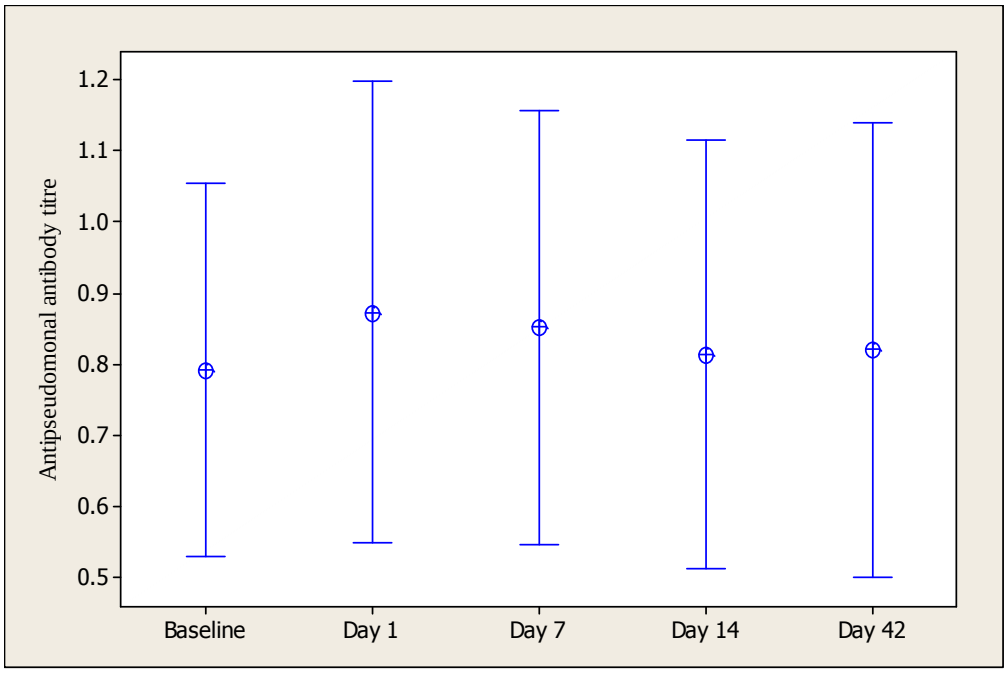

Figure 9.1: Interval plots showing change in anti-pseudomonal antibody titres in serum during the course of an exacerbation. Variables on the x-axis are the visits during the study when anti-pseudomonal antibodies were measured i.e. baseline, Day 1,7,14 and Day 42. The y axis variable is the mean (95%CI) anti-pseudomonal antibody titre. There was no significant change in the anti-pseudomonal titres during the course of the exacerbation, n=10 p=0.996.

9.5 Discussion

The purpose of this study was to investigate the prevalence of antipseudomonal antibody titres in the study cohort and measure change in their levels during the course of an exacerbation.

At baseline, in stable state bronchiectasis, eleven out of 31 patients (35%) had elevated antipseudomonal antibody titres. Our cohort was relatively stable clinically. This may explain the infrequent need for antibiotic treatment and hospital admissions in the preceding year. Eight patients had elevated levels of antibodies despite not having isolated *Pseudomonas aeruginosa* in the corresponding sputum sample. Two patients had *Pseudomonas aeruginosa* in sputum with negative antibody titres. Indeed during the course of the exacerbation, *Pseudomonas aeruginosa* was cleared by day 7 of antibiotic treatment in both patients. This indicates possible early infection. Others continued to isolate *Pseudomonas aeruginosa* despite antibiotic treatment. Whilst one of these patients was on oral Amoxicillin as antibiotic prophylaxis, the other was antibiotic free. Positive IgG antibody titres may predate isolation of P aeruginosa, but in some patients are present soon after acquisition of infection. A positive titre indicates significant exposure to P aeruginosa and could be used to detect infection in patients unable to produce sputum. The presence of a systemic immune response above the control range indicates tissue invasion and hence infection [163].

Antipseudomonal antibody titres were not significantly higher at the onset of an exacerbation in our patient cohort. There was no significant change in antibody titres over the course of the exacerbation ($p=0.996$). This may be due to the small number of patients in our study.

Detection of anti pseudomonal antibodies at an early stage, in non-CF bronchiectasis may help in differentiating between early infection and harmless colonization. It may also be a useful monitor of the progress of infection and the response to antibiotic treatment in the early stages of infection.

Chapter 10

Serum Inflammatory markers and

Capillary blood gas analysis in bronchiectasis – the COBEX study

10.1 Introduction

Systemic markers of inflammation are raised in bronchiectasis and correlate with disease severity. The level of inflammation determines disease progression and health status [181]. In Cystic fibrosis and community acquired pneumonia serum inflammatory markers rise during an exacerbation and fall on treatment with antibiotics [181, 248, 249]. The persistent local inflammation in patients with bronchiectasis and the host response may be reflected in systemic markers of inflammation. Some markers correlate closely with the anatomical extent of the disease while others correlate more closely with the lung function. No significant difference has been found in the levels of systemic inflammatory markers between patients who are on long term prophylactic antibiotics (either oral or nebulised) and those who are not on any antibiotics [181].

In respiratory conditions such as Chronic obstructive pulmonary disease (COPD), systemic inflammation is raised in stable state [250]. In exacerbations of COPD, systemic biomarkers are not useful in predicting the severity of exacerbations [81].

Plasma biomarkers are easily accessible. While absolute values may be less important, change in values may be useful in predicting onset of an exacerbation and recovery therefrom.

10.1.1 C-reactive protein (CRP) and bronchiectasis

C reactive protein is an acute phase pentameric protein produced by the liver in response to IL-6 stimulation. It is thought to help in removing autogenous and exogenous material from the circulation. This may be its main role in infection, when it acts by recognising products of pathogenic microorganisms. A raised concentration of this protein is considered to be evidence of active tissue destruction [251, 252]. Relevance in a particular disease is less well defined [81].

In patients with stable COPD, frequent exacerbators (> or = 3/year) are indistinguishable from infrequent (< or =2/year) exacerbators in terms of serum levels of C reactive protein [253]. In exacerbations of COPD, plasma CRP alone has not been found to be a useful biomarker. CRP in the presence of a major exacerbation symptom has been found to be useful in the confirmation of COPD exacerbations. When measured with a major symptom, CRP becomes 95% specific in predicting an exacerbation [81].

High CRP values are frequently found in patients with bacterial respiratory infection but may also be seen in viral illness. In a prospective study of patients with upper respiratory tract infections, higher CRP values were found in those infected with influenza A and B [254].

In stable bronchiectasis, CRP remains high signifying persistent infection/inflammation. In one cohort the CRP was raised above normal levels in 30% of patients whilst in stable state [181]. In another study measurement of the C-reactive protein (CRP) in patients suffering from bronchiectasis, clinically judged to be in

remission, showed a major on-going acute phase response. Such a response could predispose these patients to the development of reactive secondary amyloidosis [255].

In an exacerbation of bronchiectasis, the CRP is raised and decreases significantly on treatment with antibiotics [83]. In a prospective study of exacerbation in bronchiectasis more than 2/3 of patients had a raised CRP and 75% improved on completion of treatment [80].

The CRP also correlates significantly with the extent of bronchiectasis as measured by a HRCT score. This would signify local inflammation that could be measured systemically [181]. Impaired activity and morbidity are central issues in the lives of patients with bronchiectasis. CRP is significantly correlated with the activity and total scores of the St Georges Respiratory questionnaire [181]. Finally the CRP is thought to be an independent predictor of hospitalisation and mortality in patients with end stage respiratory failure [256].

10.1.2 Erythrocyte sedimentation rate and bronchiectasis

The ESR is a nonspecific measure of inflammation used commonly in clinically practise. Of little use in diagnosis, it often helps to monitor disease progress or treatment. Its major impact lies in its ability to help reinforce or lessen diagnostic probabilities [257].

In stable state bronchiectasis, when compared to healthy controls the ESR is significantly raised [258] . In one cohort, up to 33% of patients had a raised ESR when in stable state [181].

In exacerbations of bronchiectasis, the ESR has been shown to fall significantly following a two week course of intravenous antibiotics. However this was a modest improvement and fell only 1.8 fold [80].

The ESR correlates significantly with the severity of bronchiectasis as defined by an HRCT score and also the activity score as measured on the St Georges respiratory questionnaire [181]. The ESR also correlates significantly with measures of lung function including forced vital capacity (FVC), peak expiratory flow rate (PEFR), alveolar volume and the transfer
factor of lung for carbon monoxide [181].

Colonisation with bacteria is common in bronchiectasis. There is conflicting evidence with regards to ESR and CRP when different pathogenic microorganisms are isolated in the sputum. Some reports suggest no difference in the levels of these inflammatory markers in different groups [181]. Others suggest a significant association with the ESR when patients are chronically colonised with *P aeruginosa* [258].

10.1.3 Total White cell count and bronchiectasis

In stable state bronchiectasis, the total white cell count (WCC) is elevated above normal levels in up to 15% of patients [181, 259]. While neutrophils are mostly reported, a differential white cell count shows that the elevation is distributed in most cell types [259].

