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CHRONIC INFLAMMATORY LUNG DISEASE IN HUMAN IMMUNODEFICIENCY VIRUS (HIV)-INFECTED CHILDREN. EPIDEMIOLOGICAL CONSIDERATIONS, AETIOLOGICAL DETERMINANTS AND THE EFFICACY OF LOW DOSE ERYTHROMYCIN IN BRONCHIECTASIS

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In this research, the statistical planning and analyses, and recommendations arising from these analyses, have been done in consultation with Prof PJ Becker of the Institute of Biostatistics of the Medical Research Council of South Africa, as well as Prof P Rheeder of the Clinical Epidemiology Unit of the University of Pretoria.

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DECLARATION

This thesis is the candidate's own original work, performed in the Department of Paediatrics and Child Health, University of Pretoria.

R. MASEKELA

ABSTRACT

Human immunodeficiency virus (HIV) infection has reached epidemic proportions in South Africa. The availability of highly active anti-retroviral therapy (HAART) prolongs life in HIV-infected persons, who may subsequently present with chronic manifestations of HIV-infection. The respiratory morbidity attendant to HIV-infection, even in the presence of HAART is high, the aftermath of which is lung tissue destruction and bronchiectasis. As a consequence of the political decision not to offer HAART to HIV-infected children, a number of children in South Africa have been left with severe consequences of uncontrolled HIV-infection. Bronchiectasis is one of those and because children with this devastating condition were numerous in the Pretoria region, the author and her colleagues began a Chronic Lung Disease Clinic in that region. This prompted the idea of investigating both the epidemiological profiles of these children and an attempt to intervene with both standard bronchiectasis guideline care and the use of a form of therapy commonly employed in other forms of bronchiectasis. This thesis explores those ideas.

Important new and novel findings that were consequent were; that bronchiectasis is diagnosed late in HIV-infected children at a mean age of 6.9 years. The predominant organisms cultured from the airways are *Haemophilus influenzae* and *parainfluenzae* in 49% of samples. *Pseudomonas aeruginosa* (PA), common in cystic fibrosis (CF)-bronchiectasis is an uncommon pathogen in HIV-related bronchiectasis; isolated in only 2% of specimens. Tuberculosis (TB), at least as reported, is a significant antecedent of bronchiectasis, reported in 48.5%of children. A further 21.2% of the patients had received more than two courses of anti-TB treatment. However, proof of TB infection has been lacking. Respiratory morbidity is significant with the mean forced expiratory flow in one second (FEV_1) of 53%, in this cohort at the time of presentation. Thirty-six percent of all children were exposed to environmental tobacco smoke, although this was not correlated with disease severity or HIV-disease progression. There is elevation of immunoglobulins in HIV-related bronchiectasis, with a mean IgE of 79 kU/l. This was not, though, associated with HIV disease progression as previously described in adult studies, nor with the presence of allergic bronchopulmonary aspergillosis (ABPA). The elevation in IgE

was also not associated with an elevation of T helper-2 mediated cytokines, confirming the lack of association with atopy.

The predominant cytokine, identified is interleukin (IL)-8, both systemically and locally (in airway secretions). There was elevation of other T helper-1 driven cytokines, reflecting an ability to mediate adequate inflammatory responses, which was independent of the level of immunosuppression. With the presence of HAART, there was a decline in the pro-inflammatory cytokines over time, which may be attributed to the ongoing effect of HAART that ties in to, or goes beyond the restoration of T cell numbers.

Soluble triggering receptor expressed on myeloid cells (sTREM), an innate immune marker, is elevated in children with HIV-related bronchiectasis when compared to a control group of children with cystic fibrosis-related bronchiectasis. sTREM is not associated with the presence of exacerbations and the level of immunosuppression. The use of an anti-inflammatory drug erythromycin also did not impact the sTREM values. There was also no relationship between sTREM and pro and anti-inflammatory cytokines and chemokines.

Fluorine-18-fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET) could not reliably predict the presence of pulmonary exacerbations. Its diagnostic value was limited to identifying disease activity in acute pneumonia. ^{18}F-FDG PET also had no significant correlation with CRP, inflammatory cytokines or markers of HIV disease activity.

In a randomised controlled trial of erythromycin, a cost-effective immunomodulatory drug, compared to placebo, erythromycin was ineffective in reducing the number of pulmonary exacerbations. Erythromycin also failed to demonstrate any effect on systemic and local pro- and anti-inflammatory cytokines/chemokines. With access to anti-retroviral therapy, airway clearance, nutritional rehabilitation and vigilant follow

up there was an improvement in pulmonary function parameters and stability of the degree of bronchiectasis that we propose is probably in keeping with an organ system disease modifying effect that may be, an as yet, undefined and undescribed byproduct of HAART.

Keywords

Paediatrics

Microorganisms

Biomass fuels

Highly active antiretroviral therapy

Atopy

Positron emission tomography

Macrolides

Cytokines

Chemokines

Soluble triggering receptor expressed on myeloid cells.

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DEDICATION

I would like to dedicate this thesis to my mother Dikeledi, who has been a constant source of support and has given me the faith that everything is possible if you work hard enough for it. Next I would like to thank my father David for the legacy to think outside the box and to be the best.

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CHAPTER I

INTRODUCTION

Human immunodeficiency virus (HIV) infection has reached epidemic proportions in South Africa, with the current estimate of over 5 million people living with HIV and AIDS in this country [1]. The respiratory tract is a common target for infection in HIV-infected persons, with tuberculosis (TB) being a major role player [2,3]. The consequence of recurrent or destructive pulmonary infections is tissue destruction and development of bronchiectasis.

Bronchiectasis outside the context of cystic fibrosis (CF) is an "orphan" lung disease with little funding devoted to research of this condition [4,5]. The current evidence base for non-CF related bronchiectasis is from small single centre cohort studies, which include patients with bronchiectasis from a diverse group of conditions [6-12]. Small sub-group analyses from the literature suggest that post-infectious bronchiectasis results in higher morbidity when compared to bronchiectasis from other causes [12,13]. The current research focus from developing countries is mainly on epidemiologic and clinical features of non-CF bronchiectasis, with a minor component being devoted to mechanistic and therapeutic interventions [6]. It is therefore imperative that research be conducted in this field. Such research should, in addition, focus on more cost-effective interventions that take into account the unique socioeconomic challenges of developing countries.

The management of non-CF related bronchiectasis is complicated by over-reliance on data from CF bronchiectasis. This has previously led to devastating consequences, with interventions that are effective in CF, resulting in harmful effects in non-CF bronchiectasis [14]. The differences in the local innate and adaptive pulmonary immune responses, as well as the anatomical localisation of areas of lung destruction in the two conditions may account for the variability in therapeutic

responses. Hence, studies focused on interventions in non-CF bronchiectasis are obligatory.

Previous studies identified TB, recurrent chest infections and lymphocytic interstitial pneumonitis as the chief initiators of bronchiectasis in HIV-infected individuals [15-19]. There is however, after an extensive literature review, no study that has sought to identify potential risk factors, including exposures to pollutants, in children with HIV-related bronchiectasis. In the context of a developing country there is also no data on the innate and adaptive immune markers (both systemic and pulmonary) in children with HIV-related bronchiectasis.

Macrolides are currently used in CF for their immunomodulatory properties, with successful outcomes, both in improving pulmonary function parameters, as well as improving the quality of life of affected individuals [20-23]. The evidence for the use of macrolides in non-CF bronchiectasis is less robust, with small studies suggesting their potential benefit [24-28]. In studies of macrolides in non-CF bronchiectasis, the newer macrolides are under investigation, but the limitation of use of these agents in the developing world is their higher cost, which is prohibitive for low-income countries. Erythromycin is a cheap macrolide, which has been studied both in non-CF bronchiectasis and other chronic inflammatory lung diseases and has been found to be an effective intervention [26, 29-31]. This indication of erythromycin therefore prompted the randomised, controlled trial to assess the effect of this agent in HIV-related bronchiectasis.

CHAPTER II

BACKGROUND AND LITERATURE REVIEW

2.1 Human Immunodeficiency Virus infection in South Africa

Human immunodeficiency virus (HIV) is a lentivirus, which in early infection, primarily results in a rapid and irreversible depletion of mucosal $CD4^+$ memory T cells, particularly those expressing the HIV co-receptor CC chemokine receptor 5 (CCR5) [32]. The consequence of this process is depletion in the number of $CD4^+$ T cells due to increased apoptosis of infected cells and a decreased generation of $CD4^+$ T cells [33]. The HI-virus has consequent secondary effects whose end-product is immune depletion of cells other than T cells involved in innate and adaptive immune responses. Therefore, the immune deficiency affects T-cells, B-cells, macrophages, complement, phagocytes and neutrophil activity and function [34].

There are two types of HIV, namely, HIV-1 and HIV-2. HIV type-1 is the more ubiquitous type, resulting in more serious infections [35,36]. There are nine subtypes or clades of HIV-type-1, namely A, B, C, D, F, G, H, J and K [37,38]. These subtypes can be further divided into subtype A1, A2, A3, A4, F1 and F2. F1 and F2 and subtypes are found mostly in Central and West Africa [38]. Recombination can occur between the different HIV clades to form circulating recombinant forms (CRF) and unique recombinant forms (URF). These may be identified with full-genome sequencing [39]. These recombinations between subtypes can occur within a dually infected person, from whom the recombinant forms can then be passed to other individuals. In sub-Saharan Africa clade C is the most common subtype, as opposed to subtype B that is found more commonly in Europe and the United States [39-41]. Previous studies have indicated preferential in-utero transmission of HIV-1 subtype C when compared to subtypes A and D and hence higher rates of mother-to-child transmission from this clade [42,43].

The worldwide incidence of HIV infection has increased since the first identified cases of HIV in the United States in 1981 [44]. The number of people living with HIV

in 2009 was estimated at 22.5 million in sub-Saharan Africa [1]. South Africa has been at the epicentre of the HIV epidemic, since the first reported case in 1988 [45]. Thereafter, the antenatal infection rates have increased exponentially. In 1990 the prevalence of HIV was 0.4% and this rose to 29% in 2009 [46-48].

Children acquire HIV via three possible routes, namely, perinatal (in utero), intrapartum (during delivery) and postpartum (via breastfeeding). Perinatal transmission accounts for more than 90% of all childhood infections [49]. The natural history of untreated HIV infection is either rapid progression with death by one year of age, accounting for 25–30% of cases, a milder course with death by age five years, accounting for 50–60% of cases, or long-term survival beyond the age of 8 years, accounting for 5-25% of cases [49,50]. These long-term survivors of un-treated HIV-infection have been referred to as the "slow-progressor" phenotype.

In the search for a strategy to prevent perinatal mother-to-child transmission (PMTCT) of HIV infection in developed countries, initial trials of monotherapy with azidothymidine administered to pregnant women and their newborn infants (in the 1994 Paediatric AIDS Clinical Trial Group protocol 076), revealed a significant reduction in mother-to-child transmission of HIV, from 25% to 8% [51]. Prior to the availability of HAART in 1997, 25% of patients survived 5 years after diagnosis of acquired immunodeficiency syndrome (AIDS) [52]. This has now improved, with the use of HAART, to more than 75% of children living 9 years after a diagnosis of AIDS in the United States [52].

The use of HAART together with other interventions such as elective caesarean section, avoidance of breastfeeding and treatment of concurrent sexually transmitted diseases can result in a reduction in transmission of HIV to as low as to 1-2% [53-56]. The use of antiretroviral therapy for PMTCT alone can also decrease the perinatal infection rates significantly. In order for this strategy to be implemented, expectant mothers need to attend antenatal clinics. Studies in the United States have shown that HIV-infected mothers have lower ANC attendance rates, with 15%

of HIV-infected mothers having no prenatal care [57]. This is in contra-distinction to only 2% non-attendance rates in the general population. In South Africa 56% of expectant mothers attend antenatal clinics, this despite the service being freely available since 1995 in the public health sector [58]. Myer et al, described the barriers to antenatal clinic attendance in a rural setting and concluded that these include the perception that pregnancy poses no threat to health [59]. Expectant mothers in South Africa have on average one or two visits per pregnancy, whilst the World Health Organization (WHO) recommends at least four goal-directed visits in resource limited settings [59,60]. Caesarean section is not offered to all mothers, as an HIV prevention strategy, in South Africa due to cost-constraints; this despite caesarean section having a proven track record, with one meta-analysis revealing a reduction in vertical transmission rates from 7.3% down to 2% in patients offered this intervention [61].

Postnatal acquisition of HIV is another important mode of transmission, with reported transmission rates of 16% in breastfed infants [62]. This mode of transmission may be as high as 29% during acute maternal infection [62,63]. Between 200 000 of the 500 000 new HIV infections that occur each year in children, a majority are accounted for by infection through breast milk [64]. In the developing world, breastfeeding rates are high. A Malawian study revealed that roughly two thirds of HIV-infected women breastfeed beyond 6 months of an infant's life [65]. In a resource-limited country, such as South Africa, breastfeeding is known to be one of the most effective interventions to improve childhood survival [66,67]. This poses a challenge in balancing the risk of increased mortality from diarrhoeal disease, respiratory tract infections and malnutrition, with the risk of HIV transmission to an already vulnerable population of infants [66]. Kunh et al, demonstrated (in a Zambian trial) that abrupt cessation of breastfeeding at 4 months was associated with an increased risk of death in HIV-infected infants [65]. The use of replacement feeding has also been shown to significantly increase mortality in HIV-exposed and -infected infants, where clean water sources are not guaranteed [68]. The benefits of peri-partum prophylaxis and a short course of anti-retroviral therapy in this context, is negated by the continued breastfeeding, as prophylactic therapy does not usually extend beyond 4 to 6 weeks. Due to these challenges, and despite availability of

HAART for PMTCT, the number of infections in children has remained high in Sub-Saharan Africa.

The South African National Department of Health (which provides services to over 80% of the population) first published the 'Operational plan for comprehensive HIV and AIDS care', for management and treatment of HIV infection in South Africa in November 2003 [69]. Universal access to single dose nevirapine and later combination of single dose nevirapine together with 6 weeks azidothymidine for PMTCT has been available from 2003 and 2008 respectively (Figure 1) [69,70]. Delays in provision of HAART for PMTCT have resulted in high HIV perinatal and intra-partum transmission rates. With the availability of HAART, a majority of these infected children survive into childhood and adolescence, but present with chronic manifestations occurring as a result of HIV infection.

The package for PMTCT and access to HAART has resulted in a significant decline in new infections in developed countries, but data from sub-Saharan Africa has shown the exact opposite, with new HIV infection rates in adults and children not declining. This can be attributed to a wide variety of factors which include lack of access to health care, poor antenatal attendance rates, unavailability of adequate testing facilities, poor or slow governmental response to the epidemic, unavailability of affordable antiretroviral drugs and socio-cultural factors that result in high breastfeeding rates in communities. Despite the challenges of HIV infection in South Africa, there is significant cause for optimism. Research into vaccine development, new anti-retroviral agents and therapeutic strategies are proceeding.

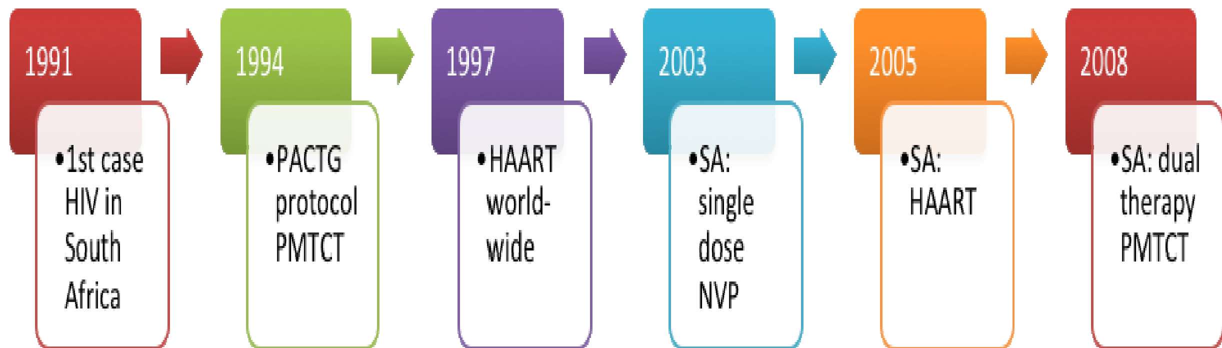

Figure 1. Timeline for human immunodeficiency virus infection and prevention of mother-to-child (PMTCT) interventions

SA: South Africa; NVP: nevirapine; HAART: highly active anti-retroviral therapy; PACTG: Paediatric AIDS Clinical Trial Group protocol 076[51]; PMTCT: prevention of mother-to-child transmission.