In exacerbations of bronchiectasis the total white cell count is raised. The mean white cell count was significantly reduced on treatment with antibiotics. Some report a significant fall in the WCC while others suggest a smaller difference of 1.5 fold decrease that is thought to be less relevant than other markers of inflammation [80, 83].

The total white cell and the neutrophil count are significantly correlated to disease severity as measured by an HRCT score, activity and total scores of the St George's Respiratory Questionnaire [181].

Lung function measures are correlated to the WCC [181]. An inverse correlation between the peripheral WCC and FEV1 percentage predicted has also been reported. This may suggest that the host peripheral leukocyte response may be a factor in the determination of lung function [121, 259].

10.1.4 Albumin and bronchiectasis

Protein in the plasma is made up of albumin and globulin. Serum albumin is the most abundant and comprises about half of the blood serum protein. It is produced in the liver and is soluble and monomeric. It has a half-life of approximately 20 days. Albumin has a number of essential physiologic effects necessary for normal health such as vasodilatation, inhibition of endothelial cell apoptosis, antioxidant effects and reduced platelet aggregation. The causes of hypoalbuminemia are malnutrition, reduced synthesis by the liver, renal losses, and chronic inflammation

In stable state bronchiectasis, serum albumin levels are lower than age matched controls [259]. In lung disease, the acute phase response starts with a local reaction at the site of injury characterized by activation of granulocytes and mononuclear cells, which in turn release acute phase cytokines (interleukin-1 [IL-1], interleukin-6 [IL-6], tumour necrosis factor-alpha [TNF-α], and interferons). IL-6 acts via specific hepatic receptors and inhibits the synthesis of albumin [260, 261]. Hypoalbuminemia is thus a marker of on-going inflammation.

A graded relationship between albumin and mortality is described. Reductions as little as 0.5g/dL in serum albumin concentration has been associated with a 54% increase in mortality rate [261]. Administering albumin to patients with hypoalbuminemia has not been shown to improve survival or reduce morbidity, suggesting that the underlying cause of hypoalbuminemia, rather than low albumin levels per se, is responsible for the increased mortality [261-263].

Albumin enters the lung by passive diffusion and can be measured in sputum and bronchial secretions. Since albumin in the sputum is derived from the blood plasma by diffusion alone, it is also a relatively sensitive marker of lung inflammation. A ratio of sputum to serum albumin is a guide to transudation into the lung. Levels of this ratio fall with treatment and rise again on cessation of treatment [264, 265]. Bronchial secretions in patients with Haemophilus influenzae secretions have higher albumin levels, indicating active local inflammation [266]. .Multivariate analysis has indicated that lower serum albumin is significantly associated with worse lung function. Persistent inflammation plays an important role in the deterioration of lung function in bronchiectasis [121].

10.1.5 Capillary blood gas and bronchiectasis

Impaired gas exchange in bronchiectasis causes hypoxemia and occasionally hypercapnia. In stable state bronchiectasis, Oxygen saturations as measured by a digital pulse oximeter shows reduced levels when compared to control subjects [258]. The cause of this is multifactorial.

In Cystic fibrosis, impaired pulmonary gas exchange is seen as measured using the multiple inert gas elimination technique. Improvement in this gas exchange is seen post treatment with intravenous antibiotics. This could be secondary to improvement in mucus plugging of small airways [267]. Airway clearance techniques (ACTs) also significantly improves mucociliary clearance and thus gas exchange [268]. In asthmatic patients who die of ventilatory failure, profound mucous plugging has been found on autopsy [269].

Significant arterial hypoxemia occasionally present in patients with extensive bronchiectasis is thought to be due to V/Q abnormalities. Right-to-left shunts are increased but contribute to a smaller extent to hypoxemia. Perfusion of unventilated alveoli is thought to be partly responsible for the intrapulmonary right-to-left shunts observed. New vascular communications in the bronchial wall and granulation tissue might shunt blood from the pulmonary arterioles to pulmonary veins. These shunts have been described previously by Liebow, Hales, and Lindskog in 1949 [56, 270].

Bronchiectasis is commonly disabling but a rarely fatal disease. However hypoxemia and hypercapnia are closely related to the mortality and described as significant risk factors [21]. This may reflect the severity of disease in these patients. Little is known about gas exchange in acute exacerbations of bronchiectasis.

Gas exchange can be measured in different ways such as arterial blood sampling or ventilation perfusion imaging. Arterial blood sampling is painful, and even with local anaesthesia the procedure causes more pain than sampling from the ear lobe [55]. Capillary blood gas (CBG) samples may be used in place of samples from arterial punctures to estimate adequacy of ventilation. A puncture is made with a lancet into the cutaneous layer of the skin at a highly vascularised area (heel, finger and toe).

10.2 Aims

The aim of this study was to establish the level of systemic markers of inflammation in stable state bronchiectasis and measure change during an acute exacerbation. We wished to define clear end points towards which future interventions can be directed.

10.3 Methods

Samples for venous and capillary blood were taken at each visit.

10.3.1 Venous blood

Two ten ml samples of venous blood were collected in sterile vacutainers

One sample was centrifuged and the serum stored at -70^{o}C for analysis of inflammatory mediators later.

The other was used to measure markers of systemic inflammation on the same day: C-reactive protein (CRP), Erythrocyte sedimentation rate, total white cell count and serum albumin.

10.3.2 Capillary blood

A capillary blood sample was used for blood gas analysis. A proprietary rubefacient [Transvasin- Hexyl Nicotinate (2%w/w), Ethyl Nictotinate (2%w/w), Tetrahydrofurfuryl Salicylate (14%w/w)] was rubbed on the ear lobe of each patient ten minutes prior to the procedure. A stab was made with the point of a lancet (2x1.5mm) in the fleshy part of the ear lobe. We collected the blood in a heparinised

capillary tube held horizontally against the puncture site. The blood gas analysis was analysed immediately (i-STAT® handheld system). Gases were measured while breathing room air with the patient at rest and in sitting at least 10 min before extraction.

10.3.3 Statistical analysis.

Variables are expressed as Mean (Standard Deviation) when distribution was normal and Median (Interquartile range) for non-parametric data. A global test for repeated measures (ANNOVA) has been used to measure change over time and paired t-test to make within group comparisons. A significant result was inferred when $p \leq 0.05$.

10.4 Results

Stable state bronchiectasis

Normal ranges of each of the systemic markers of inflammation along with median measurement values (interquartile range) for the study population in stable state is summarised in Table 10.1.

Table 10.1: Systemic markers of inflammation in stable state bronchiectasis.

Values are median (Inter quartile range).

	Bronchiectasis patients	Reference ranges	Number of patients
CRP $mg.L^{-1}$	3(2)	0-10	53
Total white cell count $x10^9.L^{-1}$	6.20(5.4)	4-11	54
ESR $mm.h^{-1}$	14(6)	1-15	49
Albumin	39(37)	30 to 50	55
PaO_2 kPa	10.8(9.9)	9.3-13.3	45
$PaCO_2$ kPa	4.5(4.1)	4.7-6.0	45

In stable state, correlation between all measured markers of inflammation and spirometric indices FEV1 % predicted and FVC % predicted was sought. Only partial pressure of Oxygen while breathing room air was significantly correlated to FEV_1 % predicted (p=0.023) and FVC % predicted (p=0.000). Table 10.2 lists the Pearson's correlation coefficient for each of these comparisons.

Table 10.2: Pearson's correlation coefficients between lung function measures and systemic markers of inflammation of patients with stable state bronchiectasis.

	FEV_1 % predicted	FVC % predicted
CRP (mg/L)	-0.017	0.080
ESR (mm/hour)	0.001	0.257
WCC ($x10^9$/L)	0.044	0.060
PaO_2 (kPa)	0.339**	0.371**
$PaCO_2$ (kPa)	-0.108	-0.140

FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; ** p<0.01.

10.4.1 C-reactive protein

The CRP increased significantly between baseline and Day 1 (p= 0.025). This trend was reversed by the end of treatment at Day 14 (Baseline v Day 14 p= 0.584, n=20). The CRP varied significantly during the course of an exacerbation (n= 20, p=0.008). Table 10.3 lists the median (interquartile range) values of CRP at all visits during the study. Figure 10.1 is a box plot demonstrating change in the median CRP levels during the course of an exacerbation.

Table 10.3: CRP levels during the course of an exacerbation of bronchiectasis.
Values are Median (inter quartile range). All values are expressed as $mg.L^{-1}$.

	Baseline	Day 1	Day 7	Day 14	Day 42
Number of patients	21	21	20	21	17
Median	3.00	9.00	3.50	3.00	3.00
IQR	2.00	16.00	4.50	3.00	9.50

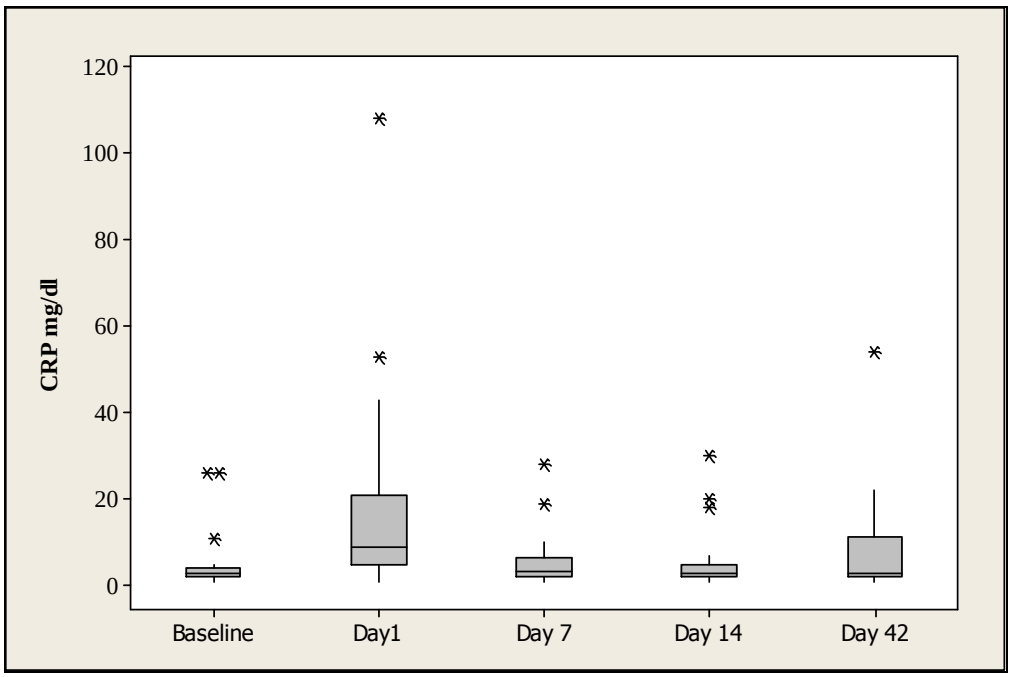

Figure 10.1: Box plot demonstrating change in median (IQR) C-reactive protein levels during the course of an exacerbation. Variables on the x-axis are the time points during the exacerbation when CRP was measured i.e. baseline, Day 1, 7 and 14 during the exacerbation and Day 42 on recovery from the exacerbation. Variable on the y-axis is CRP levels in mg/L. Extreme outliers are marked with an asterisk. There was a statistically significant change over time, p=0.008.

10.4.2 Erythrocyte sedimentation rate

There was a significant increase in the ESR between baseline and Day 1 (p= 0.017, n=14). This trend was reversed at the end of treatment at Day 14 (n=11, p=0.08). However there was no significant change in the ESR during the course of an exacerbation (p=0.227). Table 10.4 lists the median (interquartile range) values of ESR at all visits during the study. Figure 10.2 is a box plot demonstrating change in the median ESR levels during the course of an exacerbation.

Table 10.4: ESR during the course of an exacerbation of bronchiectasis.

Values are Median (inter-quartile range). All values are expressed as mm/hour.

	Baseline	Day 1	Day 7	Day 14	Day 42
Number	16	18	19	17	14
Median	13.5	17	16	13	15
IQR	10	18.5	24	16	22.2

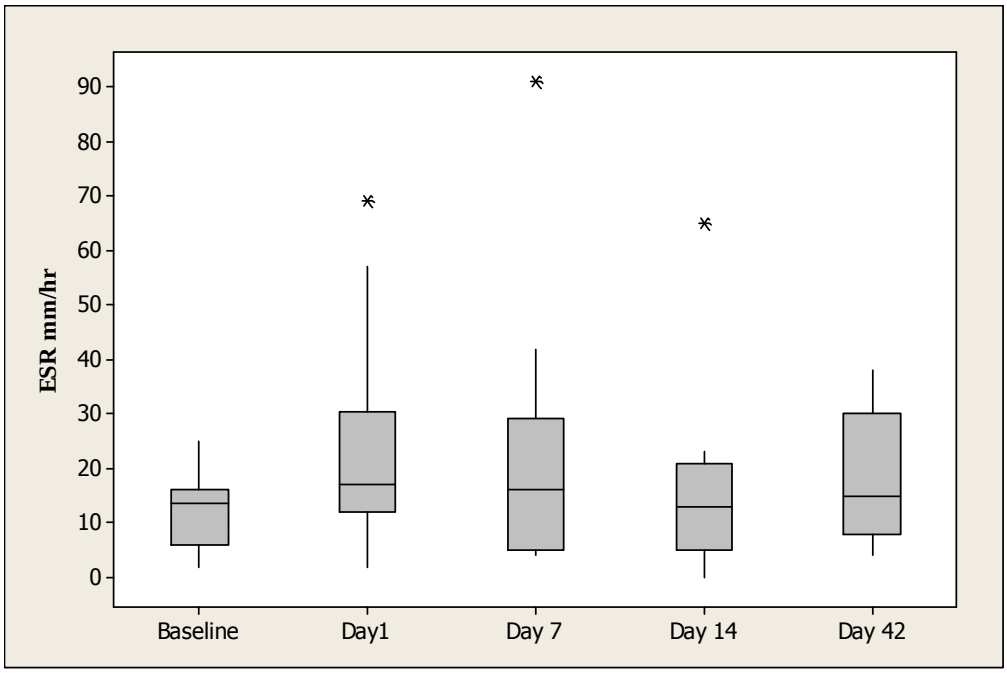

Figure 10.2: Box plot demonstrating change in median (IQR) ESR levels during the course of an exacerbation. Variables on the x-axis are the time points during the exacerbation when ESR was measured i.e. baseline, Day 1, 7 and 14 during the exacerbation and Day 42 on recovery from the exacerbation. Variable on the y-axis is ESR levels in mm/hr. Extreme outliers are marked with an asterisk. There was no significant change over time, p=0.227.

10.4.3 Serum albumin

There was a significant fall in serum albumin levels between baseline and Day 1 (n=21, p=0.026). This fall was sustained and did not recover even by Day 42, recovery from the exacerbation (p=0.022). The Body mass index (BMI) and serum albumin levels were not significantly correlated (p=0.07). The serum albumin levels did not correlate significantly with number of exacerbations that patients had suffered in the preceding year (r=0.18). Table 10.5 lists the median (interquartile range) values of serum albumin at all visits during the study. Figure 10.3 is a box plot demonstrating change in the median serum albumin levels during the course of an exacerbation.

Table 10.5: Serum albumin levels during the course of an exacerbation of bronchiectasis. Values are median (inter quartile range) in g/L.

	Baseline	Day 1	Day 7	Day 14	Day 42
Median	41	38	37	37.5	37
Inter quartile range (IQR)	4.0	5.2	3.7	5	3
Number	21	22	20	22	19

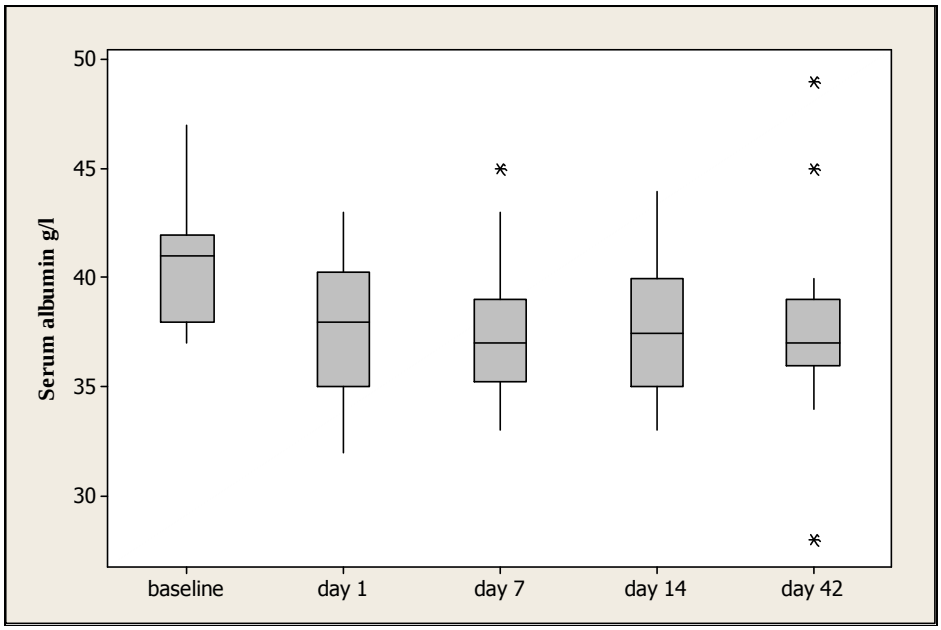

Figure 10.3: Box plot demonstrating change in median (IQR) serum albumin levels during the course of an exacerbation. Variables on the x-axis are the time points during the exacerbation when serum albumin was measured i.e. baseline, Day 1, 7 and 14 during the exacerbation and Day 42 on recovery from the exacerbation. Variable on the y-axis are serum albumin levels in mg/L. Extreme outliers are marked with an asterisk. There was significant change in serum albumin levels during the course of an exacerbation, p=0.022.

10.4.4 Total White cell count

The white cell count was higher at the onset of the exacerbation but this was not statistically significant (n= 20, p= 0.533). The white cell count did not vary significantly during the course of an exacerbation (n=15, p=0.845). Table 10.6 lists the median (interquartile range) values of total white cell counts at all visits during the study. Figure 10.4 is a box plot demonstrating change in the median total white cell counts during the course of an exacerbation.

Table 10.6: Total white cell counts during the course of an exacerbation of bronchiectasis. Values are Median (inter quartile range). All values are expressed as $x10^{9}.L^{-1}$.