2.2 Lung diseases and HIV infection

HIV is listed in South African statistical data as one of the ten leading causes of death, with respiratory tract infections being the third most common cause of death in all ages [71]. The worldwide prevalence of community acquired pneumonia (CAP) is unknown, but one global report estimates almost 2 million children less than 5 years of age, die annually from acute lower respiratory tract infections (LRTIs), and accounting for one-fifth of all childhood deaths [72)]. The prevalence of this condition is estimated to be 2-10 times greater in Africa and Asia when compared to the United States of America [72,73]. HIV and AIDS have had a significant impact on both the prevalence and severity of CAP, posing a threat to all the gains made on impacting childhood mortality in the last decade [74-76]. CAP in children accounts for between 30 to 40% of all admissions and has a case fatality rate of 15-28% [77,78]. The natural consequence of the HIV epidemic and increase in childhood pneumonia prevalence and severity is therefore an increase in numbers of hospitalisations for LRTIs and an increase in disease-related morbidity and cost. Costs would be

dictated by, not only, increased numbers of admissions but also increased utilisation of diagnostic and therapeutic services for more severe disease.

HIV has also impacted on the organisms that cause CAP in children. Besides the common organisms implicated in CAP in HIV-uninfected children, i.e. *Streptococcus pneumoniae, Haemophilus influenzae*, *Haemophilus parainfluenzae* and *Staphylococcus aureus,* gram-negative pathogens like *Escherichia coli* and *Salmonella spp. and PA* are pathogenic in HIV-infected children [78,79]. There is also evidence of higher rates of antibiotic resistance to pathogenic organisms in HIV-infected children [78,80]. This further contributes to greater morbidity and mortality in this population.

Viral respiratory tract infections are also a common occurrence in HIV-infected individuals. Immunological responses to viral infections depend on intact antibody responses, which are impaired in HIV-infected individuals. This is even more so in younger children who may have had no prior exposure to these viruses [81]. The common viruses causing LTRIs i.e. *Respiratory syncytial virus (RSV)*, *Influenza, Rhinovirus, Adenovirus* and *Human metapneumovirus* are common in HIV. There is however, evidence of prolonged shedding of viruses in HIV-infected children with shedding up to 120 days for RSV and nine months with *Parainfluenzae* virus infection [82-84].

Pneumocystis jirovecii pneumonia (PCP) is a common opportunistic LRTI in HIV-infected children. The presence of PCP is known to be commonly associated with *Cytomegalovirus* co-infection [85,86]. Access to critical care facilities and HAART allows for survival of HIV-infected children with severe acute respiratory distress syndrome. Wolff et al, demonstrated a more than two-fold risk of developing bacterial pneumonia in subjects previously treated for PCP [87]. PCP also seems to be an independent risk factor for lung function impairment later in life [88].

South Africa has one of the highest burdens of TB in the world, with rates exceeding 500/100 000 population [89]. Co-infection of TB and HIV has been well described, with HIV being a driver of increased TB prevalence [90,91]. Unfortunately, the real co-infection rates are unknown in the paediatric HIV population, due to the lack of

acceptable diagnostic tools. The radiological picture and tuberculin skin test have a low diagnostic yield in children, with the majority of children also being unable to expectorate sputum [18,19,92]. Unlike, HIV un-infected children, the presentation of TB can mimic acute pneumonia in HIV-infected children, complicating the ability to distinguish it from other causes of CAP [93]. With these challenges in TB diagnostics in HIV-infected children, attempts at TB prevention strategies have also yielded disappointing results, with current misgivings about the safety of the BCG vaccine in this population [94,95]. There has also been contradictory evidence in the literature on the role of isoniazid chemoprophylaxis (IPT). A trial by Zar et al, demonstrated benefit of IPT, with statistically significant reduction in mortality in their study population. On the other hand, a multicentre trial by Madhi et al, showed no benefit of IPT on over five hundred HIV-infected infants less than one year of age [96,97]. These challenges in diagnosis and prevention of TB result in an under-recognition and possibly over-diagnosis of TB in children infected with HIV.

The utilisation of HAART has impacted the respiratory infectious burden in HIV-infected children, particularly on the pulmonary opportunistic infections [98,99]. HIV-infected, but untreated, children have an incidence rate of 11.1 per 100 child years of acquiring acute LTRIs, and with HAART this decreases to 2.2 per 100 child years [18,19]. Therefore, a significant pulmonary morbidity, associated with LRTIs in HIV-infection, still persists when compared to HIV-uninfected children. This increased burden, coupled with malnutrition, exposure to pollutants and limited access to health care, may result in catastrophic airway destruction and subsequent development of bronchiectasis in children.

2.3 Bronchiectasis

The term bronchiectasis is derived from the Greek words *bronkia* (bronchial tubes), *ek* (out) and *tasis* (stretching). The earliest description of bronchiectasis was by Laennec in 1819 [100]. There are two anatomical classification systems used for the diagnosis of bronchiectasis, namely, the Reid and Whitwell classifications [101,102]. The Reid classification is an anatomical system based on the bronchographic appearance of the conducting airways, namely, cylindrical (tubular), varicose and

saccular (cystic) (Figure 2). In cylindrical bronchiectasis there is loss of bronchial tapering. With increasing severity of bronchiectasis, the airways take on a beaded appearance with areas of dilatation and constriction, producing a varicose appearance. Finally, the end-stage is that of irreversible ballooning of the bronchi with or without fluid accumulation causing saccular bronchiectasis. The Whitwell classification is a pathological classification based on over two hundred surgical specimens of bronchiectasis subjects, in whom three main forms were described, namely, follicular, saccular and atelectatic types.

In the past few years the diagnostic criteria for bronchiectasis have changed, with the diagnosis being based on the less invasive high-resolution computerised tomographic (HRCT) features (Box 1). HRCT scanning has revolutionised the field of pulmonology and has led to a less invasive procedure that allows for early detection of bronchiectasis. HRCT is also an accessible tool for follow up of disease progression. Unfortunately due to the high radiation burden attached to HRCT, it is an unattractive tool for regular follow-up particularly in growing children where the risk of malignancies is high.

There are a number of validated bronchiectasis CT scoring systems that are used to monitor structural changes in the lungs. These scoring systems can be utilised in the follow up of disease progression, survival prediction models, as well as a research tool to monitor therapeutic response [104-107]. The modified Bhalla is a qualitative CT scoring system has been validated for use in the context of CF-bronchiectasis [107]. This scoring system is based on nine morphologic changes such as; peri-bronchial thickening, mucous plugging, abscesses or bronchiectatic sacculations, emphysema, bullae and consolidation or collapse (Appendix D).

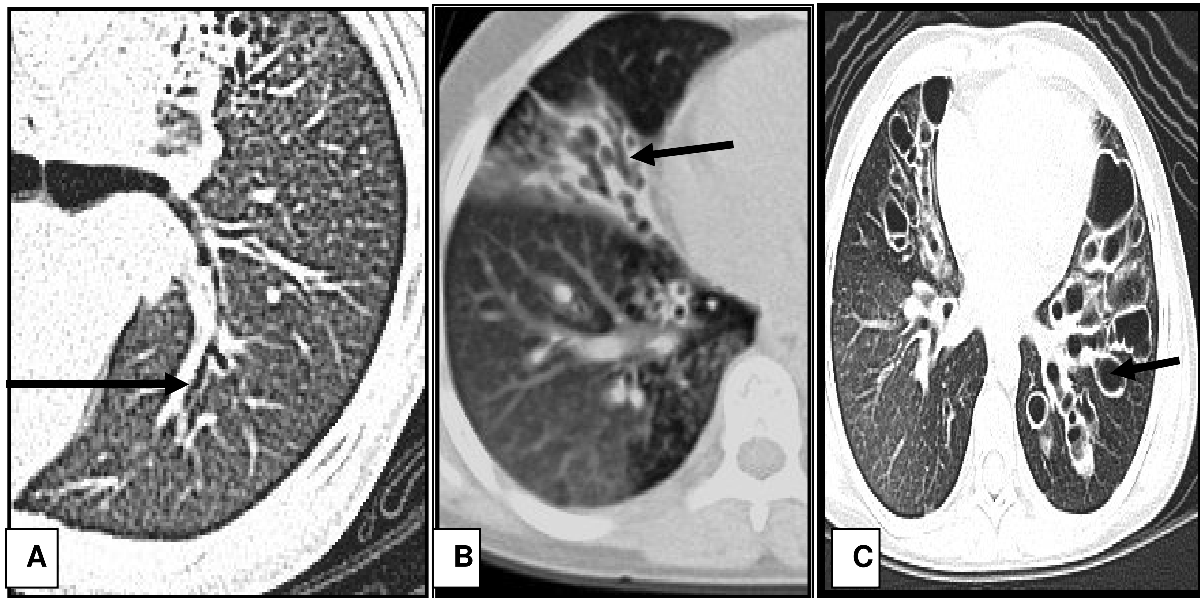

Figure 2. Stages of bronchiectasis according to the Reid classification system

High resolution computed tomography views of different stages of bronchiectasis indicated with black arrows the abnormalities indicated labelled A: cylindrical bronchiectasis; B: Varicose bronchiectasis; C: Saccular bronchiectasis

Box 1. High resolution computed tomographic features of bronchiectasis [103]

1) Signet ring sign: internal diameter of the bronchi larger than accompanying vessel
2) Bronchial dilatation
3) Failure of tapering of the bronchi
4) Presence of dilated peripheral airways at the CT periphery
5) Bronchial wall thickening with mucous plugging or impaction with tree-in-bud pattern
6) Mosaic perfusion
7) Air trapping on expiratory films

Magnetic resonance imaging (MRI) has the advantage of being radiation free. Its use in the diagnosis of bronchiectasis is limited by the poor spatial resolution, long acquisition times and cost. A recent study in children comparing HRCT and MRI

showed excellent agreement between the two study modalities in the quantitative assessment of lung damage, although MRI was found to be less sensitive than HRCT in the diagnosis of bronchiectasis and performed poorly in the detection of bullous lesions and localised emphysema [108].

Bronchiectasis is regarded as an “orphan” lung disease, as very little funding and research is devoted to this condition [4,5]. Childhood bronchiectasis has declined in affluent populations due to effective immunisation programmes, avoidance of overcrowding, adequate access to medical care, better hygiene and nutrition. Reported rates of 0.49 per 100 000 population occur in Finland [109,110]. Certain groups in industrialised countries, such as the Alaskan natives of the Yokun Kuskokwim Delta, the New Zealand Maori and the Aborigines of Australia, have inordinately high bronchiectasis rates, ranging from 3.5 to 16 per 10 000 [7,8,11].

The common causes of bronchiectasis in the developed world, excluding CF, are impaired local and systemic immune defences with post-infectious causes accounting for up to 29% of all cases, whilst primary ciliary dyskinesia, primary immune deficiencies, congenital malformations and aspiration account for the rest (Table 1) [6]. This is in contra-distinction to developing countries where infectious causes are more common, with post adenoviral bronchiolitis obliterans described as a common cause in Brazil [111]. Infections such as TB also account for a majority of cases in developing countries [16,17]. Despite the advances in genetics and diagnostic tools to determine causes of bronchiectasis, there are still a large number of children with bronchiectasis without a definite cause, both in the developed, and developing worlds.

Table 1. A summary of studies documenting aetiology of bronchiectasis in both developed as well as developing countries [6]

Country	Immunodeficiency	Post-infection	PCD	Congenital	Aspiration	Idiopathic
UK	25%	30%	1%	9%	3%	18%
Australia	23%	13%	3%	13%	3%	40%
Italy	10%	7%	24%	-	4%	55%
Turkey	15%	30%	6%	3%	4%	38%
Taiwan	10%	28%	3%	-	7%	31%
Tunisia	10%	10%	10%	-	-	50%

PCD: primary ciliary dyskinesia; UK: United Kingdom.

Risk factors associated with bronchiectasis are overcrowding, poverty, damp housing, macro- and micro-malnutrition, indoor pollution with biomass fuels (BMF) and environmental cigarette exposure (ETS) [8,11,112]. In HIV-infected children, TB and lymphocytic interstitial pneumonitis (LIP) have been found to be the most common predisposing factors for bronchiectasis [15].

BMF are those fuels that are commonly used in relatively poor communities. They include wood, charcoal, leaves and dung. These substances are used for cooking and heat generation. Most commonly, where these fuels are used for cooking purposes, people particularly women and children, are subjected to prolonged indoor exposures [113]. The effects of BMF on lung health are well documented [114-116]. The socioeconomic status of caregivers that include, the type of housing, number of people in the house and type of cooking fuel all play a critical role in the interplay between the host and environment in determining respiratory health.

The prevalence of tobacco smoking has been on the decline in many developed countries. There is however, a disproportionate increase in tobacco smoking in females worldwide, and more concerning, in people in developing countries [117]. Studies have also demonstrated higher smoking rates in HIV-infected individuals when compared to un-infected individuals [118,119]. The World Health Organization (WHO) has estimated that the annual consumption of cigarettes for South Africa is between 1500 and 2499 per person; with an estimated 1000 million smokers worldwide [117]. Tobacco smoke contains more than 4000 compounds, as well as over 50 known carcinogens, irritants and toxic agents that have significant damaging effects on the respiratory system. The primary components of ETS are the sidestream emitted from the smouldering of the tobacco between puffs, as well as the exhaled smoke. Sidestream smoke is produced at a lower temperature than mainstream smoke, with many more carcinogens and toxic substances being generated in this by-product [119]. ETS has toxic as well as irritative effects within the airway. The exposure to ETS also results in an alteration of mucociliary clearance of the airways via inhibition of both chloride and potassium conductance in bronchial epithelial cells [120]. Some studies have demonstrated that tobacco smoke increases mortality and morbidity in HIV-infected individuals [121].

The respiratory pathogens implicated in exacerbations of bronchiectasis in both developed and developing countries are similar to the common pathogens causing CAP i.e. *Streptococcus pneumoniae*, *Haemophilus influenzae*, gram negative *Enterobacteriacae* and respiratory viruses [122].

The exact pathophysiological mechanisms involved in the initiation of bronchiectasis are unknown. The currently accepted theory is the ‘vicious circle’ theory, proposed by Cole, in the mid-eighties (Figure 3) [123]. Cole’s theory evolves around an initial “hit” or “trigger” that results in airway inflammation. The inflammatory process is established such that, with subsequent lung infections, persistent airway inflammation occurs. This is associated with release of pro-inflammatory cytokines IL-6, IL-8 and neutrophil elastase [124-126]. These cytokines recruit inflammatory mediators, whose end-product is mucous gland hypertrophy and mucus hyper-production. Excess mucus compromises the mucociliary escalator, which further

perpetuates microbial invasion of the airway. Mucus performs an innate immune function in the lungs by acting as the first barrier in the airways. Mucus is made up of mucin proteins, water, surfactant phospholipids, peptides and defence proteins. There are many changes that occur to the mucus properties of patients with chronic inflammatory lung disease [127]. Goblet cell hyperplasia contributes to excessive mucus production. In the presence of infection, epithelial cells modulate the recruitment of inflammatory cells by the production of chemokines, cytokines, adhesion molecules and the modulation of expression of receptors. The presence of persistent infection, impairment of the protective mucociliary escalator as well as the presence of enzymes such as elastase, produces damage to the airway and lung tissue [128].