	Baseline	Day 1	Day 7	Day 14	Day 42
Median	5.8	6.7	6.3	6.3	6.0
Inter quartile range (IQR)	1.95	3.30	3.25	2.75	1.90
Number	21	21	21	22	17

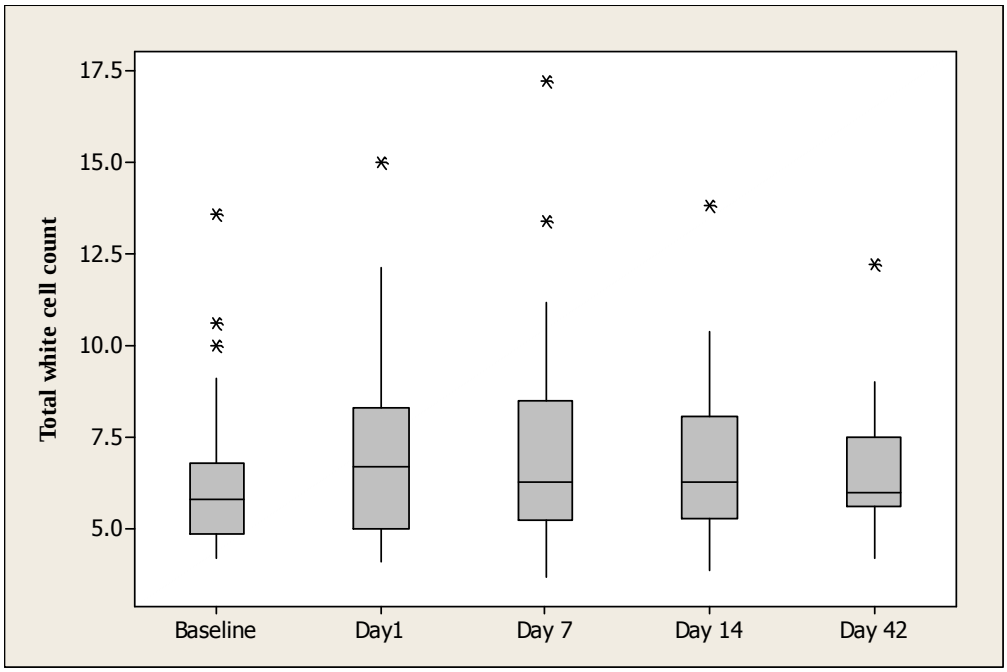

Figure 10.4: Box plot demonstrating change in Median (IQR) total white cell counts during the course of an exacerbation. Variables on the x-axis are the time points during the exacerbation when total white cell counts in blood were measured i.e. baseline, Day 1, 7 and 14 during the exacerbation and Day 42 on recovery from the exacerbation. Variable on the y-axis are total white cell counts ($x10^9$). Extreme outliers are marked with an asterisk. There was no significant change in total white cell counts during the course of an exacerbation, p=0.845.

10.4.5 Capillary blood gas

10.4.5.1 PaO_2 - Partial pressure of Oxygen in capillary blood

The PaO_2 did not vary significantly between baseline and Day 1 (p= 0.158, n=16) or during the course of an exacerbation (p=0.229). However there was a trend with the PaO_2 falling from baseline to Day 1 but recovered by Day 7. Table 10.7 lists the mean (SD) values of PaO_2 at all visits during the study. Figure 10.5 is an interval plot demonstrating change in the mean PaO_2 during the course of an exacerbation.

Table 10.7: Capillary blood PaO_2 levels during the course of an exacerbation of bronchiectasis. Values are mean (Standard deviation) in kPa.

	Baseline	Day 1	Day 7	Day 14	Day 42
Mean PaO_2	10.8	10.3	11.5	11.4	12.2
Standard Deviation	2.657184	3.272743	3.206107	2.05669	2.815402
Number	16	22	19	20	19

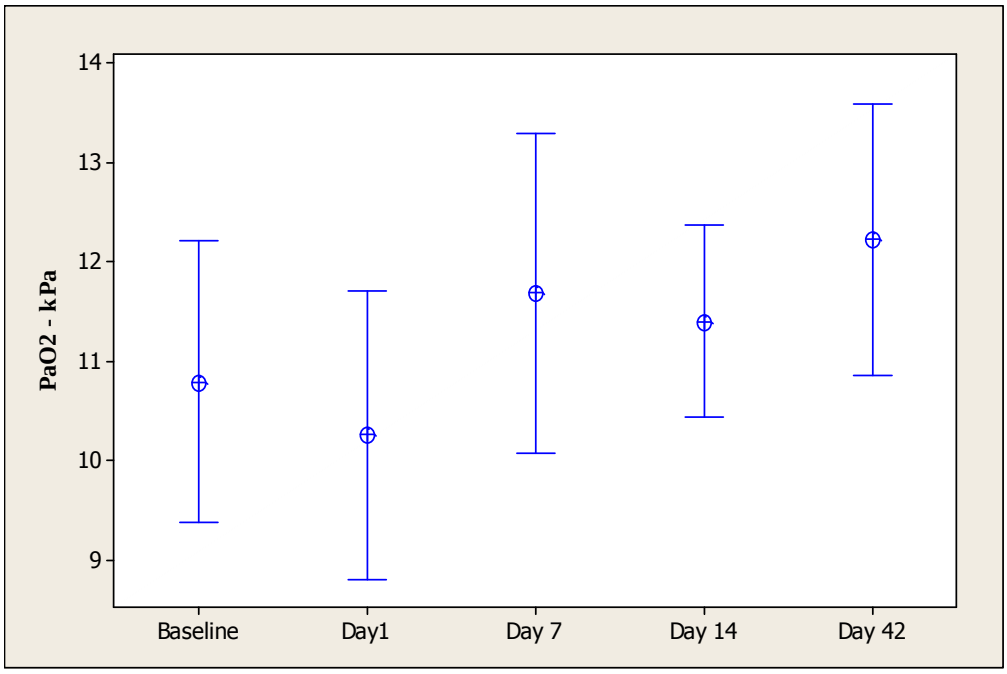

Figure 10.5: Interval plot demonstrating change in mean (95% CI) PaO2 during the course of an exacerbation. Variables on the x-axis are the time points during the exacerbation when PaO_2 in capillary blood was measured i.e. baseline, Day 1, 7 and 14 during the exacerbation and Day 42 on recovery from the exacerbation. Variable on the y-axis is PaO_2 in kPa. Extreme outliers are marked with an asterisk. There was no significant change in PaO_2 during the course of an exacerbation, p=0.288.

10.4.5.2 PaCO2 - Partial pressure of Carbon dioxide in capillary blood

The $PaCO_2$ did not vary significantly between baseline and Day 1 (p= 0.754, n=16) or during the course of an exacerbation (p=0.288).

Table 10.8: Capillary blood $PaCO_2$ levels during the course of an exacerbation of bronchiectasis. Values are Mean (Standard deviation) in kPa.

	Baseline	Day 1	Day 7	Day 14	Day 42
Number	16	21	20	20	19
Mean $PaCO_2$ kPa	4.7	4.6	5.8	4.8	4.6
Standard Deviation	0.614783	0.67637	4.018693	0.475616	0.452414

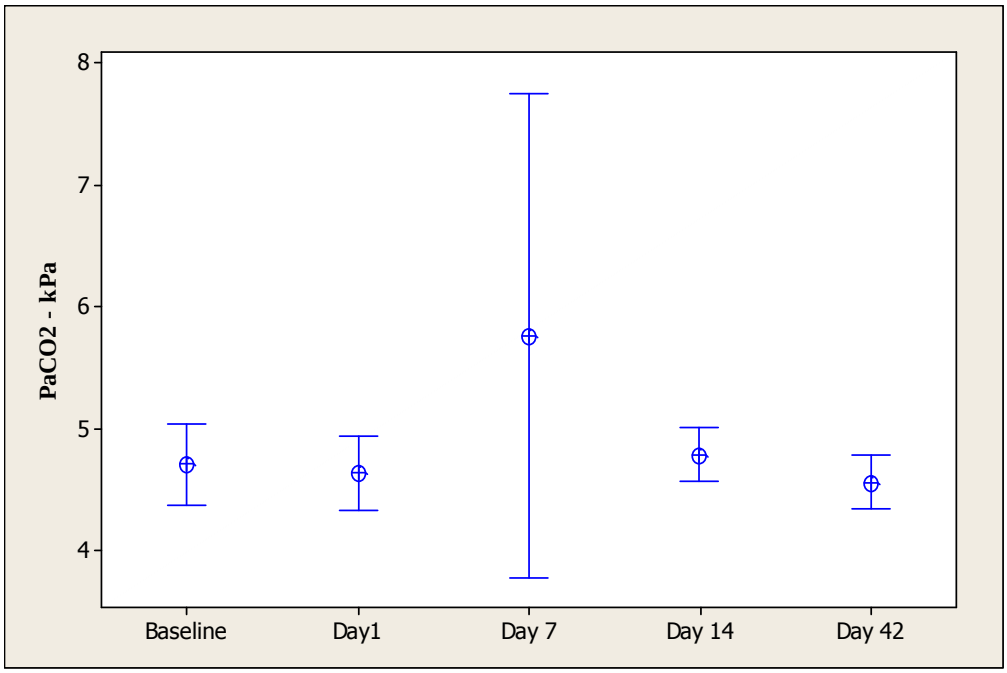

Figure 10.6: Interval plot demonstrating change in mean (95% CI) PaCO2 during the course of an exacerbation. Variables on the x-axis are the time points during the exacerbation when $PaCO_2$ in capillary blood was measured i.e. baseline, Day 1, 7 and 14 during the exacerbation and Day 42 on recovery from the exacerbation. Variable on the y-axis is $PaCO_2$ in kPa. There was no significant change in $PaCO_2$ during the course of an exacerbation, p=0.229.

10.5 Discussion

We hypothesised that markers of inflammation within serum increase during an exacerbation and may be used as biomarkers of disease. There are currently no validated markers in bronchiectasis to assess response to treatment [80, 98].

CRP has previously been shown to be high at the onset of an exacerbation and reversing on treatment within 14 days [80, 83]. This fall on treatment was very significant (9 fold) [80]. The median CRP at the onset of exacerbation in our cohort was 9mg/L which is lower than that reported by Murray et al of 67mg/L [80]. However patients in Murray et al cohort had failed a course of oral antibiotics and were likely to be a sicker cohort. Most other reports have studied patients requiring hospital admission whilst ours was a purely outpatient study. Our patients may have had milder disease as we have identified a bias in recruitment. Sixteen per cent of patients in our study showed no improvement in CRP after treatment as compared to 9% of patients in other reports [80]. The purpose of our study was to validate the change in CRP during an exacerbation and we were able to do this. The CRP increased significantly between baseline and the onset of an exacerbation. This trend was reversed on treatment with antibiotics. The CRP also does not correlate to lung function parameters [181] . We were able to confirm this in our cohort ($r= 0.125$ and $p=0.437$).

The mean ESR in our cohort was 58mm/hr at the start of an exacerbation and this is comparable to other reports of 40mm/hr [80]. The ESR increased significantly at the onset of the exacerbation and reversed on treatment. Fifty three per cent of patients had improved their ESR by Day 7 of treatment and this rose significantly to 86% of patients by Day 14. We were unable to show a significant correlation between ESR and spirometric indices.

Serum albumin levels have not previously been assessed in the setting of an acute exacerbation of bronchiectasis. We were able to demonstrate a significant fall in serum albumin levels at the onset of an exacerbation. However treatment did not reverse this effect. Recovery from exacerbation is thought to occur by 4 weeks. The serum albumin levels had not yet recovered at this time. Serum albumin levels may reflect local inflammation in lungs and it is possible that this inflammation takes longer to recover to normal levels. While elevated markers of inflammation are reduced on treatment with antibiotics they are clearly inadequate in dealing with persistent airway inflammation [181, 248, 249]. Presence of elevated systemic markers of inflammation is independent of the presence of infected sputum [181].

The mean total white cell count was 6.7($x10^9$/L) in our study. This is lower than other reports of 10.8±7.1($x10^9$/L). The white cell count is above normal limits in only 0.03% of patients in stable state. [80]. We found that all our patients had a total WCC within normal limits at baseline. Other workers have also acknowledged the change in WCC to be marginal although significant [80]. We confirm this in our study. While noting a trend of increased WCC at the start of an exacerbation and a fall on treatment we were unable to prove statistical significance. The WCC has also been shown to be significantly correlated to lung function parameters but this was not true in our cohort [181].

The PaO_2 was within normal limits for 80% and $PaCO_2$ was within normal limits in all our patients. This is comparable to other reports wherein the PaO_2 was below normal limits in 15% of patients while the $PaCO_2$ was above normal limits in 0.02% of patients while in stable state [181]. Again the mean PaO_2 in our cohort is comparable to other reports of 10±6 kPa [181]. The PaO_2 and $PaCO_2$ in our study did not vary significantly during the course of the exacerbation.

In steady state patients colonized by *Pseudomonas* have significantly lower PaO2 than patients not colonized [109]. There was no significant difference in our cohort between patients carrying *Pseudomonas* and those that were not (p=0.617). Perhaps the number of patients in our study was too small to make this comparison.

CRP and ESR are robust markers of inflammation in blood and can be used as biomarkers in therapeutic trials. Serum albumin, WCC, PaO_2 and $PaCO_2$ are not useful parameters to predict the onset of an exacerbation or thereafter measure response to treatment.

Chapter 11

Cytokines in sputa and sera of patients with bronchiectasis in the COBEX study

11.1 Introduction

The pathogenesis of bronchiectasis remains poorly understood. Three distinct pathogenic elements, namely infection, inflammation and enzymatic actions, interact with each other and have been implicated in the pathophysiology of bronchiectasis . When exposed to bacterial endotoxin, bronchial epithelial cells release inflammatory mediators Some of these mediators are proinflammatory and others anti-inflammatory. Among the proinflammatory mediators involved, IL-8, IL-1β, and TNF-α play a role favouring the trafficking of activated neutrophils through the bronchial wall into the bronchial lumen. The anti-inflammatory mediators IL-6 and IL-10 act as a counterpart of proinflammatory mediators by promoting the synthesis of natural antagonists IL-1β and TNF-α [127]. Intense neutrophil infiltration into the tracheo-bronchial tree occurs as a result which further aggravates the release of inflammatory mediators. Human airway epithelial cells also produce ET-1, which promotes neutrophil adhesion to endothelial cells [166].

Airway inflammatory response triggered by bacterial stimulation may be excessive in relation to the bacterial burden indicating. It continues to reverberate even after the infection is controlled. The altered homeostasis of airway inflammatory response to

bacterial infection in the dynamic process of host–pathogen interaction dictates the clinical manifestations of the lung disease [166, 167].

Finally neutrophil toxic products impair the structure and functioning of the airway mucosa by digesting airway elastin, basement membrane collagen and proteoglycan, contributing in this way to the progression of the disease.

11.2 Tumour necrosis factor (TNF α) and Interferon γ (IF γ) in bronchiectasis.

TNF-α is a proinflammatory cytokine synthesized and secreted by monocytes and macrophages. It has a range of properties, such as chemo attraction of neutrophils at the site of inflammation and up regulation of the expression of other chemokines with a function similar to that of IL-8.It is generally accepted that TNF-α is an essential mediator in the inflammatory cascade of a range of lung diseases and that it is able to serve as a marker of progression or prognosis in certain diseases such as pneumonia and cystic fibrosis [258, 271-274]. In steady state bronchiectasis, sputum neutrophil elastase levels correlate with the per centage of neutrophils, TNF-α and 24-h sputum volume that is a marker of disease activity [166, 169]. Neutrophilic infiltration into bronchiectatic airways is also mediated by tumour necrosis factor –α [6, 172]. This causes release of toxic products such as neutrophil elastase (NE), metalloproteases and reactive oxygen species. Because of the large number of neutrophils present, lung defences are overwhelmed [2, 172, 173]. Patients with more advanced disease have more inflammation within the airway. Levels of TNF-α are higher in broncho-alveolar lavage fluid of these patients [127].

IFN-γ is a cytokine that is critical for innate and adaptive immunity against viral and intracellular bacterial infections. Aberrant IFN-γ expression is associated with a

number of auto inflammatory diseases. The importance of IFN-γ in the immune system stems in part from its ability to inhibit viral replication directly and most importantly from its immunostimulatory and immunomodulatory effects. IFN-γ is produced predominantly by natural killer (NK) and natural killer T (NKT) cells as part of the innate immune response, and by cytotoxic T lymphocyte cells once antigen-specific immunity develops. Non-typable Haemophilus influenzae is a major cause of respiratory tract infection especially in chronic obstructive pulmonary disease and bronchiectasis. The primary factor that determines whether clinical disease occurs or not is the nature of the lymphocyte response. When challenged with non-typable *H. influenzae patients with* bronchiectasis put out lower levels of IF γ levels, suggesting that CTL and NK cell responses are important in preventing disease from non-typable *H. influenzae* infection [275]. Interferon-gamma “trafficking" is also thought to play a central role in non-tuberculous mycobacterial infection associated with bronchiectasis [276].

11.3 IL-1β, IL-6, IL-8, IL-10, IL-17 and bronchiectasis

Interleukin-1β (IL-1β) is a cytokine produced by activated macrophages as a proprotein, and is an important mediator of the inflammatory response involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis.

Interleukin-6 (IL-6) is an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine. It is secreted by T cells and macrophages to stimulate immune response to inflammation. In terms of host response to a foreign pathogen, IL-6 has

been shown, in mice, to be required for resistance against *Streptococcus pneumoniae* [277].

Interleukin-8 (IL-8) is a cytokine produced by macrophages and other cell types such as epithelial cells. Its primary function is the induction of chemotaxis in its target cells (e.g. neutrophil granulocytes).

Interleukin-10 (IL-10) is an anti-inflammatory cytokine. It is produced primarily by monocytes and to a lesser extent by lymphocytes. This cytokine has pleiotropic effects in immunoregulation and inflammation. It enhances B cell survival, proliferation, and antibody production. It is capable of inhibiting synthesis of pro-inflammatory cytokines like IFN-γ, IL-2, IL-3, TNFα and GM-CSF made by cells such as macrophages and regulatory T-cells. IL-10 also displays potent abilities to suppress the antigen presentation capacity of antigen presenting cells.