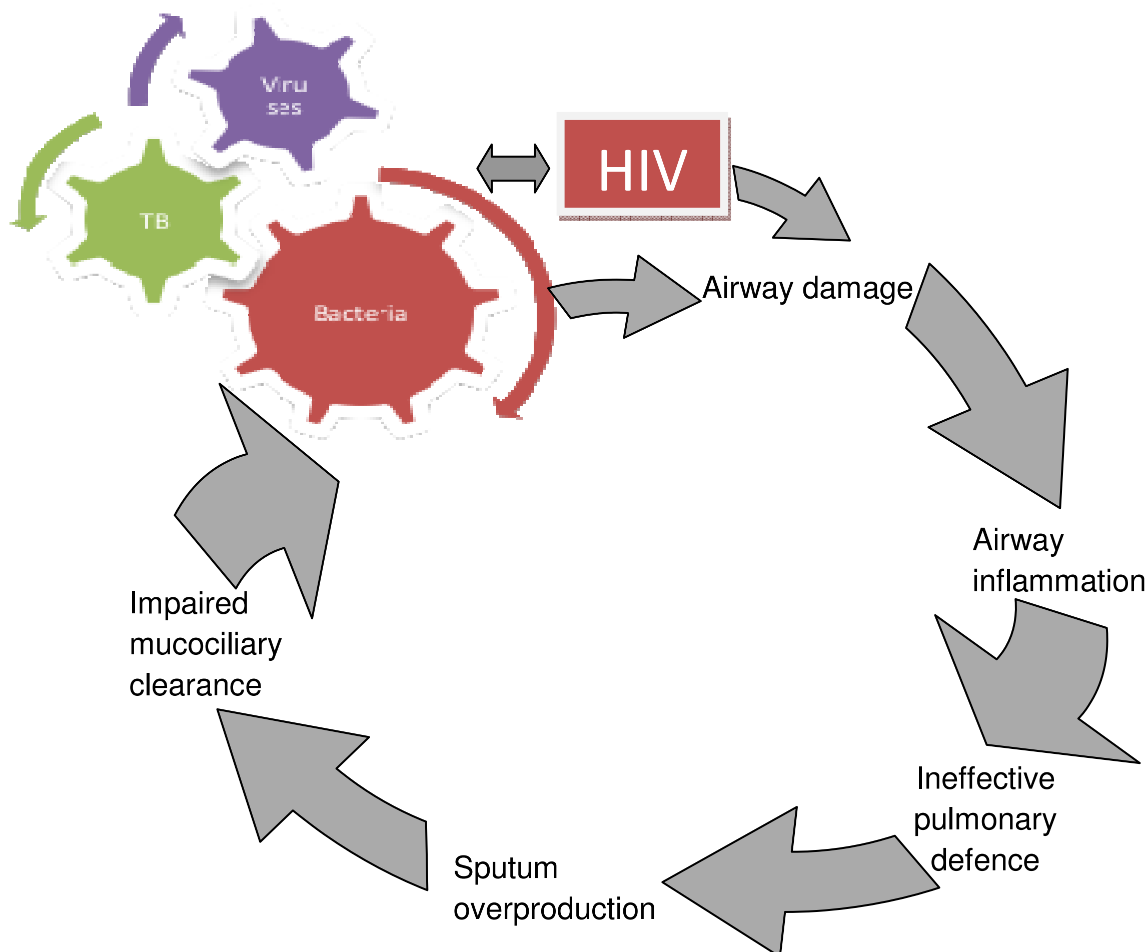

Figure 3. Proposed pathophysiology of bronchiectasis in HIV- infection

Bronchiectasis is characterized by periods of quiescence and exacerbations. The current definition of a pulmonary exacerbation in children is based on historical information and clinical criteria of onset of new symptoms. Currently used definitions include the presence of two or more of these symptoms, namely, increased tachypnoea or dyspnoea, change in frequency of cough, increase in sputum productivity, fever, chest pain and new infiltrates on the chest x-ray [129,130]. This definition of an exacerbation has limitations, as it was extrapolated from data on adults with chronic obstructive pulmonary disease (COPD) [131]. In the context of cystic fibrosis exacerbations are used as an outcome parameter in clinical trials [130]. In the paediatric setting, the validity of the definition is further compromised by the fact that a second hand history is obtained from a caregiver.

2.4 Immunological markers and bronchiectasis

The human airway is continuously exposed to airborne pathogens, which are cleared by interactive processes, involving both the innate immune system and mechanical clearance mechanisms in the lung. Bronchiectasis is thought to occur due to the de-regulation of the innate and adaptive immune system, with uncontrolled recruitment and activation of inflammatory cells in the airway [132]. HIV infection results not only in depletion of immune cells, but also in qualitative defects of immune cells. These abnormalities involve both the innate (macrophages, complement, phagocytes and neutrophil activity and function) and adaptive immunity functions (T and B-cells). The Langerhans cells and the $CD4^+$ T lymphocytes are the initial targets of the HI virus, although other dendritic cells also play a role [133]. It is thought that HIV infects resting $CD4^+$ T lymphocytes resulting in homing of CD4 cells into the lymph nodes [134]. During this homing process apoptosis occurs only after secondary signals are activated through the homing receptors. This process results in generalised lymphadenopathy, which occurs in an orderly fashion, both in simian HIV, and in humans [133]. The order of the lymph node involvement occurs in a cranio-caudally fashion with initial involvement of the upper torso followed by the lower limbs, and finally, gastrointestinal lymph nodes [134]. There is also decreased generation of $CD4^+$ T cells [133]. This depletion in the immune system results in the increased susceptibility to infections, particularly in the respiratory tract, where active immune

surveillance by the innate immune system, in particular neutrophils and macrophages, is required.

The depletion in T cells results in immune dysregulation that mediates a switch from T helper-1 (Th1) mediated (cellular) immune responses (which are involved in activity against infectious antigens); to a B cell dependent T helper 2 (Th2) mediated (humoral) immune activity (Table 2) [134,135]. Cytokines are intracellular signalling molecules whose function is to regulate the proliferation, differentiation and activation of immune cells [136]. Cytokines have many physiological functions, which assist in an organism's response to microorganisms, as well as playing a pivotal role in inflammatory and anti-inflammatory responses. Changes in cytokine levels in HIV-infected individuals may influence HIV viral control and $CD4^+$ T cell homeostasis, both potentially, negatively or positively. There is evidence in the adult literature, which suggests that the switch to a Th2 cytokine production, with hyperglobulinaemia, is associated with a more rapid progression to AIDS [137,138]. Even prior to $CD4^+$ T cells depletion, there is a qualitative defect in $CD4^+$ Tcells, which results in loss of antigen and mitogen-induced IL-2 and interferon gamma (INF-γ) production [139]. These are key cytokines in the Th1 pathway.

Table 2. Inflammatory and anti-inflammatory cytokines and chemokine involved in chronic inflammation adapted from [135]

	Cytokine	Source	Mechanism
Th-1	IL-1β	Macrophages	↑neutrophil production bone marrow, ↑ TNF-α and IL-6 production, ↑MMP production, ↑ COX-2 production and ↑ adhesion molecules and chemokines and ↑histamine release.
	IL-6	Mononuclear phagocytes, T-cells	↑Liver production of APR, ↑growth factors for mature B-cells and increase IL-2 expression.
	IL-8	Macrophages, activated T- cells	Chemotactic migration and activation of neutrophils, monocytes, eosinophils and lymphocytes to inflammatory site. ↑neutrophil adherence to endothelium by ICAM-1 upregulation.
	TNF-α	Activated macrophages and monocytes	PGE2 synthesis, induction of APR production by liver.
	INF-γ	Activated T cells and NK cells.	↑MHC class I and II expression on nucleated cells,↑ effector functions of mononuclear phagocytes. Activation of macrophages to kill intracellular pathogens.
	G-CSF	Monocytes, fibroblasts and endothelial cells	Stimulates neutrophils, perpetuates eosinophil activation and survival.
	GM-CSF	Monocytes, fibroblasts and endothelial cells	Stimulates neutrophils, perpetuates eosinophil activation and survival.
Th-2	IL-2	Activated T helper cells	Growth factor/activator for T cells, NK cells, and B cells. Promotes the development of LAK cells. Increased lymphokine secretion of IFN-γ, IL-3, IL-4, IL-5 and GM-CSF.
	IL-4	$CD4^+$ T cells, mast cells and basophils	Induces $CD4^+$ T cells to differentiate into Th2 cells, promotes immunoglobulin class switching to IgG1 and IgE, stimulates collagen and IL-6 production.
	IL-5	$CD4^+$ T helper cells and NK cells	Eosinophil differentiation and activation and stimulation of immunoglobulin class switching to IgA, stimulates IgE production and mast cell /eosinophil stimulation.
	IL-13	Th2 lymphocytes	Increases CD23 expression and induces IgG4 and IgE class switching.
	IL-17	Activated T lymphocytes	Stimulation of IL-6 and IL-8 production and ↑ ICAM-1 expression.

	Cytokine	Source	Mechanism
Chemokines	MIP-1β	Monocytes	Chemotactic migration and activation of monocytes, lymphocytes to inflammatory site.
	MCP-1	Monocytes	Chemotactic migration and activation of neutrophils, monocytes, eosinophils, lymphocytes to the inflammatory site.
	IP-10	Monocytes, fibroblasts and endothelial cells	Chemoattractant of activated T cells, NK cells, dendritic cells and monocytes.
Anti-inflammatory	IL-4	T cells	Inhibits production of pro-inflammatory cytokines: IL-1, IL-6, IL-8, and TNF-α.
	IL-6	Phagocytes and T cells	Inhibits TNF-α and IL-1 and increases IL-ra.
	IL-10	$CD4^+$ T cells, activated $CD8^+$ T cells, and activated B cells	Inhibits IFN-γ production by NK cells, inhibition of IL-4 and IFN-γ induced MHC class II expression on monocytes and reduction of antigen-specific T cell proliferation.
	IL-1ra	Immune complexes, neutrophils, macrophages	Inhibits IL-1 by competitive binding to the IL-1 receptor and induces IL-6 synthesis.
	IL-13	Th2 lymphocytes	Inhibiting the production of inflammatory cytokines, such as IL-1β, IL-6, IL-8 and TNF-α.

↑; Increase; Th: T helper; IL: Interleukin; MHC: major histocompatibility complex; MMP metalloproteinase; TNF-α: tumour necrosis factor alpha; MIP-1β: macrophage inflammatory protein-1 beta; MCP-1: monocyte chemotactic protein-1; IP-10: interferon gamma inducible protein-10; IL-1ra: interleukin 1 receptor antagonist; APR: acute phase reactants; lymphokine-activated killer cells (LAK); IgG1: Immunoglobulin G 1; PGE2: prostaglandin E2; NK cells: natural killer cells; GMCSF; granulocyte colony stimulating factor; ICAM: intracellular adhesion molecule; COX: Cyclooxygenase; Ig: immunoglobulin;

A reduction in IL-2 levels results in a switch to IL-4 production, a critical step in the switch to a Th2 mediated response [139]. IL-4 drives the development and expansion of Th2 cells and mediates downstream effector functions, such as B-cell activation, in particular increased major histocompatibility complex (MHC) class II expression and isotype switching to IgE production [140]. Although the exact mechanism is not well understood, a possible role of HIV antigens, gp120 and HIV-1-trans-activating protein (Tat protein) are suspected to be integral to this process. Gp120 is thought to act as a super-antigen, stimulating the immune system with a bias toward Th2 cytokine production via release of IL-4 and IL-13 from human F epsilon R positive cells ($F_{c\varepsilon}R1$) [141]. With declining $CD4^{+}$ Tcells, there is polyclonal hyperglobulinaemia, which also involves immunoglobulin G (IgG). In CF-related bronchiectasis, IgG levels have been found to be associated with a poorer prognosis, which is postulated to be related to higher antigenic exposure from systemic presentation of antigens through damaged airway mucosa [142,143].

Tat protein may also act as a chemoattractant for $F_{c\varepsilon}R1$ positive cells and may also upregulate CCR3 expression [144]. Some pro-inflammatory cytokines INF-α, INF-Y and granulocyte macrophage colony stimulating factor (GM-CSF) have been found to decrease HIV replication in tissue culture whilst IL-2 and tumour necrosis factor alpha (TNF-α) contribute to enhanced replication [145]. The plasma activation marker, soluble TNF receptor II (sTNFRII) has been found to correlate with AIDS progression, independent of HIV viral load and $CD4^{+}$ T cells [146]. The chemoattractant interferon gamma induced protein (IP-10) was also found to correlate independently with HIV viral load [146]. This chemokine plays an important role in viral infections, including influenza [147]. In the presence of HAART pro-inflammatory cytokines, IL-1β, IL-6, IL-8, IL-12, IL-17, GM-CSF and TNF-α were found to be similar in HIV-uninfected women when compared to HIV-infected women with adequate HIV viral suppression on HAART [145].

The prototype for bronchiectasis, CF, is a genetic disorder caused by a defect on chromosome 7; resulting in an abnormal cystic fibrosis transmembrane regulator

(CFTR) gene. This results in an abnormal chloride secretion by the apical epithelial cells. The accumulation of aberrant CFTR in the endoplasmic reticulum is thought to result in calcium release and stimulation of NFκβ. NFκβ causes the release of IL-8 and inflammation of the airway. As the inflammatory process becomes chronic; there is histotoxic inflammation with an increase of lymphocytes and monocytes. This process occurs in the CF airway with a continued predominance of neutrophils [148,149]. It is thought that the chronic infections that occur in CF, cause an increase in granulocyte colony stimulating factor (G-CSF) and GM-CSF, with signalling of reduction in cellular apoptosis, causing this persistence of neutrophilic airway inflammation.

Pathogens interact with the host's immune system via specific pattern recognition proteins (PRP), whose function is to mediate rapid clearance of the organism, through downstream activation of chemokines and cytokines. The innate immune system is activated by pathogen associated molecular patterns (PAMPs), which are recognised by pattern recognition receptors such as toll-like receptors (TLR) [150]. TLR activation triggers a cascade resulting in the activation and nuclear translocation of nuclear factor κβ (NFκβ) with subsequent release of pro-inflammatory cytokines IL1β, IL-8 and TNF-α [151]. IL-8 is a potent chemoattractant for neutrophils [152]. Neutrophils are integral to the innate immune mechanisms in the lung, with neutrophilic inflammation central to the pathogenesis of bronchiectasis. Elevated levels of neutrophil derived products IL-6, IL-8 and TNF-α have been found in the sputum of adults with stable bronchiectasis [153]. Transepithelial migration of neutrophils from the intravascular compartment occurs in a co-ordinated fashion with interplay of various adhesion molecules. Three families of adhesion molecules are involved; i.e. the selectins, integrins (CD11/CD18) and the immunoglobulin superfamily (intravascular adhesion molecule 1 (ICAM-1) and vascular adhesion molecule (VCAM)-1 [138]. These adhesion molecules are upregulated in the presence of IL-1, IL-8 and TNF-α. Both VCAM-1 and ICAM-1 have been found to be elevated in bronchiectasis subjects [134]. Adherent neutrophils migrate to the inflammatory site under the direction of the neutrophil chemoattractant IL-8. Activated neutrophils produce neutrophil elastase (NE) and matrix metalloproteinases (MMP)-8 and MMP-9. NE is an omnivorous enzyme produced

during phagocytosis and neutrophilic cell death. NE has three main mechanisms of action. Firstly, it has a proteolytic effect. Toxic products digest the airway elastin, basement membrane collagen and proteoglycans [138]. Secondly, it induces the release of cytokines IL-6, IL-8 and GM-CSF [132]. Finally, it's a powerful secretagogue inducing expression of the mucin gene MUC5AC, via the generation of reactive oxygen species [132]. In CF, free elastase has been found to be associated with reduced opsonisation of pathogens, thus acting as a potent stimulator for IL-8 production [154]. This elevation, coupled with the elevated proteases released from neutrophils, namely NE, MMP-2, MMP-6 and MMP-9; overwhelm the anti-protease defence mechanisms rendering the lung vulnerable to destruction [104,154,155]. MMP-9 levels have been found to correlate with IL-8 and pulmonary function reduction, in children with CF [156].

GM-CSF is a potent chemokine that allows prolonged survival of neutrophils in the airway. The intensity of the pro-inflammatory cytokine and chemokine responses; IL6, IL-8 and GM-CSF, is higher in subjects with airway colonisation by microorganisms. The use of antibiotics reduces the production of these pro-inflammatory mediators [157-160].

There is still no ideal marker to distinguish between infection and inflammation in bronchiectasis. The triggering receptor expressed on myeloid cells (TREM)-1 holds promise as such a biomarker for acute infection in this context. TREM is a 30k-Da glycoprotein of the immunoglobulin superfamily that is expressed on myeloid cells. It is coded for by genes residing on chromosome 6. TREM-1 has been found to be critical in the innate immune system and causes an amplification of the host's response to microbial agents, in the presence of TLR2 or TLR4 ligand mediated responses. TREM-1 has a short intracellular domain and when bound to these ligands, it associates with a signal transduction molecule, DAP12, which triggers secretion of inflammatory cytokines (IL-6, IL-8, GMCSF, TNF-α and macrophage chemotactic protein [MCP]-1) that amplify the host's response to microbial agents. There is also then reduction in the production of the anti-inflammatory cytokine IL-10 [161-165]. TREM-1 is mainly expressed in blood neutrophils, alveolar macrophages

and monocytes. It also triggers degranulation of neutrophils, calcium mobilisation and tyrosine phosphorylation of mitogen-activated proteins (ERK1 and ERK2) [164]. The membrane bound form of TREM-1 is liberated by the proteolytic cleavage of its extracellular domain by MMPs to produce a soluble form (sTREM-1). sTREM is a 27kDa protein that can be identified in biologic fluids and is upregulated on phagocytic cells, in the presence of bacteria (especially PA, *Staphylococcus aureus* (S. aureus) and fungi such as *Aspergillus fumigatus* [166]. TREM-1 has also been implicated in neutrophil/platelet interactions, with subsequent mediation of platelet-induced activation of neutrophils [167]. In contrast sTREM-1 is not upregulated in non-infectious inflammatory diseases such as ulcerative colitis and psoriasis [163].

sTREM-1 can be measured in serum, sputum and pleural fluid. sTREM-1 is demonstrating promise as an inflammatory biomarker of acute infection in various pulmonary conditions including CAP, ventilator associated pneumonia, non-tuberculous mycobacterial infection and COPD [168-170]. A previous in vitro study in CF, has demonstrated contradictory results with low levels of sTREM demonstrated in CF monocytes, suggesting that in CF-bronchiectasis, sTREM levels are reduced, and this is postulated to being due to a down-regulation of monocytes to endotoxin challenge [171].

2.5 Treatment of bronchiectasis

Interventions in the management of HIV-related bronchiectasis include medical as well as adjunctive therapies. The therapeutic goals of treatment include the following: restoration of the immune system, promotion of mucociliary clearance, prevention of further lung damage, promotion of normal growth, avoidance of toxins, identification and management of complications, and treatment of exacerbations to retard disease progression [172]. The use of HAART is critical to the reconstitution of the immune system to reduce the risk of additional lung infections in HIV-infected individuals.

Although airway clearance with chest physiotherapy is universally recommended, the evidence for benefit is limited. A Cochrane review demonstrated no improvement in pulmonary function parameters in patients who had regular multi-modality airway clearance techniques [173]. The benefit to individuals seems to lie in the reduction of cough frequency and improvement in quality of life [174]. The technique used does not appear to have any impact on the outcome, although in patients with gastroesophageal reflux, care should be taken when instituting techniques that use the head down position. This is particularly important in young children.

In bronchiectasis, the rheological properties of mucus are abnormal, with variations in rheology depending on the cause of bronchiectasis. In childhood post-infective bronchiectasis mucus is less viscous and more transportable than that of children with CF [175]. The agents used for airway clearance are either mucolytics or airway hydrators. Mucolytic agents reduce mucus viscosity and promote clearance of secretions. They do this via several mechanisms, which include; disruption of disulphide bonds and liquefying proteins that degrade DNA filaments and actin. This modality of treatment is attractive in a condition where increased mucus tenacity and viscosity is a problem. Recombinant DNAse (rhDNAse) has been used with excellent results in CF. However; in non-CF bronchiectasis, the results have been disappointing. In a large multi-centre trial by O'Donnell et al, rhDNAse was found to have detrimental effects on participants, with accelerated decline in pulmonary function [14]. Forced vital capacity (FVC) was reduced by 3.1% in the rhDNAse group when compared to placebo group. Patients also experienced increased exacerbations in the intervention group. This finding is in contra-distinction to the benefits documented in CF. This may have several explanations; firstly, there are differences in rheological properties of mucus in the CF airway when compared to the non-CF bronchiectatic airway [175]. Secondly, in CF, the pathology is mostly in the upper lobes, and the use of mucolytics may therefore facilitate mucus clearance with gravity, whilst in non-CF bronchiectasis the lower lobes are affected and this may thus hamper the effective clearance of thin secretions against gravity [9,14]. Due to the harm demonstrated in this study there have been no paediatric studies conducted using rhDNAse in non-CF bronchiectasis. Therefore, the use of rhDNAse is strongly discouraged in patients with non-CF bronchiectasis. The uses of mucus

hydrators, such as hypertonic saline and mannitol have been studied in non-CF bronchiectasis. Hypertonic saline has shown benefit in one small adult study when used in conjunction with chest physiotherapy [176]. A Cochrane review and a recent trial of the use of mannitol, demonstrated benefit of this agent, in changing the physical properties of mucus in fourteen adults with bronchiectasis [177,178]. Larger trials are needed to assess the efficacy of mucus hydrators.

Antibiotic therapy forms the cornerstone of bronchiectasis treatment. The use of antibiotics can prevent airway damage by treating infections, maintain and improve pulmonary functions, as well as improve quality of life. PA infection is rare in children with non-CF bronchiectasis [129]. Inhaled antibiotics have been extensively studied in the context of CF. This route of drug delivery has the benefit of targeted drug delivery, limitation of systemic drug absorption and reduction in side effects. This therefore, renders inhaled therapies a more attractive option in the treatment of bronchiectasis. The drug doses required for oral and intravenous antibiotics, to achieve bactericidal levels in airway secretions, require plasma levels to be between 10 and 25 times above the mean inhibitory concentration. For optimal benefit of inhaled drugs, they need to be at a pH above 4.0 and have an osmolarity between 100-1100 mOsmol. Several antibiotics, including tobramycin, ceftazidime and gentamycin, have been studied, especially in the context of CF in subjects colonised with PA[179-181]. There is currently insufficient evidence for the recommendation of the use of inhaled antibiotics, especially since pseudomonas colonization is rare, in non-CF bronchiectasis in children, although small studies with inhaled tobramycin, colistin and aztreonam have suggested some benefit [179].

The management of exacerbations entails the use of broad-spectrum antibiotics that cover the common CAP pathogens. In HIV-infected children it is important to regularly survey for fungi and mycobacteria (both MTB and atypical mycobacteria).

Anti-inflammatory drugs such as corticosteroids, are natural candidates in the management of bronchiectasis, as they can play a pivotal role in breaking the cycle

of inflammation. Steroids mediate their anti-inflammatory effects by reducing inflammatory cytokines, inhibiting prostaglandins, reducing adhesion molecules and inhibiting nitric oxide in the airway. Regrettably, systemic corticosteroids can not be used long term due to their of their unfavourable side-effect profile. Inhaled corticosteroids (ICS) have been shown, in randomised trials, to reduce the number of exacerbations, reduce sputum volume and improve quality of life in bronchiectasis [182-184]. One randomised trial of eighty-six adults demonstrated that subjects colonised with PA derived the most benefit from the use of ICS [182]. In a systematic review fluticasone was found to cause adrenal suppression and Cushing's syndrome when used in combination with ritonavir over the long-term [185]. HIV-infected children, who are at risk of fungal infections and require itraconazole, often reveal systemic side effects when this drug is used in combination with inhaled corticosteroids [172].

Bronchodilators, principally the short acting beta agonists are used where there is a demonstrable bronchodilator response test on pulmonary functions. These drugs offer symptomatic relief to patients, however, no long-term studies have been performed on the value of these agents on other outcome measures in bronchiectasis.

With the failure of medical therapy, surgical intervention may be considered. Indications include localised disease, focal disease with failure to thrive and life threatening haemorrhage. Subjects who derive the most benefit from surgery are those with excessive sputum impaction (demonstrated on CT scan) and persistent airway obstruction. There have been no long-term follow up studies post pneumonectomy in HIV-infected children.

2.6 Immunomodulators and bronchiectasis

A. Macrolides and bronchiectasis

Macrolide antibiotics are a group of antibiotics that contain a macrocytic lactone ring with a number of sugar moieties attached to the ring. Macrolides are further sub-classified according to the number of lactone rings into the 14, 15 and 16-member ring macrolides (Table 3). The oldest of these drugs is erythromycin. Erythromycin is a 14-member macrolide, which was first isolated by McGuire, and colleagues in 1952 from *Streptomyces erythreus,* found in soil samples, in the Philippines. The other macrolides are semi-synthetic agents.

Table 3. Types of macrolide antibiotics

14 member ring macrolide	Erythromycin
	Troleandomycin
	Clarithromycin
	Roxithromycin
15 member ring macrolide	Azithromycin
16 member ring macrolide	Josamycin
	Spiramycin
	Midecamycin

Azithromycin is an azalide with an added methyl-substituted nitrogen atom onto the lactone ring, to form the 15-member ring. Clarithromycin is formed by the methylation of the hydroxyl group at position 6 of the lactone ring. These structural modifications confer on azithromycin and clarithromycin a slightly better side effect profile when compared to erythromycin. These modifications reduce the interaction of these drugs with drugs metabolised by the cytochrome P450 system. There are also significantly fewer gastrointestinal side effects with these agents. Azithromycin and clarithromycin

have a far superior tissue penetration in vitro and a longer elimination half-life; and thus require only once or twice daily dosing respectively. The drawback of the use of these agents is their significantly higher cost when compared to erythromycin. Erythromycin is a relatively inexpensive and effective drug. Macrolide concentrations are at least 10-fold higher in epithelial lung fluid than in serum [186].

The anti-bacterial mode of action of macrolides is by reversible binding to the 50s subunit of the ribosome in prokaryocytes. This results in prevention of ribosomal translation and thus prevention of bacterial replication. Macrolides are bacteriostatic for staphylococci, streptococci and *Haemophilus* but they may exert bactericidal effects at very high concentrations. Macrolides do not have bactericidal effects against PA but do result in inhibition of biofilm formation and also inhibit the organism's ability to produce alginate and other extracellular polysaccharides [187,188]. Macrolides are commonly used as first-line therapy for treatment of acute bacterial infections such as CAP in adults. The potential use of macrolides for their immune modifying effects was first discovered in patients with severe steroid dependent asthma [189]. The concomitant use of troleandromycin was found to result in significant improvement in asthma control in patients and also led to dose reduction of steroids without loss of asthma control. These immunomodulatory effects of macrolides are limited to the 14- and 15-membered ring macrolides.

The use of low dose macrolides in the management of chronic inflammatory lung disease was initially described in Japanese patients with diffuse panbronchiolitis (DPB) [190-192]. DPB a common condition in Japan and South East Asia, and is a progressive inflammatory disorder whose sufferers present with chronic productive cough, wheezing, exertional dyspnoea, chronic sinusitis, mucoid PA colonisation, mixed restrictive and obstructive pulmonary functions and diffuse chronic inflammation involving the bronchiolar and centrilobular regions of the airway. Untreated, DPB has a very poor prognosis; in 1984 the five-year survival rate was 26%. With the use of low dose erythromycin, the mortality of these patients was dramatically reduced, with 10-year survival rates increasing to 92% [193]. This was coupled with an improvement in pulmonary functions and improved quality of life. The immunomodulatory effects of macrolides include reduction in sputum volume,

inhibition of virulence factors produced by bacteria, diminished neutrophil influx, down regulation of IL-8 production, inhibition of NFκβproduction, reduction in ICAM-1 and reduced NE [30,31,194,195]. These immunomodulatory effects cause a reduction in pulmonary exacerbations, improved pulmonary function parameters and improved quality of life [25,26,28,190,192,193]. The clinical improvement of subjects may take up to three months to demonstrate an effect.

The use of macrolides is not only limited to DPB. In the late 1990s there was rekindled interest in the use of macrolides in the treatment of other chronic inflammatory lung disorders, including CF. In the setting of CF, azithromycin has been consistently found to result in a reduction in the number of pulmonary exacerbations, time to first exacerbation and improvement in nutritional parameters [20,22,196]. In CF, macrolides form part of the cornerstone of therapy in subjects colonised with PA*,* with emerging evidence of their benefit in CF subjects without PA[197]. With initiation of macrolides there is a modest initial improvement in pulmonary functions.

There are a few studies looking at the immunomodulatory role of macrolides in the management of patients with non-CF bronchiectasis (Table 4). One adult study by Tsang et al, studied the effect of erythromycin in patients with severe idiopathic bronchiectasis. They found a significant improvement in FEV_1, FVC, and sputum volume over a period of 8 weeks in 11 patients when compared to 10 controls [30]. In this study there was no change in the pro-inflammatory mediators (IL-1β, IL-8, TNF-α, and leukotriene B4). Only one retrospective observational study in children demonstrated improvements in small airway function (maximal mid-expiratory flow) and a reduction in IL-8 [195]. Trials conducted on macrolides in bronchiectasis are limited in patient numbers and length of treatment. However, universally all have shown a consistent reduction in the frequency of exacerbations and sputum volumes [22,28-30,198].

Table 4. A summary of clinical trials of the use of macrolide therapy in bronchiectasis

Author	Year	Study drug	Study design	Age group	Benefit
Tsang[30]	1999	Erythromycin	RDBPCT	Adult	↑FEV_1,↑FVC ↓sputum volume
Yalcin [28]	2006	Clarithromycin	RPCT	Paediatric	↓sputum volume,↓sputum cytokines
Koh[31]	1997	Roxithromycin	RDBPCT	Adult	↓airway reactivity to methacholine
Davies[194]	2004	Azithromycin	Prospective open-label	Adult	↓symptoms and ↑ D_{LCO}
Cymbala[198]	2005	Clarithromycin	Randomised open-label, crossover	Adult	↓sputum volume
Serisier[29]	2011	Erythromycin	Retrospective RCT	Adult	↓exacerbations ↓antibiotic use
Coeman[18]	2011	Erythromycin	Retrospective observational	Adult	Improved symptom scores
Anwar[24]	2008	Azithromycin	Retrospective observational	Adult	↑FEV_1 ↓exacerbations

Abbreviations: ↑, increased, ↓, decreased, D_{LCO} ,pulmonary diffusion capacity for carbon monoxide; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; RCT, randomised controlled trial; RDBCT, randomised double blind controlled trial; RDBPCT, randomised double blind placebo controlled trial

B. Macrolide resistance and safety

Long term use of macrolides results in resistance, particularly to streptococci, *Haemophilus* and staphylococci. There are three mechanisms by which resistance occurs [199]. Firstly, this may be due to ribosomal target modification mediated by methylases encoded by the *erm (*B) gene. Secondly, this may be due to mutations of the 23S rRNA or ribosomal proteins L4 and L22, which lead to conformational changes in the binding site of macrolides. Finally, active drug efflux occurs due to the

membrane bound efflux protein *mef* (A) gene. Phaff et al, found increasing resistance of *S. aureus* to macrolides in CF patients, with resistance levels reaching 17.2% in those consuming macrolides, versus 3.6% in those not using macrolides [200]. Tramper-Stranders et al, also found an exponential increase in staphylococcal resistance to macrolides, from 83% in the first year of therapy, to 100% in the third year of macrolide use [201].

There are safety concerns with regards to the long-term use of macrolides. This is based on the known cardiac side effects (*torsades de pointes*) with the use of macrolides, particularly erythromycin. The use of macrolides in conjunction with drugs that inhibit the CYP3A pathway may potentiate this effect. Fortunately, post-marketing surveillance of the long-term use of erythromycin in Japan, indicates this side effect to be extremely rare [199]. The prime concern with the use of macrolides for long-term anti-inflammatory therapy is the development of macrolide resistant non-tuberculous mycobacteria (NTM), which are commonly found in bronchiectasis patients. The newer macrolides azithromycin and clarithromycin form the backbone therapy for NTM management. The carriage of NTM is particularly high in patients with bronchiectasis, and more so in those co-infected with HIV. A multi-centre trial of CF subjects recovered NTM in 13% of over 900 subjects studied [202].

2.7 Metabolic imaging and bronchiectasis

The management of bronchiectasis involves aggressive antibiotic treatment of exacerbations, physiotherapy and optimal vaccinations to prevent pulmonary infections [203]. Current tools to assess disease severity and progression of bronchiectasis include; pulmonary function testing, sputum culture surveillance, chest imaging and measurement of lung inflammatory biomarkers. All of these tools have their limitations since there is currently no “gold standard” test for these assessments. In order to halt progressive lung tissue damage, careful attention to early identification and treatment of exacerbations is vital.

The definition of an exacerbation is based on new onset symptoms, which in paediatric patients is limited by the reliability of anamnesis provided by the caregiver or young child. Physiological measurements of pulmonary functions are useful in clinical follow-up of patients but can vary with time even in the absence of treatment and are usually not a specific measurement of the therapeutic intervention being studied. Reliable spirometry can also not be accurately performed in young children. Chest radiography is insensitive and provides gross anatomical localisation of pathology; whilst HRCT is the gold standard for diagnosing bronchiectasis and can be used for monitoring of structural lung changes. It does not however, provide any information on disease activity. There is also a concern for the patient’s radiation burden from HRCT, especially if serial scanning is performed, making this an unattractive option for regular follow-up. The use of newer ultrafast low dose HRCT may hold some promise but more data on this test is needed.

The current gold standard method to assess lung inflammation includes analysis of airway neutrophils obtained from bronchoalveolar lavage samples [204]. This procedure is invasive, but can provide information on specific lung segments and does not encapsulate the activity levels in the whole lung. However, the lung parenchyma, as well as the intravascular compartment, is inaccessible with these techniques. Sputum cultures are useful to guide antibiotic therapy but do not differentiate between chronic colonisation and acute infection.

Functional imaging using positron emission tomography with 2-[F-18]-fluoro-2-deoxy-D-glucose as a tracer (^{18}F-FDG-PET), is widely used in the diagnosis of oncological diseases, and frequently meets the criteria for evidence-based medicine in that context [205,206]. ^{18}F-FDG differs from glucose by the substitution of the hydroxyl group with a fluorine atom on the second carbon of the glucose. When injected intravenously, ^{18}F-FDG rapidly diffuses into the extracellular spaces throughout the body. It is transported into living cells by the same mechanism as glucose, via the D-glucose transporter (GLUT-1), and is phosphorylated by hexokinase to fluoro-deoxyglucose-6-phosphate [207]. The deoxy- substitution at the second carbon position prevents further metabolism and the product accumulates in the cell at a

rate that reflects glucose metabolism. Tracer uptake is enhanced in activated inflammatory cells including neutrophils, lymphocytes and macrophages. The use of ^{18}F-FDG, for in vivo cancer imaging, is based on high metabolic turnover of saccharides by tumour tissue. In the inflammatory response, neutrophils have an increased expression of the glucose transport protein, with up-regulation of the hexokinase activity, a feature that was first described by Warburg several decades ago [208]. Elevated ^{18}F-FDG accumulation in inflamed tissues is not only related to increased glucose metabolism in inflammatory cells, but also by macrophage proliferation and recruitment. ^{18}F-FDG, a glucose analogue in which the oxygen molecule in position 2 is replaced by a positron-emitting ^{18}fluorine, undergoes the same uptake as glucose, however, its first metabolite FDG-6-phosphate, cannot be further metabolised in the glycolytic pathways. As most tumours have low phosphatase activity, FDG-6-phosphate will be accumulated in the cell, resulting in so-called ‘metabolic trapping’ [206,209].