Interleukin 17 (IL-17) is a cytokine that acts as a potent mediator in delayed – type reactions by increasing cytokine production in various tissues to recruit monocytes and neutrophils to the site of inflammation, similar to Interferon gamma. IL- 17 is produced by T helper cells. IL-17 functions as a proinflammatory cytokine that responds to the invasion of the immune system by extracellular pathogens and induces destruction of the pathogen's cellular matrix. IL-17 acts synergistically with tumour necrosis factor α and Interleukin-1.

Neutrophilic infiltration into bronchiectatic airways is mediated by various agents such as host complement factor 5a (C5a), leukotriene B_4 (LTB_4), IL-8 and IL1, tumour necrosis factor –α and leukotriene (LT) B_4 [6, 172]. These cause release of toxic products such as neutrophil elastase (NE), metalloproteases and reactive oxygen species. Because of the large number of neutrophils present, lung defences are

overwhelmed [2, 172, 173]. Activated neutrophils do not differentiate between bacteria and bystander lung tissue. While individual cytokines have different actions, there may well be an overlap in function. IL-8 is one of the most potent chemoattractants which also degranulates neutrophils in bronchiectatic airways [71]. TNF-α and IL-1 have been shown to induce the breakdown of tight junctions in the blood/brain barrier in vivo [174]. A combination of anti-TNF and anti-IL-1 antibodies completely neutralized cell separation in the vascular endothelium that is induced by *Streptococcus pneumoniae* [175].

Exacerbations are associated with elevated inflammation in the form of augmented cytokine expression, cellular infiltrate, adhesion molecule expression, loss of lung function and symptomatic deterioration [177]. in exacerbations of (COPD), the systemic inflammatory response is proportional to that occurring in the lower airways and greater in the presence of a bacterial pathogen. In particular serum IL-6 is correlated significantly to sputum IL-8 [179]. This has not previously been studied formally in bronchiectasis. Most studies have evaluated these mediators in stable state disease. Investigating these biomarkers has the potential to yield information about underlying mechanisms of disease and aid development of therapeutic strategies [71, 81].

11.4 Aims

We wished to investigate the interrelationship between concentrations of serum and sputum inflammatory markers in steady state bronchiectasis.

The following markers were studied: IFN-γ, TNF-α IL-6, IL-8, IL-10, IL-17 and IL-1β.

We hypothesised that the inflammatory burden would increase at exacerbation and wished to follow the inflammatory markers longitudinally through the exacerbation.

11.5 Methods

Serum and sputum samples were collected at the same time during the visit. Not all patients were able to provide sputa at the time of their study visit. When provided, samples were not always adequate for microbiological and cytokine analysis. Samples analysis is described in Appendix iv.

Supernatant cytokine was analyzed with the Bioplex Protein Array system using beads specific for IFN-γ, TNF-α IL-6, IL-8, IL-10, IL-17 and IL-1β according to the manufacturer's instructions. The detection limit for IL1B, 6, 8, 10, 17 and TNF-α was 1pg/ml. This was kindly done for us by Mrs Karen Brown at the Immunology Department laboratory at Papworth Hospital.

The limits of detection were 0.7pg/ml (IL-6), 10pg/ml (IL-8), 3.9 pg/ml IL-Iβ, and 15.7 pg/ml TNF-α.

11.6 Statistical analysis

Normally distributed data is presented as mean ± standard deviation, whilst non-parametric data were expressed as median and inter quartile ranges. Normally distributed continuous variables were compared by *t* test; otherwise the differences were assessed by the Mann-Whitney U test. Variance was measured using ANOVAs and general linear model. Correlation was analysed using Spearman's Rho. Analysis was performed using SPSS and Minitab 15 software. Significance was assumed if $p<0.05$.

11.7 Results

Stable state bronchiectasis

Thirty six patients had sputa analysed while only 30 patients had simultaneous serum samples analysed in stable state.

We investigated the interrelationship between serum inflammatory markers using Spearman rank correlations, corrected for multiple comparisons. This resulted in a matrix of 135 correlations.

Sputum IL-1β was significantly correlated to the ESR (r= 0.525, p=0.004) and CRP (r=0.680, p=0.000). We were unable to obtain any other significant relationship between sputum and serum inflammatory markers.

There was a significant interrelationship between many serum inflammatory markers.

TNF-α was significantly correlated to serum IL-8 (r=0.375, p=0.045), IL-6 (r=0.946, p=0.000) and IL-1β (r=0.366, p=0.047).

Serum IL-8 was also significantly correlated to IL-6 (r=0.506, p=0.005) and IL-17 (r=0.605, p=0.001).

Serum IFN-γ was significantly correlated to serum IL-17 (r=0.725, p=0.000)

There was a significant interrelationship between many sputum inflammatory markers.

Sputum IL-8 was significantly correlated to sputum TNF-α (r=0.339, p=0.043), IL-10 (r=0.471, p=0.004) and IL-1β (r=0.432, p=0.009)

Sputum TNF-α was significantly correlated to sputum IL-6 (r=0.479, p=0.003), IL-10 (r=0.473, p=0.004), and IL-1β (r=0.457, p=0.005).

Sputum IL-6 and IL-10 were significantly correlated (r=0.346, p=0.039).

In particular EBC pH did not significantly correlate to any marker of inflammation. Sputum IL-10 was significantly correlated to PaO_2 at room air (r=0.388, p=0.042).

Acute exacerbation of bronchiectasis

Twenty-two of these patients were screened at the time of an acute exacerbation. No significant change was noted in any of the inflammatory markers in serum globally during the exacerbation.

Table 11.1 summarises the change in soluble markers of inflammation in serum during the course of the exacerbation - IFN-γ; TNF-α; IL-6; IL-8; IL-10; IL-17; IL-1β.

TNF-α, IL-8 and IL-1β levels in sputum significantly changed during the course of the exacerbation. Levels of these markers increased at Day 1 and fell to normal levels only by Day 14 on completion of treatment.

Table 11.2 summarises the change in markers of inflammation in sputum during the course of the exacerbation - IFN-γ; TNF-α; IL-6; IL-8; IL-10; IL-17; IL-1β.

There was no significant correlation between markers of inflammation is serum and sputum either in stable state or during the exacerbation. Table 11.3 lists the Spearman's correlation coefficient and significance levels for each of the measured inflammatory markers in serum and sputum.

Table 11.1: Changes in median (IQR) levels of cytokines in serum during an exacerbation of bronchiectasis. Bioplex (pg/ml).

Cytokine	Baseline	Day 1	Day 7	Day 14	Day 42	p value
	Median (Interquartile range)					
Number of patients	11	20	20	20	19	
Interferon-γ	1(7)	2(8.75)	2(2)	1(3.25)	2(9)	0.992
Number of patients	11	20	20	20	19	
TNF-α	7(1)	8[261]	8(1)	7(1.75)	8(2)	0.668
Number of patients	11	20	20	20	19	
IL-1β	1(0)	1(0.75)	1(0)	1(0)	1(0)	NA
Number of patients	11	20	20	20	19	
IL-6	3(9)	7(18.25)	4(4.75)	3(4)	4(4)	0.181
Number of patients	10	20	20	20	19	
IL-8	12(11.5)	15(20.2)	15(14.2)	10(7.2)	12(2)	0.067
Number of patients	11	20	20	20	19	
IL-10	5[261]	5.5(12.5)	5(3.75)	5(2)	5(4)	0.184
Number of patients	11	20	20	20	18	
IL-17	1(4)	2(3.75)	1.5(3.5)	1(4.25)	1(6.75)	0.989

Table 11.2: Change in median levels of cytokines in sputum during the course of an exacerbation. [Bioplex (pg/ml)]. NA- not applicable

Cytokine	Baseline	Day 1	Day 7	Day 14	Day 42	p value
	Median (Interquartile range)					
Number of patients	17	20	18	19	15	
Interferon-γ	1(0)	1(0)	1(0)	1(0)	1(0)	NA
Number of patients	17	20	18	19	15	
TNF-α	8(20)	23(93.8)	4.5(16.75)	2(9)	6(30)	0.004
Number of patients	17	20	18	19	15	
IL-1β	8(23.5)	44(134)	11(25)	6(22)	12(46)	0.030
Number of patients	17	20	18	19	15	
IL-6	47(279)	181(308)	97(168)	114(138)	88(237)	0.649
Number of patients	17	20	18	19	15	
IL-8	10814 (9902)	16319 (15888)	12714 (16110)	6590 (13480)	16400 (14393)	0.040
Number of patients	17	20	18	19	15	
IL-10	1(1.5)	1(9.8)	1(0)	1(0)	1(0)	0.472
Number of patients	17	20	18	18	15	
IL-17	1(0)	1(0)	1(0)	1(0)	1(0)	NA

Table 11.3: Pearson's correlation coefficient and significance levels for sputum and serum cytokines.

Cytokine	Baseline	Day 1	Day 7	Day 14	Day 42
Interferon-γ					
Correlation coefficient 'r'	*	-0.091	*	*	*
p value	*	0.704	*	*	*
TNF-α					
Correlation coefficient 'r'	0.498	0.042	0.474	0.450	0.240
p value	0.143	0.862	0.047	0.053	0.390
IL-1B					
Correlation coefficient 'r'	-0.157	-0.013	-0.042	0.257	*
p value	0.664	0.958	0.869	0.288	*
IL-6					
Correlation coefficient 'r'	0.185	-0.164	-0.291	0.045	-0.177
p value	0.610	0.490	0.242	0.855	0.529
IL-8					
Correlation coefficient 'r'	0.286	0.068	0.229	-0.097	-0.238
p value	0.456	0.776	0.362	0.692	0.393
IL-10					
Correlation coefficient 'r'	-0.121	-0.029	-0.227	-0.038	-0.087
p value	0.740	0.902	0.366	0.877	0.758
IL-17					
Correlation coefficient 'r'	*	-0.051	-0.078	-0.147	*
p value	*	0.830	0.757	0.562	*

*Values identical (not detectable <1pg/ml) and Pearson's correlation co-efficient not valid

11.8 Discussion

Patients with bronchiectasis show a minimal systemic inflammatory response, with poor correlations between systemic and bronchial inflammatory mediators. This suggests that the inflammatory process is mostly compartmentalized [127]. Our study was able to confirm this. We studied 7 inflammatory markers in sputa and serum. Only sputum IL-1β was significantly correlated to CRP and ESR. We found no correlation between any of the other measured individual markers in sputum and serum. In COPD the systemic inflammatory response is proportional to that occurring in the lower airways. In particular serum IL8 is significantly correlated to sputum IL-8 [179]. Although there is similar neutrophilic inflammation in bronchiectasis, we were unable to find a significant correlation between serum and sputum IL-8; r= 0.133 and p = 0.577. Also at the onset of an exacerbation in COPD Serum IL-6 has been shown to be significantly correlated to CRP [179]. We did not find this to be true in our cohort (r=0.072, p=0.720).

The pro and anti-inflammatory mediators showed a significant correlation within compartments. The IL-6 and IL-10 were significantly correlated as would be expected. The TNF-α, IL-1β and IL-8 were significantly correlated to each other.

Serum levels of most inflammatory markers other than IL-8 remained low at all times. This is in keeping with other reports [178]. During the exacerbation we found no significant change in any of the measured inflammatory markers.

Most importantly, all the 7 measured markers of inflammation in sputa - IFN-γ, TNF-α IL-6, IL-8, IL-10, IL-17 and IL-1β increased from baseline to Day 1 and fell on

treatment. However only TNF-α, IL-8, and IL-1β changed significantly during the course of the exacerbation.

We had also hypothesised that exhaled breath condensate pH may worsen during an exacerbation and may be correlated to markers of inflammation. We were unable to find a significant correlation between exhaled breath condensate pH and either sputum or serum inflammatory markers.

Inflammation in the airways that is exaggerated by the presence of PPM, and the higher the bacterial load the more intense the inflammation [127]. The number of patients in our study was unfortunately too small to detect change in levels between patients colonised with PPMs and those without and to compare markers to the total bacterial load.

Sputum TNF-α, IL-8, and IL-1β are important mediators of inflammation and change significantly during the course of an exacerbation. We would recommend their use as biomarkers in future therapeutic trials.

Chapter 12

Summary and Discussion for future work

12.1 Summary

This study was designed to allow identification of the key outcome measures in acute exacerbations and provide critical endpoint data for the powering future interventional studies.

Symptoms of cough, breathlessness, chest pain, chest discomfort, volume of sputum, colour of sputum and fatigue when measured on a visual analogue scale vary significantly during the course of an exacerbation. A simple Modified Borgs' breathlessness score was equally effective in predicting change at this time. Either or both these would be effective tools in any therapeutic trial

HR-QoL as measured by the Euroqol questionnaire is useful in predicting the onset of an exacerbation and improvement from thereof. It is time consuming but will be a very useful marker in any interventional study.

In steady state bronchiectasis Impulse Oscillometry is at least as effective as conventional spirometry and is likely to cause less distress to the patient. Forced vital capacity actual is the only spirometric index likely to be of use in the trail setting.

Sputum appearance alone changes significantly from mucoid or mucopurulent to purulent at the onset of an exacerbation. Identification of a new PPM is common and

can predict the onset of an exacerbation. Microbial clearance in sputum occurs on appropriate treatment.

C-reactive protein has once again proven itself to be a useful marker of disease. It remains an essential tool in any therapeutic challenge. ESR and serum albumin can predict the onset of an exacerbation but are not of use in assessing improvement.

Sputum levels of TNF-α, IL-8 and IL-1β are effective indicators and can be recommended for use as end points in therapeutic interventional trials.

12.2 Discussion for future work

Several studies have investigated markers of inflammation in the blood as indicators of the intensity of the host response to pulmonary infections and have used them to evaluate the efficacy of antibiotic treatment in suppurative lung disease [246, 249, 265, 278, 279]. These markers are shown to correlate to disease severity. Other studies have assessed response of clinical parameters to treatment with antibiotics [80, 83]. However there remains paucity in the literature of studies validating end points prior to engaging in a trial of therapy.

It would be prudent to start by defining the clinical state that needs investigation. Currently stable state bronchiectasis, which would be the baseline for all valedictory studies, remains poorly defined. Some authors use a six week infection free period as a definition [181] while others prefer non specific descriptions such as " less than 20% volume change in sputum in the last 24 hours with lack of decline in lung volumes and clinical parameters" [71]. Some fail to specify criteria altogether [27]. We have used a

4 week infection free period as stable state within this study. A similar ambiguity exists in defining an exacerbation of bronchiectasis. As previously described in Chapter 1 there is no standard definition to proceed on. A consensus must be reached on defining the clinical state, so trialling therapy does not fall prey to confounding factors.

Simple bedside evaluations of symptoms in our study have provided very significant results. Most studies so far have used either spirometric indices or more complicated inflammatory markers in blood to assess disease and change therein. Perhaps looking out for simpler techniques may continue to give us adequate answers in the future. We would recommend that the visual analogue score for clinical symptoms as described in Chapter 3 and a modified Borg's breathlessness score be used in all future studies looking at therapeutic interventions.

No validated HR-QoL questionnaire is currently available for exacerbations of bronchiectasis. It is assumed that a questionnaire validated for stable state disease would be useful in exacerbations. Various questionnaires have been employed in therapeutic trials. These include the chronic respiratory disease questionnaire [83].

and the St Georges respiratory questionnaire [80] A formal validation of a HR-QoL in exacerbations of bronchiectasis is desirable.

In Chapter 6, we were surprised to find that FEV_1 and FEV_1 % predicted were unchanged at the time of an exacerbation. Only FVC absolute was affected by the exacerbation. We feel that the small number of patients in our study might have hindered a more meaningful response. We would like to see a larger study in a similar fashion prior to accepting this result finally.

Impulse Oscillometry is new on the horizon and is not widely used as yet. Clearly the first step would be to promote familiarity with the test itself. We have shown that it significantly correlates to spirometric indices in stable disease. We have not validated this formally in the exacerbation cohort. But as patient comfort is very important, use of IOS frequently in the research setting is to be encouraged.

Exhaled breath condensate pH failed to prove useful in our hands. However having demonstrated a difference between the study cohort and healthy individuals, it continues to hold promise. Our methodology may have been inadequate and numbers small, we would therefore like to see a similar study with a larger population. We also await further analysis of the stored condensate in the hope that it may yield a thumbprint for bronchiectasis.

Microbial prevalence and clearance is central to bronchiectasis [17, 80]. However most descriptions are of bacterial disease and within this group of potentially pathogenic micro-organisms. There is very limited mention of commensals or upper respiratory tract flora. It is uncertain if this would add to the bacterial load quantitatively and hence to pathogenesis. The microbial balance within the lung milieu is unexplained. Also there is very little evidence of viral infections in bronchiectasis. Significant morbidity has previously been described when patients with bronchiectasis are exposed to Rhinovirus, Corona virus and Respiratory syncytial virus infections. Transmission from close contacts is well documented. Respiratory tract symptoms following exposure to the virus is comparatively severe in these patients [280].

The epithelium of the respiratory tract forms a large surface area that maintains intimate contact with the environment. In response to these challenges many strategies have evolved to protect the host. These include the barrier functions of the epithelium,

cough, mucociliary clearance, resident professional phagocytes, and the secretion of a number of proteins and peptides with host defence functions. The surface and sub mucosal gland epithelium of the conducting airways is a constitutive primary participant in innate immunity. In the near future, more research is required to better understand the signalling mechanisms for innate immune responses and the nature of any deficiencies in innate immunity associated with bronchiectasis. The capacity of cytokines to precisely control the movement of inflammatory cells into inflamed airways suggests that cytokines and their receptors might provide targets for therapeutic treatment to modulate airway inflammation, in order to prevent further deterioration in lung function and to better control symptoms [166, 281].

The presumed triggering events of bronchiectasis are more common than bronchiectasis itself. It is therefore important to understand why some individuals exposed to a triggering event develop permanent damage leading to bronchiectasis while others do not. Similarly, identification of risk factors related to disease progression, particularly in children, is of particular clinical importance since this can allow for early intervention and so minimise long-term morbidity and mortality [166].