Elevated ^{18}F-FDG accumulation in inflammatory tissues is related to increased glucose metabolism that is produced by stimulated inflammatory cells, macrophage proliferation, and healing [210,211]. ^{18}F-FDG uptake in acute inflammation occurs primarily by activated neutrophils whose metabolism, during the respiratory burst, is triggered by rolling and adhesion phases and is heavily dependent on anaerobic glycolysis. On activation, resting lymphocytes switch to glycolysis and increase their glucose uptake, up to twenty fold, by increasing the expression of glucose transporters. Multiple cytokines and growth factors facilitate this glucose transport [212].

The role of ^{18}F-FDG PET has been explored in the context of inflammatory diseases in both HIV-infected and HIV-uninfected individuals. It’s role in the staging of HIV-infection, both in animal models as well as in adults, has demonstrated orchestrated lymph node involvement, which occurs in a cranio-caudal fashion [213,214]. In addition, it has shown promise in aiding the early diagnosis of both pulmonary, and extra pulmonary TB in HIV-infected and un-infected individuals [215,216]. Although CT has been found to be highly predictive of low-density lymph nodes with peripheral

enhancement for the diagnosis of TB, delayed ^{18}F-FDG PET images captured two hours post-injection, were found to identify fifty percent more lymph nodes than conventional CT alone [216]. The utility of ^{18}F-FDG PET extends to diagnosis of "cold" abscesses in TB that demonstrate moderate peripheral and low central activity because of a lack of an accompanying inflammatory reaction. In TB pulmonary lesions, there is high uptake of ^{18}F-FDG that is related to the high glycolytic rate of inflammatory cells. There are also case reports of the value of ^{18}F-FDG PET to detect opportunistic infections such as *Mycobacterium avium intracellulare* lesions in HIV- uninfected individuals [217].

^{18}F-FDG PET has been found to quantitatively delineate lung infection and inflammation in a diverse group of lung diseases including CF, pneumonia, pulmonary fibrosis, sarcoidosis and interstitial pneumonitis (Table 5) [218-222]. In CF, a well-described cause of bronchiectasis, Chen et al*,* demonstrated a positive correlation between the rate of ^{18}F-FDG uptake in the lung field, and the number of neutrophils present on bronchoalveolar lavage fluid [222]. ^{18}F-FDG PET has also been shown to demonstrate areas of enhanced uptake during pulmonary exacerbations in CF; these disappeared after antibiotic therapy [221]. In CF subjects with the highest signal on PET scanning, those with the most rapid pulmonary function decline, correlated well with the number of neutrophils present in bronchoalveolar lavage fluid [221]. Chen et al*,* also demonstrated ^{18}F-FDG PET to be a useful in assessing anti-inflammatory therapy after challenge with endotoxins. These authors postulated that PET scan may therefore serve as a platform for assessing response to therapy, particularly anti-inflammatory therapies [222]. Therefore, it appears that ^{18}F-FDG PET is a highly sensitive tool for assessing inflammation and infection, although it still lacks specificity for the accurate diagnosis of lung disease. ^{18}F-FDG PET can also localise sites of infection by various pathogens including PA, and *Staphylococcus* at various sites.

Table 5. Summary table of clinical trials using ^{18}F-FDG PET for chronic pulmonary diseases

Author	Year	Condition (number)	Finding
Jones[218]	1997	Acute pneumonia (5) Bronchiectasis (5)	↑uptake in pneumonia Diffuse minor uptake
Labiris[219]	2003	Cystic fibrosis (10)	No change in glucose utilisation
Jones[220]	2003	COPD (6) Asthma (6)	↑uptake in controls No uptake
Chen[222]	2006	Cystic fibrosis (20)	↑uptake correlated with BAL neutrophils and disease severity
Klein[221]	2009	Cystic fibrosis (20)	↑uptake during exacerbation which disappeared after antibiotics

↑uptake: increased ^{18}F-FDG uptake; BAL: bronchoalveolar lavage; COPD: chronic obstructive pulmonary disease

CHAPTER III

SCOPE OF RESEARCH AND HYPOTHESIS

Antenatal infection rates of HIV remain high in South Africa, with transmission infection rates having currently reached a plateau at 29% [46,47]. Due to the slow response to the HIV epidemic by the South African government (which provides healthcare to the majority of South Africans), measures to prevent MTCT have also lagged behind [69,70]. This has resulted in a large number of children with vertically transmitted HIV in the population. These HIV-infected children have a high risk of acquiring respiratory tract infections [18,19]. Even with the availability of HAART, HIV-infected children still have a higher background risk of lower respiratory tract infections when compared to un-infected children [19]. The end product of recurrent or destructive lung infections is the development of bronchiectasis, with the airway “locked” into an inflammatory cycle [123].

Worldwide the most common cause of bronchiectasis in children is CF. Bronchiectasis outside the context of CF is very poorly studied and is regarded as an “orphan” lung disease, with insufficient funding and research devoted to this condition. The pathophysiologic and immune mechanisms of bronchiectasis have been shown to be different in CF when compared to other forms of bronchiectasis [173].

The data available in non-CF bronchiectasis is limited by the fact that the majority of studies are from small cohort studies, and include subjects with bronchiectasis from heterogeneous causes [6]. There are previously identified risk factors for bronchiectasis, which have been described in both the developed world and developing world. These factors have not been previously studied in children with HIV-bronchiectasis in the developing world, where barriers to accessing health care, and socioeconomic risk factors, may enhance the burden of respiratory tract infections. Hence the importance of this study and thesis. The author therefore undertook to study the epidemiologic factors associated with HIV-related

bronchiectasis in a developing world context. In addition this research sought to uncover the potential risk factors for development of bronchiectasis.

The focus of research in developing countries is usually on epidemiologic data with little attention to mechanistic or therapeutic aspects of non-CF bronchiectasis. The hallmark of HIV-infection is the immune system dysregulation, which affects both innate and adaptive immune system functions [133]. There are limited data available on the systemic and pulmonary immune markers in children with HIV-related bronchiectasis. Knowledge of the immune functions in HIV-related bronchiectasis may help in improving understanding, not only of the pathophysiology of bronchiectasis, but may inform new therapeutic strategies in this condition.

There is a lack of a standardised definition of an exacerbation of bronchiectasis with various tools and definitions being used even in research settings. Some authors have used clinical definitions; whilst others have attempted to use both clinical and objective biomarkers to define exacerbations. An ideal for a biological marker would be one that is easy to perform, rapid, reliable and inexpensive. This would therefore guide antibiotic therapy, reduce overuse of antibiotics, reduce the risk of antibiotic resistance and reduce side effects. This is even more important in the context of HIV infection where there is a high pill burden and the risk of drug-to-drug interactions. sTREM-1 has shown promise as an ideal marker for diagnosis of acute inflammatory lung diseases.

Metabolic imaging is emerging as a diagnostic technique for inflammatory diseases. The co-registration of PET scanning and HCRT also has the added benefit of providing both anatomical localisation of pathology, as well as an appraisal of metabolic disease activity. This makes ^{18}F-FDG PET an attractive tool for the diagnosis and management of inflammatory diseases, particularly chronic inflammatory pulmonary disease. There are limited data on the use of PET/CT for the diagnosis of exacerbations in bronchiectasis, with one study in a cohort of CF subjects, suggesting its utility for this application [221].

The management of non-CF related bronchiectasis is complicated by over-reliance on data from CF. With the limitations of currently available therapies and lack of progress in new drugs to manage non-CF bronchiectasis, there is a need for novel approaches and study of interventions that can modulate or retard the "inflammatory cycle" in bronchiectasis. Macrolides are the natural candidate drugs for this process, as they have a proven track record in both CF-bronchiectasis and other chronic inflammatory lung diseases. There is, however, a lack of large randomised placebo-controlled trials in non-CF bronchiectasis [28-30]. The current available data on macrolides under study are the newer macrolides, which are expensive and would generally be inaccessible for patients in developing countries. There is therefore a need for the study of macrolides which are cost-effective and affordable in developing countries where HIV and bronchiectasis are common. The newer macrolides also form a critical component of the treatment of NTM, organisms that are prevalent in HIV-infected persons [223,224]. With the increased use of these drugs in bronchiectasis treatment, there is concern for development of resistance to these agents. Erythromycin fulfils the criteria for the unmet needs in the management of HIV-related bronchiectasis, as it is both inexpensive and does not form part of the therapy for NTM infections.

This thesis was therefore embarked on, with the following research questions:

a. What are demographics of children with HIV-related bronchiectasis?

b. What are the predisposing and aggravating factors for the development of HIV-related bronchiectasis?

c. What are the organisms found in the airways of children with HIV-related bronchiectasis? What role does TB play in the population of children with HIV-related bronchiectasis?

d. What are the local and systemic inflammatory and anti-inflammatory cytokines/chemokines in HIV-related bronchiectasis?

e. What is the role of the innate immune marker, sTREM-1, in HIV-related bronchiectasis in comparison to a control group of children with CF?

f. Does ^{18}F-FDG PET have the ability to detect sites of active inflammation in children with HIV-related bronchiectasis, with or without exacerbations?

g. Is there agreement between ^{18}F-FDG PET and local and systemic inflammatory biomarkers and markers HIV disease activity?

h. What is the efficacy of erythromycin versus placebo, in reducing the number of pulmonary exacerbations in children with HIV-related bronchiectasis over a period of 52 weeks (1 year)?

i. Does erythromycin have an impact on pulmonary function parameters, pro-inflammatory and anti-inflammatory cytokines/chemokines and sTREM-1 when compared to placebo in HIV-related bronchiectasis?

CHAPTER IV

SUBJECTS AND METHODS

A brief overview of the study subjects and methodology is provided here. The methodology for each of the components in the research will be described in subsequent chapters.

SUBJECT SELECTION

Due to the high number of children being referred to the tertiary Paediatric Chest Clinic, Steve Biko Academic Hospital, Pretoria, South Africa, for chronic chest symptoms, an HIV-related bronchiectasis clinic was set in motion at this hospital.

Bronchiectasis was suspected in children if they presented with a chronic suppurative cough, clubbing and halitosis. This cohort of children was screened for inclusion in the study and underwent CT chest for detection of bronchiectasis. The study subjects were enrolled between January 2009 and June 2010. Subjects were regarded as eligible if they met the inclusion criteria and had no exclusion criteria.

Inclusion criteria

- Subjects between the age of 6–18 years;
- Ability to perform reliable pulmonary function tests;
- Able to attend monthly follow up clinics for 52 weeks;
- Informed consent given to participate in the study;
- Assent given for all children over the age of 7 years;
- Confirmed to be HIV-Elisa positive if diagnosed over the age of 18 months or a confirmed HIV-PCR positive test if diagnosed under the age of 18 months;
- Bronchiectasis confirmed on high resolution computed tomography.

Exclusion criteria

- Lack of informed consent for HIV testing;
- Inability to perform reliable pulmonary function tests;
- Bronchiectasis related to cystic fibrosis or other identifiable cause for bronchiectasis;
- Subjects on the following medications that could interact with erythromycin: the anti-epileptic carbamazepine, anti-coagulants e.g. warfarin, ergotamine tablets for migraine headaches, long term midazolam use or cyclosporin;
- Poor compliance to medication due to mental impairment in the caretaker/guardian;
- Subjects already enrolled in another clinical trial.

Study entry

The following baseline (Visit 1) investigations were performed in all the subjects (Appendix A):

1. Detailed clinical assessment including anthropometry and detailed clinical examination.
2. Baseline chest radiograph with lateral view.
3. Combination ^{18}F-FDG PET/CT scan.
4. Baseline blood testing including full blood count, liver function testing, urea and electrolytes, CD4 count, HIV-viral load, Radio Allergo Sorbent Test (RAST) test for *Aspergillus*, RAST test for Phadiatop and Paediatric food mix (FX5), Immunoglobulins (Ig) - IgA, IgG, IgG subclasses, IgM and IgE.
5. HIV ELISA after written informed consent following appropriate counselling if not previously performed.
6. Serum sample for cytokine assays.*
7. Induced sputum for microbiology and TB culture.

8. Induced sputum for cytokine assays.*

9. Pulmonary function test with bronchodilator response test.

10. Screening sweat test.

*Circulating cytokines/chemokines, as well as those in induced sputum measured were: IL-1β, IL-ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17, GM-CSF, MCP-1, INF-γ, TNF-α, IL-1β, IP-10, G-CSF, GM-CSF, sTNFRI, elastase and sTREM-1.

Subjects were randomised, in a double-blind study, to receive either placebo or erythromycin (125mg per os daily if <15kg or 250mg per os daily if >15kg once per day for the study duration). All subjects were followed up monthly (Visit 2-11) for a period of 52 weeks. At the baseline visit all the subjects were given a daily medication diary to complete for the study medication.

Subjects were instructed to report to the clinic immediately if an exacerbation (per protocol) was noted.

Follow up visits

At the monthly visits the following study related procedures were performed.

- Detailed clinical examination and anthropometric measurements;
- History of any exacerbations and instituted treatment over the previous month;
- Noting of any medication taken besides normal treatment and study medication;
- Pulmonary function tests;
- Induced sputum for microbiology;
- Induced sputum for TB where suggestive symptoms or suspicion of new infection;

- Review of medication diary and assessment of compliance via pill-count and interview;
- Collection of medication diary.

Study end

At the end of the study on week 52 of the study (Visit 12) the following study procedures were performed:

- Detailed clinical examination and anthropometric measurements;
- Pulmonary function tests;
- Combination ^{18}F-FDG PET/CT scan;
- Induced sputum for microbiology and tuberculosis;
- Induced sputum for cytokine assays;
- Serum sample for cytokine assays;
- Serum for IgA, IgE, IgG, IgG subclasses and IgM;
- Serum for full blood count, liver function test, urea and electrolytes, $CD4^+$ T cell count and HIV-viral load.

STATISTICAL ANALYSIS

The statistical evaluations were performed with the help of statisticians from the Medical Research Council of South Africa as well as the Clinical Epidemiology Unit of the University of Pretoria. The statistical software package used was Stata Release 10 and 11 (Stata Corp LP, College Station, TX, USA), and elaboration of all the relevant statistical methods used will be included in the relevant sections of the thesis.

CHAPTER V

DEMOGRAPHIC CHARACTERISTICS AND EPIDEMIOLOGIC DETERMINANTS OF CHILDREN WITH HUMAN IMMUNODEFICIENCY VIRUS-RELATED BRONCHIECTASIS

5.1 OBJECTIVES

The objectives of this study were to determine the demographic findings of children with HIV-related bronchiectasis. Additional objectives were to document the potential predisposing and aggravating factors in the development of bronchiectasis. In addition it sought to explore the pulmonary pathogens, including tuberculosis, cultured in the airways of children with HIV-related bronchiectasis.

5.2 SUBJECTS AND METHODS

5.2.1 SUBJECTS

All children with HIV-related bronchiectasis attending the Paediatric Chest Clinic at the Steve Biko Academic Hospital, Pretoria, South Africa, from January to November 2009, were invited to participate in the study. Patients were enrolled if they exhibited any symptoms suggestive of bronchiectasis, namely chronic productive cough, clubbing or halitosis. In addition, all children must have had both radiological and chest computed tomographic confirmation of bronchiectasis. HIV diagnosis was confirmed to be present through: a positive HIV-ELISA for children diagnosed at age greater than 18 months, or a confirmed positive HIV-PCR test if diagnosed under the age of 18 months. An important inclusion criterion was that only children aged 6-18 years who were able to perform reliable lung function tests be included in the study. After screening, fifty-six children were confirmed to have HIV-related bronchiectasis; however, thirteen were excluded due to their age (being less than 6 years) (Figure 4). Forty-three participants (77%) were eligible for inclusion. In one participant parental consent was not granted and this subject was therefore excluded. Seven participants were lost to follow up, with a total of 35 children included in the final analysis.

5.2.2 METHODS

Clinical investigations

All participants had demographic variables recorded on a data collection sheet, which included: age, gender, weight (kg), height (m), body mass index (kg/m^2) and socioeconomic status (indices used were receipt of a social grant and type of household cooking fuel) [Appendix A]. The WHO growth charts were used to calculate the weight, height and BMI z-scores [225]. The following were also recorded: age at HIV diagnosis, timing of initiation of HAART, home exposure to ETS, type of method used for cooking in the household and prior and current treatment for TB.

Pulmonary function testing performed included forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC and forced expiratory flow ($FEF_{25–75}$). Spirometry was measured using the Viasys SpiroPro Jaeger Spirometer (Hoechberg, Germany) by an experienced lung function technologist.
An exacerbation was defined clinically as the presence of at least two of the following parameters: increased tachypnoea, dyspnoea, change in frequency of cough, increased sputum productivity, fever, chest pain and new infiltrates on chest x-ray [226].

Laboratory investigations

A study dedicated physiotherapist performed sputum induction with 5 ml, 5% hypertonic saline administered via a facemask with an ultrasonic nebuliser (Goodwish KWC 6Td Nanjing City, Jiangsu province, China), followed by postural drainage with percussions and vibrations. The sputum samples obtained were sent for analysis for respiratory bacteria (including TB)and respiratory viruses (*Respiratory syncytial virus*, *Influenza A* and *B*, *Parainfluenza*1–3, *Adenovirus* and *Cytomegalovirus*). No specific testing was requested for mycology unless indicated. Monthly sputum samples were sent for microbiological testing. Of these, 17.8% were collected during an exacerbation.

Serum samples were collected for the following investigations: $CD4^+$ T lymphocytes, HIV viral load, C-reactive protein (CRP) using turbidimetry (UniCel DxC 880i, Beckman Coulter analyser). Circulating concentrations of IgA, IgE, IgG and IgM, as well as those of the IgG subclasses, IgG_1, IgG2, IgG_3 and IgG_4, were assayed by nephelometry (Siemens Healthcare Diagnostics, BN Prospec Nephelometer, Newark, NJ, USA) using materials and controls supplied by Siemens Healthcare Diagnostics. Serum samples were sent for ImmunoCAP® RAST testing for *Aspergillus fumigatus* (Phadia AB, Uppsala, Sweden).

Screening sweat chloride conductivity (Nanoduct™ Neonatal Sweat Analysis System, Wescor, Inc., South Logan, UT, USA) was performed in all participants.

Statistical analysis

Data analysis was performed using Stata Release 10 (Stata Corp LP, College Station, TX, USA) and the Spearman's rank correlation coefficient test was used to assess the correlation between markers of HIV disease activity, pulmonary function testing, TB and socioeconomic status indices. The Wilcoxon rank sum test (Mann-Whitney test) was used for the comparison between the participants with and without HIV viral load suppression. The Mann-Whitney test was also used for the comparison of groups exposed and un-exposed to ETS and for comparison of bacterial culture positive and negative participants. Testing was performed at the 0.05 level of significance.

Ethical Clearance

Ethics approval, to conduct the study, was granted by the Research Ethics Committee of the Faculty of Health Sciences, University of Pretoria (protocol number 100/2008 [Appendix B]). Signed informed consent was obtained from the parents/guardians of all enrolled participants. Assent was obtained from all children over the age of 7 years (Appendix C). The study was conducted in accordance to Good Clinical Practice Guidelines and the Declaration of Helsinki.

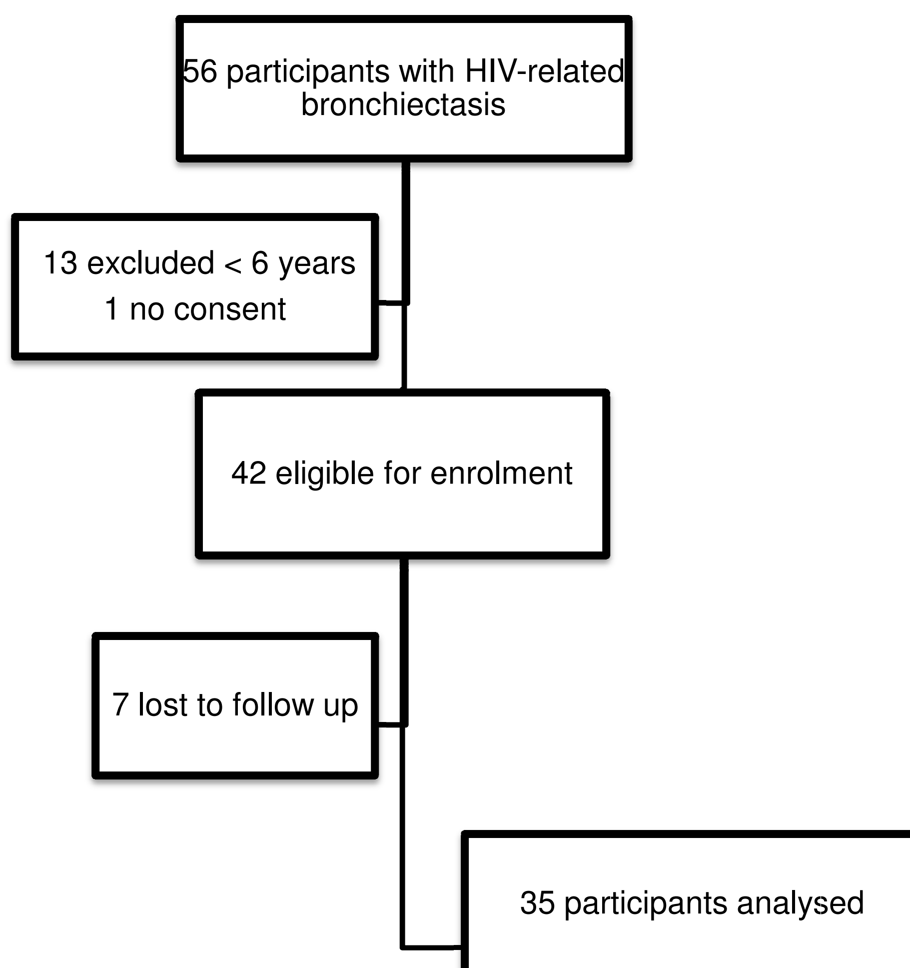

Figure 4. Enrolment and follow-up plan of children with HIV-related bronchiectasis

5.3 RESULTS

The baseline demographic characteristics of all the participants are reflected in Table 6. Thirty-five participants were enrolled, with a male/female ratio of 57%: 43%. The mean age of the study population at study entry was 8.2 ± 2.3 years. The diagnosis of HIV was made at a mean age of 6.9 years (range 6 - 11.1 years). The mean weight z-score and height z-scores were -1.8 and -1.2 respectively. The mean BMI z-scores for the cohort were 0.7. The mean BMI for the study population was 15.3 kg/m^2 (range 12.2 - 21.3 kg/m^2). Two patients died, two and four months after enrolment in the study respectively; both presented with severe bilateral lung disease and were oxygen dependent.

The mean total and percentage CD4$^+$ T cell count of the subjects was respectively 569 x10^9 cells/l and 18.3% (Centre for Disease Control stage 2). The mean HIV viral load was <25 copies/ml: 19 (54%) subjects had HIV viral suppression with undetectable HIV viral load <25 copies/ml, and 16 (46%) were non-suppressed. All but one of the participants had received HAART at enrolment. The mean number of months on HAART was 18.8 ± 18.8 months. There was no statistically significant difference in the number of months on HAART between the participants with HIV viral suppression compared to those without suppression (17.5 ± 16.2 months and 20.4 ± 21.9 months; p=0.80), respectively (Table 7). There was also no statistically significant difference in the weight and height of participants with and without HIV viral suppression (21.8 ± 6.3 kg and 22.5 ± 8.5 kg; p=0.77) and (118.9 ± 13.6 cm and 118.0 ± 15.5 cm; p=0.86), respectively.

A total of 161 sputum cultures were performed over the follow up period (multiple samples were collected from all 35 patients; Figure 5). At presentation, 42.9% of the subjects had a positive culture for a bacterial pathogen. The most common organisms were *H. influenzae* and *parainfluenzae,* which accounted for 51% of all cultures. *Moraxella* spp., accounted for 4% of the cultured organisms. PA *and S. aureus* accounted for a minority with only 2% and 1% of all cultures, respectively. *PA* was cultured in only one participant on repeated specimens. RAST testing for *Aspergillus fumigatus* was negative in all the participants tested (less than 0.35 kU/l).

Table 6. **Baseline characteristics of children with human immunodeficiency virus-related bronchiectasis**

Parameter	Mean	SD
Age (years)	8.2	2.3
Weight (kg)	22.1	7.3
Height (cm)	118.0	14.3
HAART (months)	18.8	18.8
HIV viral load (RNA copies/ml)	<25*	337762.7
CD4 count (total X10^6)	569.0	456.2
CD4 count (%)	18.3	9.1
FEV_1 (% predicted)	53.0	18.9
FEF_{25-75} (% predicted)	52.0	35.3
FVC (% predicted)	46.4	14.3

FEV_1: Forced expiratory volume in 1 second; $FEF_{25–75}$: Forced expiratory flow; HIV: Human immunodeficiency virus; Ig: Immunoglobulin; C-RP: C-reactive protein, CD4: Cluster differentiation cells $CD4^+$ T cells; HAART (months): Number of months on highly active antiretroviral therapy; *: Subjects with HIV viral suppression undetectable HIV viral load.

Table 7. Comparison of children with HIV-related bronchiectasis, with and without HIV viral suppression

Parameter	*Suppressed N=19	† Non-suppressed N=16	P-value
Weight‡ (kg)	21.8	22.5	0.77
Height‡ (cm)	118.9	118.0	0.86
FEV_1‡ (% predicted)	53.0	46.6	0.20
HAART‡ (months)	17.5	20.4	0.80
IgA‡ (g/l)	2.9	3.3	0.43
IgE‡ (kU/l)	57.0	159.0	0.09
IgG‡ (g/l)	27.7	34.9	0.26
IgM‡ (g/l)	1.6	2.1	0.19
CRP‡ (mg/ml)	25.4	55.2	0.41

* Viral load <25 copies/ml; † Viral load >25 copies/ml; ‡ Mean values; FEV_1: Forced expiratory volume in 1 second; Ig: Immunoglobulin; HAART (months): Number of months on highly active antiretroviral therapy; CRP: C-reactive protein

Two participants were culture positive for MOTTs, namely *M. fortuitum* and *M. avium intracellulare* with these organisms being cultured in more than one sputum sample. Of the study population, 48.5% had previously received one course of anti-tuberculosis treatment, 21.2% two courses and 6% three courses or more. In total 75.7% had received at least one or more courses of anti-TB therapy. Only one participant had positive viral identification on sputum (*Parainfluenza* type 2 virus). Only two subjects had histologically confirmed lymphocytic interstitial pneumonitis. One patient presented with an interstitial pattern on chest x-ray and a ground glass appearance of CT chest, had a biopsy to rule out TB as a cause of lung disease. The

second patient underwent lung biopsy due to pneumonia not responding to antibiotics, TB therapy and anti-fungal therapy, in an attempt to identify the cause of the pneumonia.

With respect to pulmonary function testing, the mean FEV_1 and FVC were 53.0 %predicted (range 5 - 86) and 46.4 %predicted (range 15-71), respectively. The mean $FEF_{25–75}$ was 52 %predicted (range 11 - 165). Only eight (22.8%) children had a positive bronchodilator response, defined as a 15% increase in FEV_1 after administration of a bronchodilator. When comparing the FEV_1 of those with positive or negative microbiological sputum culture at enrolment, the groups did not differ significantly (p= 0.52). There was a lack of correlation between months on HAART and FEV_1 or $FEF_{25–75}$ (r = -0.13 and r = 0.04), respectively. There was also no statistically significant difference between participants with HIV suppression and non-suppression with respect to FEV_1 or FEF_{25-75} (53.0 ± 17.5 %predicted and 46.6 ± 19.9 %predicted; p=0.20) and (64.5 ± 37.5 % predicted and 51.2 ± 32.7 %predicted; p=0.30), respectively.

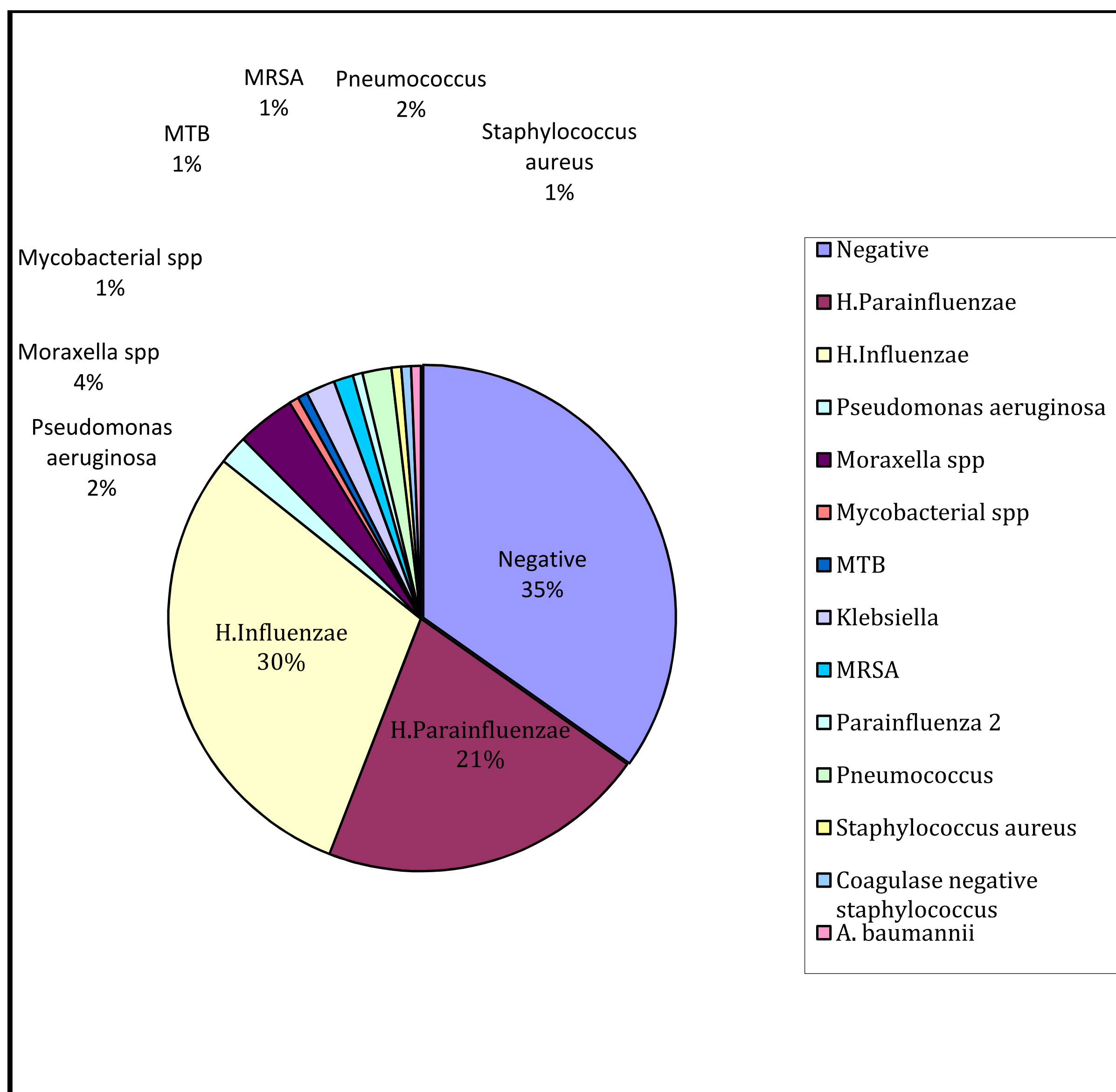

Figure 5. Sputum microbiology results of children with HIV-related bronchiectasis - cumulative data for 161 samples (N= 35).

IgE was the most significantly elevated Ig with a mean value of 79.0 ± 279.0 kU/l for the study population. IgE appeared to be significantly higher in the group of participants without HIV-viral suppression, although this did not reach statistical significance (mean ±SD) [57.0 ± 223.5kU/l and 159.0 ± 316.9kU/l; 95% CI 143.6 to 341.8; p=0.09]. IgG was also elevated with a mean for the study population of 26.0 ± 16.0 g/l, with IgG1 being the most significantly elevated subclass (N=12). There was no statistically significant difference between IgG levels in subjects with HIV viral suppression, when compared to those with no viral suppression (27.7 ± 12.9 g/l and

34.9 ± 18.5 g/l; p= 0.26), respectively. IgA and IgM were not significantly elevated with mean values of 2.7 ± 1.3g/l (laboratory range 0.8-3.0g/l) and 1.5 ± 1.1 g/l (laboratory range 0.4 - 1.8g/l), respectively, for the study population. With respect to IgA and IgM, there was no significant difference in subjects with HIV viral suppression compared to those with HIV viral suppression (2.9 ± 1.3 g/l and 3.3 ± 1.4 g/l; p=0.43) and (1.6 ± 0.7 g/l and 2.1 ± 1.4 g/l; p=0.19), respectively.

CRP was low, with a mean value of 9.2 ± 86.6 mg/l for the study population. The mean CRP was elevated to a greater extent in the virologically non-suppressed participants (55.2 mg/l vs. 25.4 mg/l), although this was not statistically significant (95% CI; -9.3 to 119.6 and 10.5 to 40.3; p=0.35), respectively.