Therapeutic policy at our centre includes the administration of prophylactic antibiotics. This may have interfered with some of our results particularly in the microbiological aspects of our study. Perhaps a naive population may yield different or more powerful results than ours. It would be sensible for future studies to set this out in the inclusion criteria.

We acknowledge a bias in our study cohort. We think that the patients we have recruited may not be very ‘sick’ and hence our results may be underestimating the reality. We also note the small number of patients that were followed through the

exacerbation (n=22). A larger study with a mixed cohort of severe and less severe would be useful to validate our results. Also most studies are done on local populations [3, 27, 71]. However the audience remains international. It would be useful to set up a task force to facilitate multicentre studies. This will also ensure that efficacy is standard across populations.

Finally, an effort at new strategies in the management of bronchiectasis is constantly on [282]. We hope to have provided important information in establishing an effective foundation on which therapeutic trails can be built.

Chapter 13

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Chapter 14

Appendix

Ethical approval notice (august 2006)

Valid notice of substantial amendment (February 2008)

Clinical record form (including visual analogue scale)

Appendix i

Papworth Hospital NHS
NHS Foundation Trust

Papworth Everard
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CB3 8RE

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Tel: 01480 830541
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www.papworth-hospital.org.uk

01 August 2006

Dear Dr. Bilton

Characterisation of acute exacerbations in non-CF bronchiectasis to establish measures of treatment success to facilitate design of interventional studies

R&D Ref: P01096
MREC Ref: 06/Q0104/33

I am writing to confirm that the above project has been reviewed by Papworth Hospital NHS Trust and has approval to proceed. Documents reviewed were those listed below:

– REC Application	Version 5.1	Date 06.03.06
– Protocol	Version 1.0	Date 06.03.06
– Participant Information Sheet	Version 3.0	Date 25.07.06
– Participant Consent Form	Version 3.0	Date 25.07.06
– GP/Consultant Information Sheet	Version 1.0	Date 06.03.06

You are reminded that the study must follow the approved protocol and that any proposed amendments must be submitted for review by both the Trust and the Research Ethics Committee. Submission for both reviews should be made via the R&D Office (c/o Donna Griggs).

Please ensure that any unexpected serious adverse events are reported immediately by fax to the R&D Unit on 01480 831450.

Approval is subject to compliance with the Trust Policy and Procedures on Research Governance which can be found on the Intranet. You are also required to comply in a timely manner with the project monitoring and auditing requirements of the Trust and may be asked to provide non-confidential information on the outputs and impact of the research.

The Medicines for Human Use [Clinical Trials] Regulations 2004 require that medicinal trials are conducted in compliance with Good Clinical Practice as defined by the International Conference on Harmonisation (i.e. ICH GCP). Although the regulations only apply to medicinal trials, the Trust has a fundamental duty of care to all patients and the R&D Planning Group has therefore decided that all invasive studies should be conducted to GCP standard. You are therefore required to conduct this project to GCP standard. Please contact the R&D Unit if you require further information about GCP or practical support to ensure compliance.

Please sign and date the enclosed copy of this letter and return to the R&D Unit (c/o Donna Griggs) to confirm your compliance with the Trust Policy and Procedures on Research Governance.

Yours sincerely

A Wooster

Alison Wooster
Senior R&D Manager

Carbon copy: Jane Elliott (R&D Unit), Dr Supriya Sundaram

Appendix ii

COPY

NHS

National Research Ethics Service

Cambridgeshire 1 Research Ethics Committee
(formerly Huntingdon Research Ethics Committee)

Victoria House
Capital Park
FULBOURN
Cambridge
CB21 5XB

Telephone: 01223 597656
Facsimile: 01223 597645

12 February 2008

Miss Jane Elliott
Research Officer
R&D Department
Papworth Hospital
Papworth Everard
Cambridge
CB23 3RE

Dear Jane

Study title: **Characterisation of acute exacerbations in non-CF bronchiectasis to establish measures of treatment success to facilitate design of interventional studies**

REC reference: **06/Q0104/33**

Amendment number: **Amendment 2**
Amendment date: **10 January 2008**

Thank you for submitting the above amendment which was received on 06 February 2008. I can confirm that this is a valid notice of a substantial amendment, subject to receiving a notice of substantial amendment form signed by the Chief Investigator or a representative of the sponsor such as yourself, and that it will be reviewed by the Sub-Committee of the REC at its next meeting.

Documents received

The documents to be reviewed are as follows:

Document	Version	Date
Protocol	2	10 January 2008
Participant Information Sheet: Healthy volunteer	1	10 January 2008
Participant Consent Form: Healthy volunteer	1	14 November 2007
Notice of Substantial Amendment (non-CTIMPs)	Amendment 2	10 January 2008

Notification of the Committee's decision

The Committee will issue an ethical opinion on the amendment within a maximum of 35 days from the date of receipt.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval for the research.

06/Q0104/33:	**Please quote this number on all correspondence**

Yours sincerely

Robin Scovil
REC Assistant Administrator

E-mail: robin.scovil@eoe.nhs.uk

Appendix iii

Clinical Record Form – Please see attached PDF

Appendix iv

Methodology of analysis of sputa

Sputum samples were collected at each visit. The specimen was then divided into three parts – Two samples were a hundred microlitre aliquot each (Samples A&B) and the third was a three hundred microlitre aliquot (Sample C).

Sample A

A 100 micro litre aliquot of sputum was taken from the fresh sample. Three volumes of phosphate buffered saline were added to these aliquot. This mixture was gently mixed using a pipette. The sample was ultra-centrifuged at 50,000rpm for 90 min at 4^0C in a Beckman JA 25.50 fused angle rotor centrifuge (Beckman Instrument Inc., USA). The sol phase was removed with a pipette and stored at -80^0C. {Aliquots of the homogenized sputum were added to an equal volume of glycerol broth (20% glycerol in nutrient broth) and stored at –80°C for future use}.

Sample B

A further 100 micro litre aliquot was homogenised with an equal volume of 0.1% dithiothreitol (DTT, Pro-Lab Diagnostics, Neston, UK). This sample was vortexed for 5-10 seconds and then stood for 30 minutes at 37^oC. It was then vortexed again for 5-10 seconds. Using a 10 micro litre loop, a direct smear of sputum was made on a glass slide, allowed to dry and fixed in methanol for later staining and reading.

Ten microliters of sample B was then used to inoculate a Sabouraud agar. This was incubated in an atmosphere of air at 37^0C. This was read at 24 and 48 hours and then in 3 days. The homogenized specimen was further diluted to 1 in 100 in sterile distilled water (SDW). It was then inoculated by hand onto Blood Agar, Chocolate Agar, Cled

medium (Cysteine Lactose Electrolyte Deficient agar) and MSA (Mannitol Salt agar) plates each.

Identification of bacteria was done using standard methods including the 20NE test, the Oxidase test , the catalase test, tributrin test, Coagulase test, procedure for X and V factors, the optochin and bacitracin tests. Four colonies of each organism identified were selected and each individual colony was subcultured on HBA, suspended in glycerol broth and stored on sterile glass beads at –80°C. Bacteriological media were provided by Oxoid Ltd, Basingstoke, UK and the API NE used for the identification of *P.aeruginosa* was from Biomerieux, Basingstoke, UK.

Microorganisms with potential pathogenicity described include *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Stenotrophomonas maltophila*, *Staphylococcus aureus, Achromobacter xylosoxidans* and Coliform species.

The homogenized specimen was further diluted 1 in 200 and 1 in 20000 in sterile distilled water (SDW) for quantitative studies. Twenty microlitres of each dilution was then inoculated by hand onto Blood agar, Chocolate agar, PCFC and MSA plates using a hockey stick to spread the inoculums. Blood and Chocolate Agar were incubated for 48 hours in an atmosphere of air + 5%CO2 at 37°C and the Cled, PCFC and MSA in an atmosphere of air at 37degrees centigrade. These plates were read at 24 and 48 hours.

Total viable counts of bacteria were calculated from the growth on these plates. Results were expressed in colony forming units per millilitre (cfu/ml). Only pathogens with count $\geq 10^3$cfu/ml were regarded as significant.

Sample C

Three hundred microlitres of sputum was homogenised with 4 volumes of 0.1% DTT. The sample was gently mixed with a pipette. It was then left to stand for 15 minutes at 37 °C. Four volumes of Phosphate buffered saline were then added to this mixture. The sample was gently mixed again. The mixture was filtered through 60 micrometer nylon gauze. The solution was then centrifuged at 1800rpm for 10 minutes in a Heraeus Biofuge fresco centrifuge [DJB labcare UK]. Two hundred microlitre aliquots of the supernatant were then aspirated. Inhibitors were added to samples before freezing at -80°C. For Neutrophil elatase assay: To each eppendorf a 8µl of freshly prepared mixture of Nα-p-Tosyl-L-lysine chloromethyl ketone hydrochloride (TLCK) which inhibits trypsin-like activity and ethylene diamine tetra-acetic acid (EDTA) to inhibit metalloprotease activity and 3µl of z-phe-diphenlyphosphonate (inhibits chymotrypsin-like activity) was added. For Cytokine Assay: To each eppendorf a 6µl of freshly prepared mixture of TLCK and EDTA (1:1 ratio by volume), 2µl z-phe-diphenlyphosphonate and 2µl methoxysuccinylAAPV-CMK (neutrophil elastase inhibitor) was added.