Thirteen (36%) children were exposed to ETS, with at least one smoker amongst the household contacts. The mean $CD4^{+}$ T cell percentage count for children exposed, and those not exposed, to ETS was not statistically significant (24.0 ± 8.3% and 16.0 ± 8.9%; p= 0.33). There was no statistically significant difference between ETS exposed, and unexposed, children with respect to the mean HIV viral load (725.8 ± 1562.0 copies/ml and 30679.3 ± 60308.0 copies/ml; p= 0.09), respectively. With respect to FEV_1, there was also no statistically significant difference between ETS-exposed, and non-exposed, children (95% CI 40.6 to 55.5; p=0.64). The two children who died were both exposed to ETS. The use of BMF, which included paraffin oil, coal stoves, gas stove and other indoor organic heat sources, was evident in fourteen (40%) participants.

All but one of the participants received social support in the form of a government grant ranging from US$32-US$236 monthly (US$1=ZAR 7.81).

5.4 DISCUSSION

Children with HIV-related bronchiectasis are diagnosed with HIV-infection after the age of 6 years in this cohort. It is presumed that the majority of these children had

vertical transmission of HIV. This may therefore demonstrate a failure of the PMTCT program as in these individuals, HIV-infected women and their newborn children were clearly not offered HIV testing, as well as poor postnatal follow-up of HIV exposed infants. The delayed presentation may have many explanations; including the possibility of the 'slow-progressor' phenotype, failed health services or missed diagnosis due to mislabelling as tuberculosis.

The anthropometric measurements of children with HIV-related bronchiectasis in this study were within normal limits, with the majority of children having acceptable weight, height and BMI z-scores. This despite them having both a chronic inflammatory lung disease and HIV-infection, both of which can increase metabolic demands. The use of HAART has been previously shown to positively impact growth parameters in children, with a sustained increase up to 96 weeks after initiation of treatment [227, 228]. This may account for the growth parameters in this study population, as the majority of participants were on HAART. The impact of nutrition on lung morbidity is well described in cystic fibrosis, where the lower the BMI, the higher is the morbidity from lung disease [229].

H. influenzae and *-parainfluenzae* are the predominant organisms cultured in children with HIV-related bronchiectasis. In South Africa, *H. influenzae* type B (Hib*)* vaccination has been universally available for all children since July 1999. A laboratory surveillance study conducted before and after Hib vaccination showed a 65% reduction of absolute cases of Hib decreasing by in children aged <1 year, from 1999–2000 to 2003–2004; whilst rates of non-typeable *H. influenzae* have increased, especially in HIV-infected children under the age of five [230]. Although in the context of HIV-infected vaccinated children Madhi et al, found Hib vaccine to be less effective than in HIV un-infected children with modest vaccine effectiveness of 44% versus97% respectively [231]. The overall vaccine effectiveness in the reduction of invasive Hib disease in the population (both HIV- infected and - uninfected) in the Madhi cohort was 83%.

S. aureus was also not a major pathogen in this study population. McNally and colleagues found that the risk of *S. aureus* nasal carriage was 2.86 times higher in HIV-infected children presenting with CAP [232]. A systematic review of causes of community acquired pneumonia also revealed a 2.5 fold risk of *S. aureus* infection [86]. Although, this pathogen seems to have a major role in CAP in HIV-infected children, is does not seem to be a major pathogenic organism in HIV-related bronchiectasis.

More than three quarters of the study population had a prior diagnosis and treatment for TB, three of which were microbiologically confirmed. The challenges of accurate TB diagnosis in HIV-infected children are well documented, and in a high TB burden area there may be over-reliance on radiological diagnosis [18,92,233]. The limitation of this approach is that, TB may have a similar radiological picture to bronchiectasis, and this may therefore explain how bronchiectasis may have been missed in some children. Coupled with this, children with bronchiectasis may also present with MOTTs infections, which may be mislabelled as *Mycobacterium tuberculosis* [218]. Almost a quarter of children in the current study had received two courses of anti-tuberculosis treatment. This is not surprising, as current guidelines depend heavily on chest X-ray interpretations and the presence of chronic cough for more that two weeks for TB diagnosis at the primary health care level [233]. TB may have therefore been an important precedent for the development of bronchiectasis in the majority of children in this cohort. Lymphocytic interstitial pneumonitis rates in our study population were low and therefore do not explain the mechanism for bronchiectasis in this study population.

This study suggests that an important differential diagnosis to TB in HIV-infected children, with recurrent chest symptoms, is bronchiectasis and there is an urgent need for a guideline to help identify children with bronchiectasis.

Respiratory morbidity from HIV-related bronchiectasis is significant, with accelerated pulmonary function decline. In this study, the mean FEV_1 was 53% of predicted; this is in comparison to a population of New Zealand children with non-CF bronchiectasis, where the authors reported a baseline predicted FEV_1 of 66% of predicted [234]. Haidopoulou et al*,* also reported FEV_1 of 75% of predicted in a group of children with bronchiectasis secondary to primary immunodeficiency, although their study population had been diagnosed with primary immunodeficiency at a median age of 3.4 years [235]. An explanation for the lower pulmonary function measurement in the current study may be that, even with the presence of HAART, there is still a significantly higher risk of exacerbations related to the abnormal immune responses to pathogens in HIV-infected individuals [236]. Secondly, the delayed diagnosis of bronchiectasis and HIV-infection in this study population may account for this accelerated pulmonary function loss.

As with HIV-infected children with acute pneumonia [237,238], the current study documented elevated serum IgG levels. This probably reflects immune hyper-stimulation related to B-cell activation secondary to HIV infection. In another form of chronic inflammatory lung diseases CF, IgG has been found to correlate with a decline in pulmonary functional status [142,143]. This does not appear to be the case in HIV-related bronchiectasis.

ETS exposure does not explain FEV_1 or HIV viral load variability. This is in comparison to an adult study by Feldman et al*,* where there was a statistically significant difference in morbidity and mortality of smokers with HIV-infection [121]. A previous study in our population of 121 HIV-infected children, showed no difference in HIV staging in ETS exposed and un-exposed children [239]. This is consistent with our current finding.A recent study by Kabali et al*,* also found no association between cigarette smoking and HIV disease progression [240].

A significant proportion of the children in the current study were exposed to BMF. Exposure to BMF is known to impact lung health by, not only increasing the risk of acute lower respiratory tract infections, but also it's local effects on the bronchial epithelial layer with increased bronchial inflammation and reduced mucociliary clearance which in turn, increases the residence time of inhaled particles [241-243]. This may also account for the lower pulmonary functions in this cohort, and may therefore be a potential risk factor for children developing recurrent chest infections

The strength of this study is that it provides pilot data on the demographic determinants of children with HIV-related bronchiectasis in a developing country setting, with a high TB burden. The limitations were the small sample size and the exclusion of younger patients. There were also no objective measurements to quantify ETS exposure. Larger trials are needed to confirm these findings.

5.5 CONCLUSION

Children with HIV-related bronchiectasis have the diagnosis of HIV infection made late. In a setting with a high TB burden, the differential diagnosis of an abnormal chest x-ray in children with a chronic cough or previously treated TB, should include bronchiectasis. Exposure to environmental tobacco smoke and biomass fuels may be potential contributors to increased morbidity associated with HIV-related bronchiectasis, although these factors do not seem to impact markers of HIV disease progression.

CHAPTER VI

PULMONARY AND SYSTEMIC CYTOKINE/CHEMOKINE PROFILES IN CHILDREN WITH HUMAN IMMUNODEFICIENCY VIRUS-RELATED BRONCHIECTASIS

6.1 OBJECTIVES

A primary objective for this aspect of the thesis was to assess the role of local and systemic inflammatory and anti-inflammatory cytokines/chemokines in children with HIV-related bronchiectasis. A secondary objective was to assess the role of atopy in children with HIV-related bronchiectasis and to compare these children to a control group of HIV-infected children with no evidence of bronchiectasis as well as HIV un-infected children

6.2 SUBJECTS AND METHODS

6.2.1 SUBJECTS

56 children with HIV-related bronchiectasis attending the Paediatric Chest Clinic at the Steve Biko Academic Hospital, Pretoria, South Africa, from January to November 2009, were screened. Figure 4 (Chapter V) illustrates the enrolment and follow up plan of the participants. The enrolment criteria and patient numbers have been previously described (Chapter V).

A group of HIV-infected children on HAART, without evidence of bronchiectasis attending the Tshwane District Hospital HIV clinic, were invited to participate, to serve as a control group for the study. A second group of HIV un-infected children attending routine Paediatric Cardiology and Paediatric Neurology clinics were also enrolled to serve as a second control group. The participants were enrolled as a prospective convenience sample of children aged 3 months to 12 years. A total of fifty HIV-infected children with no bronchiectasis and fifty HIV un-infected children were enrolled.

6.2.2 METHODS

The following investigations were conducted on the children with HIV-related bronchiectasis.

Sputum: An induced sputum sample was also collected from each patient. Patients were nebulised with 5ml of 5% saline delivered via a facemask with an ultrasonic nebuliser (Goodwish KWC 6Td, Nanjing city, Jiangsu Province, China), followed by postural drainage with percussions. An aliquot of sputum was stored at -20°C pending measurement of sputum cytokines, prior to which the specimens were rendered less viscous by treatment with 0.1% dithiothreitol (DTT) at a ratio of 1:4 (w/v) sputum: DTT, with gentle agitation for 15minutes at room temperature. This was followed by addition of a volume of phosphate-buffered saline (0.15m, pH 7.4) equal to that of DTT. After gentle mixing for 5 minutes, the liquefied sputum was centrifuged (2250 rpm for 10 min) and the supernatants removed for determination of sputum cytokines.

Serum: Venous blood (5ml) was collected in endotoxin-free, silicone-coated vacutainers containing a gel separator. The blood samples were allowed to stand at room temperature and delivered to the laboratory within 2 hours of venepuncture, where they were immediately centrifuged (3000 rpm for 10 minutes), after which the serum was removed, aliquoted, and stored at minus 20°C until performance of the assays as described below.

Serum and sputum cytokines: These were measured using the Bio-Plex® suspension bead array system (Bio-Rad Laboratories Inc, Hercules, CA, USA), which utilises luminex® Xmap™ multiplex technology to enable simultaneous detection and quantitation of multiple different analytes in a single sample. The system uses an array of microspheres in liquid suspension, conjugated with a monoclonal antibody specific for a target protein. The beads contain different ratios of two spectrally distinct fluorophores, thereby assigning a unique spectral identity. These antibody-coupled, colour-coded beads were then incubated with the serum or sputum samples ($^{1}/_{4}$ and $^{1}/_{10}$ dilutions respectively), and washed, followed by addition of a biotinylated detection antibody, washed again, and finally incubated with

streptavidin-phycoerythrin. A wide range of standards (0.38-91756.00 picograms/ml) were used to enable quantitation of the individual cytokines using a BioPlex array reader with a dual laser detector and real time digital signal processing. The following analytes were measured: IL-1β, IL-1ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17, IFN-γ, TNF-α, G-CSF, GM-CSF, MCP-1 and macrophage inflammatory protein-1 beta (MIP-1β).

Immunoglobulins: Circulating concentrations of IgE were assayed by nephelometry (Siemens Healthcare Diagnostics, BN Prospec Nephelometer, Newark, NJ, USA) using materials and controls supplied by Siemens Healthcare Diagnostics. Screening for circulating specific IgE antibodies was performed using the ImmunoCAP® paediatric food mix and aeroallergen (fx5 and Phadiatop respectively, Phadia AB, Uppsala, Sweden), with follow-up using individual allergens where necessary. Specific IgE RAST testing was also performed for *Aspergillus fumigatus.*

Serum was also collected for CRP determination by methodology described in 5.2.2.

The following investigations were performed in the un-matched control group of HIV-infected children without bronchiectasis:

A questionnaire was administered which included an overview of the child's medical history, a personal diagnosis of atopy (asthma, allergic rhinitis and eczema), a family history of atopy and a general examination of the patient's current state of well-being was conducted. The WHO HIV clinical staging was determined for all the participants [244]. Blood was sent for $CD4^+$ T cell counts determination by flow cytometry analysis. Skin prick test (SPT) (Alk-Abello) for common aeroallergens was conducted in all patients with negative saline and positive (histamine-dihydrochloride 10mg/ml) controls. An induration of 3mm or greater than the negative control was regarded as a positive result. The allergen extracts used were: Bermuda grass, five-grass mix, tree mix, dog hair dander, cat hair dander, standard house dust mite (*Dermatophagoides pteronyssinus*), Blatana sp (cockroach).

The children in the second control group of HIV un-infected children had skin prick testing for common aeroallergens as described for HIV-infected children without bronchiectasis.

Statistical analysis

Data analysis was performed using Stata Release 10 (Stata Corp LP, College Station, TX, USA) and statistical analysis using the Spearman's correlation coefficient was used to assess for correlations between markers HIV of disease activity ($CD4^+$ T cell count and HIV-viral load) and cytokine/chemokines. The Wilcoxon rank sum test (Mann-Whitney test) to compare the cytokines/chemokines of the participants with HIV viral load suppression and those without HIV viral suppression. A Welch two sample t-test with unequal variances was employed in analysis of $CD4^+$ T cell count in relation to family history of atopy, dermatitis and asthma for the control group. A p-value of <0.05 was considered statistically significant.

Ethical Clearance

The ethical approval obtained for the thesis applied to this study component (Appendix B).

6.3 RESULTS

All thirty five participants (mean age 8.2 ± 2.3 years) contributed serum and sputum samples for analysis. Of the cytokine analysis in blood and sputum (Figure 6), IL-8, a macrophage derived cytokine, was the most significantly elevated in both the sputum and serum (400.0 ± 8656.5 pg/ml and 115.6 ± 156912.2 pg/ml), respectively. There was no correlation between $CD4^+$ T cell percentage counts or HIV viral load and IL-8 (r= -0.07 and r= -0.21), respectively. There was a lack of correlation between CRP and the serum cytokines IL-6 and IL-8, respectively (r= 0.26 and r= 0.32). INF-γ, a

Th-1 cytokine, was elevated in the serum but not in the sputum of participants (118.7 ± 342.6 pg/ml and 9.4 ± 22.8 pg/ml). The sputum IL-1β was also elevated (20.6 ± 462.4 pg/ml). Of the other Th-1 derived cytokines (IL-6 and TNF-α), very low levels were detected in the serum and none in the sputum. There was no correlation between IL-1β, IL-6, IL-8 and number of months on HAART (r= 0.27; r= 0.29 and r= 0.13), respectively. The stimulating factor, GM-CSF was elevated both in serum and sputum (48.5 ± 118.8 pg/ml and 21.8 ± 77.5 pg/ml), respectively; however, G-CSF levels were insignificant in both serum and sputum. When comparing participants with HIV viral suppression, and those without HIV viral suppression, there was no statistically significant difference for all the cytokines IL-1β, IL-6, IL-8, TNF-α, GM-CSF and G-CSF (Table 8).

The chemokine MIP-1β was elevated in the serum as compared to the sputum (47.0 ± 1489.0 pg/ml and 0.7 ± 7.6 pg/ml), respectively. There was no correlation between serum MIP-1β and $CD4^+$ T cell percentage, nor for HIV viral load (r= 0.19 and r= -1.10). Similarly, MCP-1 was only slightly elevated in the serum but not in the sputum (12.6 ± 181.7 pg/ml and 0.8 ± 7.6 pg/ml), respectively.

IL-1ra, an anti-inflammatory cytokine, was elevated to a greater extent in the serum, as compared to sputum samples (171.2 ± 500.5 pg/ml and 70.0 ± 3975.2 pg/ml), respectively. There was no correlation between IL-1ra and the number of months on HAART, CD4 count, nor HIV viral load (r= 0.17; r= -0.02 and r= -1.86), respectively. There was also no statistically significant difference in the serum IL-1ra between subjects with HIV viral suppression as compared to those without viral suppression (202.7 ± 518.0 pg/ml vs. 42.1 ± 485.7 pg/ml; p=0.44). There were undetectable levels of IL-10, the other anti-inflammatory cytokine, in both the serum and sputum of participants (2.2 ± 1.9 pg/ml and 0.3 ± 0.3 pg/ml), respectively.

The mean total IgE for the group was 79.0 ± 279.0kU/l, with only 10% of all children having a positive specific IgE on RAST testing for inhalants or foods. RAST testing for allergic bronchopulmonary aspergillosis (ABPA) [*Aspergillus fumigatus*] was

performed and was negative in all participants. Total IgE and CD4 percentage count did not reveal any correlation (r= -0.02; p=0.48) (Figure 7). There was, however, a trend towards statistical significance, when comparing virologically suppressed and non-suppressed participants with respect to IgE (p=0.09). There were low levels of the Th-2 related cytokines IL-2, IL-4, IL-13 and IL-17. There was no correlation between IL-4 and the HIV viral load (r=0.42). There was also no correlation between the cytokines IL-2, IL-4, IL-13 and IgE (r= -0.22; r= -0.21 and r= 0.06 respectively).

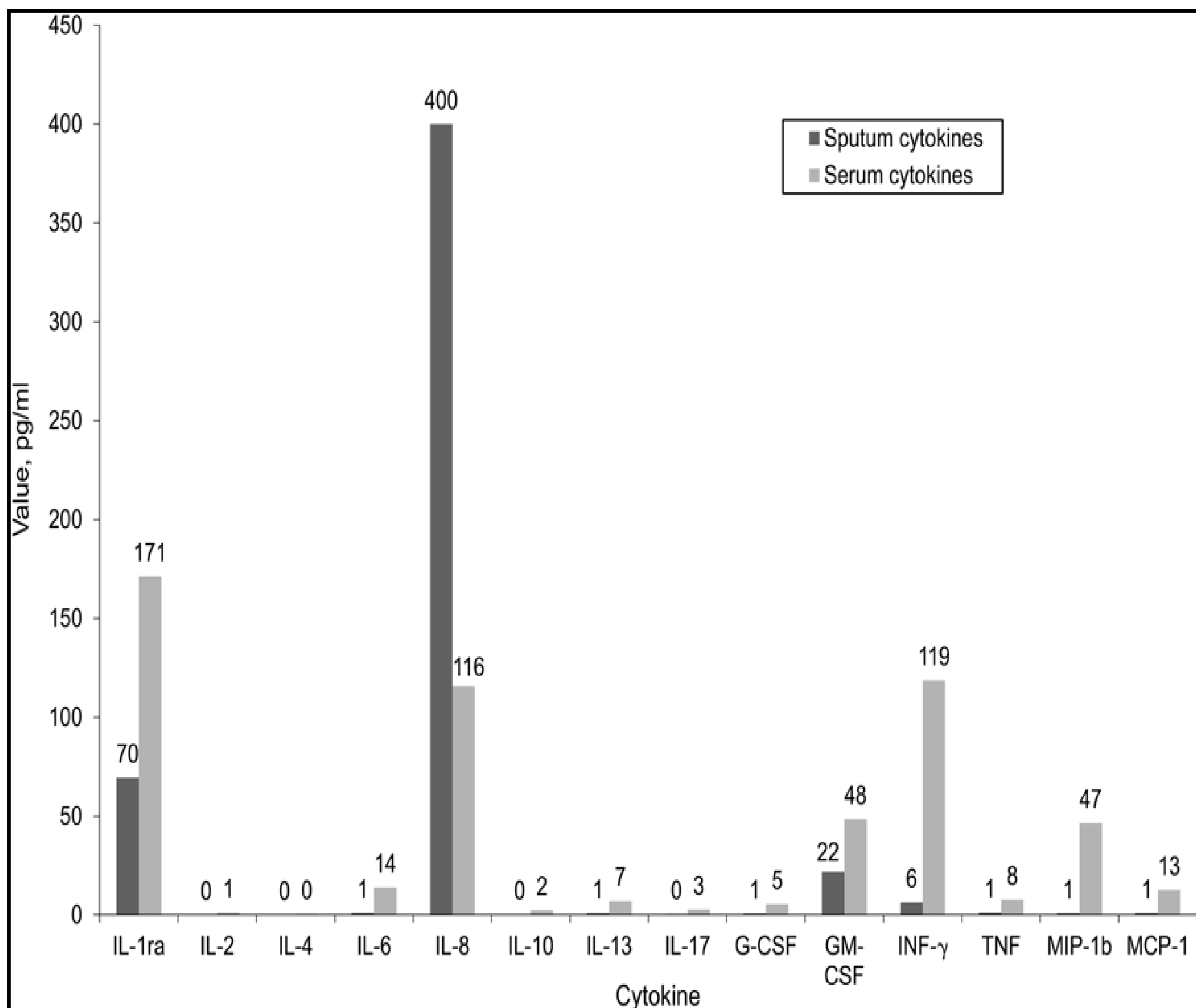

Figure 6. The baseline serum and sputum cytokine values of children with HIV-related bronchiectasis

Table 8. Comparison of serum and sputum cytokines/chemokines of children with HIV-1 related bronchiectasis, with and without, HIV-viral suppression.

Variable	Suppressed N=19	Non-suppressed N=15	P value
HAART (months)‡	17.5	20.4	0.80
Serum IgE (kU/l)‡	180.8	316.9	**0.09**
Sputum IL-4 (pg/ml) ‡	0.1	0.0	0.80
Serum IL-4 (pg/ml)‡	0.5	0.4	0.24
Sputum IL-8 (pg/ml)‡	5548.0	3294.2	0.17
Serum IL-8 (pg/ml)‡	52113.0	14667.0	0.74
Serum INF-γ(pg/ml)‡	19.1	15.0	0.17
Sputum INF-γ (pg/ml)‡	10.3	1.1	0.67
Sputum IL-1ra (pg/ml)‡	271.5	41.0	0.35
Serum IL-1ra (pg/ml)‡	202.7	42.1	0.44

IL: interleukins; HAART: Highly active antiretroviral therapy;‡: mean values reported; SD: standard deviations; IgE: Immunoglobulin E; INF- Interferon gamma; IL-ra: Interleukin receptor agonist.

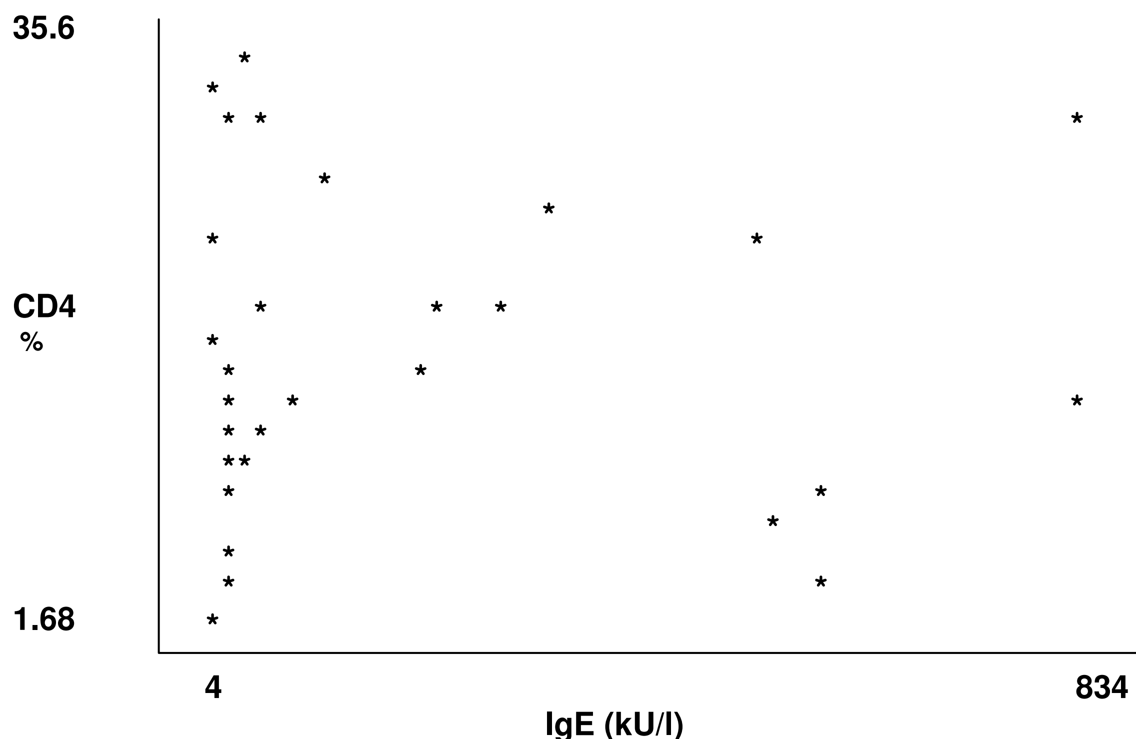

Figure 7. **Plot demonstrating Spearman correlation between Immunoglobulin E (IgE) and CD4^{+} T percentage cell count of children with HIV-1 related bronchiectasis (r=-0.02; p=0.48)**

For the control group of 50 HIV-infected children with no evidence of bronchiectasis, five children (10%) had positive skin prick test (SPT) for aeroallergens, with the most commonly identified aeroallergen being *Dermatophagoides (D.) pteronyssinus*, in three participants (Figure 8). Twelve participants (24%) had a positive family history of atopy; with only two of these having positive SPT. Eleven (22%) had been previously diagnosed with asthma, with the majority (nine) having negative SPT. Results of the WHO and CDC staging of the participants and SPT results are reflected in Table 9. Two of the asthmatic patients were WHO HIV stage 1 and only one of these had a positive SPT. All five patients with positive SPT had allergic rhinitis, with thirty (60%) children presenting with a history of allergic rhinitis. Thirty-four (68%) gave a history of itchy dermatitis, although this was not confirmed to be eczema in the majority of cases. Of the fifty HIV-uninfected control group of children, eight (16%) HIV-negative children had positive SPT for aeroallergens. *D.pteronyssinus* and grass were the most common aeroallergens identified in this group.

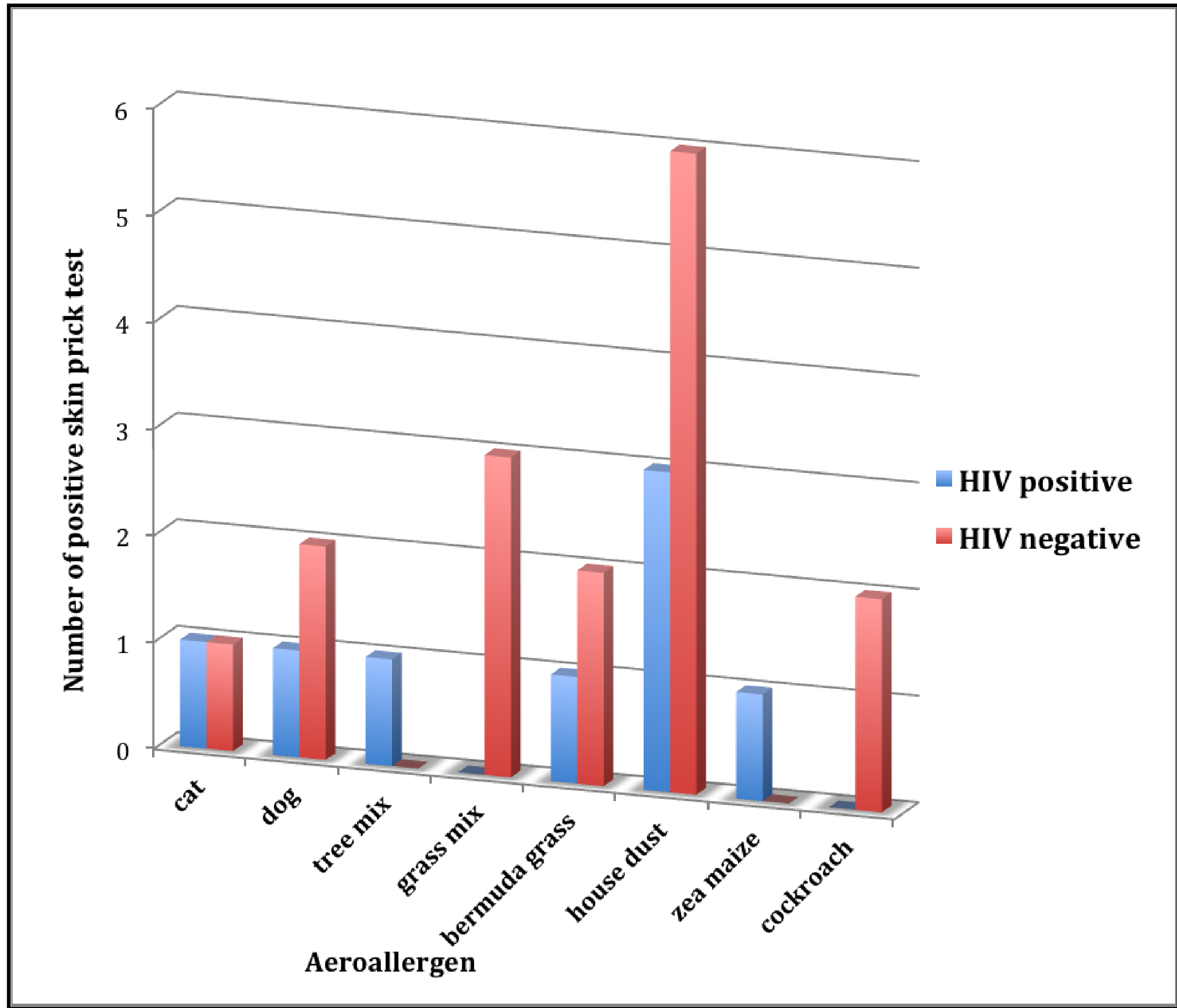

Figure 8. **Graphic presentation of specific positive skin prick tests in HIV-infected without bronchiectasis and HIV-negative children, some patients with more than one positive aeroallergen**

Table 9. Skin prick test findings of HIV infected children without bronchiectasis, according to immunological staging (N=50)

	N (%)	SPT positive (%)	Asthma (%)
WHO clinical stage			
1	17 (34)	1 (2)	2 (4)
2	16 (32)	1 (2)	3 (6)
3	14 (28)	2 (4)	4 (8)
4	3 (6)	1 (2)	2 (4)
CDC immunological stage			
1 (CD4 >25%)	13 (26)	1 (2)	2 (4)
2 (CD4 15-24%)	21 (42)	2 (4)	3 (6)
3 (CD4 <15%)	16 (32)	2 (4)	6 (12)

WHO HIV stage: World Health organization HIV clinical staging ref [244]; CDC: Centre for Disease Control staging for HIV ref [245]; SPT: Skin prick test

There was no relationship between logarithmic transformed (log) $CD4^{+}$ T cell count and SPT positivity on the Welch *t-test* (p=0.61), log $CD4^{+}$ T cells count and presence of reported asthma (95% CI -0.5 to 0.7; p=0.71), and log $CD4^{+}$ T cells count and reported presence of dermatitis (95% CI -0.6 to 0.5; p=0.84). There was also no relationship between $CD4^{+}$ T cell count and family history of atopy (95% CI -0.3 to

0.5; p=0.63). There was no statistically significant difference between participants with a family history of atopy, when compared to those without a family history of atopy, with respect to log $CD4^+$ T cell counts 6.4 (95%CI 6.0 to 6.1) and 6.5 (95% CI 6.2 to 6.8; p=0.63), respectively.

6.4 DISCUSSION

The predominant cytokine found both in the pulmonary milieu and systemic circulation was IL-8. This is similar to findings in CF-related bronchiectasis, where oxidative stress results in increased IL-8 levels [246-248]. IL-8 is a marker of neutrophil-driven inflammation, where elevation may suggest that the disease process, in HIV-related bronchiectasis, is neutrophil-dependent. There is also evidence of IL-8 having chemotactic function, particularly during acute exacerbations; but in the current study, the level of IL-8 was independent of the presence of exacerbations [152]. Whether or not, the neutrophil driven inflammatory process in HIV-bronchiectasis is dependent on the innate or adaptive immune mechanisms, requires further exploration. All potentially relevant cytokines, that may relate to inflammatory disease of this nature, and that represent Th1-driven inflammation, including IL-1β, IL-6, INF-γ and TNF-α were slightly elevated in the systemic circulation, but to a lesser extent in the lungs. As with previous studies, this study found very low levels of IL-1β, IL-2, IL-4 and IL-17 in HIV-infected individuals [136,145]. This may be related to the use of HAART, as prior studies have indicated a reduction in the pro-inflammatory cytokines when HAART is being taken. HAART may return cytokine levels to those seen in HIV un-infected individuals [136,145]. The high serum INF-γ levels in this study also confirm findings by Watanabe et al, of 35 HIV-infected adults, who demonstrated persistently high INF-γ levels. They postulated the high levels to be related to HIV viraemia [136]. Numerous studies have demonstrated high TNF-α levels to be associated with acute HIV-infection [249-251]. It is encouraging that the levels in this cohort were reduced. Although in HIV infection the dominant abnormality is immunosuppression, the systemic immunological responses, and to a lesser extent, pulmonary responses in this cohort appear to be exaggerated.

The colony stimulating factors associated with neutrophilic migration, GM-CSF and to a lesser extent G-CSF, were also elevated, reflecting neutrophilic recruitment. This may reflect an ability to mount immune responses against pathogens. However, the levels of these factors did not correlate with HIV staging or use of HAART. Cozzi-Lapri et al, [249] found low levels of GM-CSF in subjects on HAART, which decreased further, on terminating HAART, for two months. All the participants in their study had HIV viral suppression below 400 copies/ml, whereas only just over half of our population had complete viral suppression, expressed as less than 25 copies/ml. This effect may account for the differences in the results. The neutrophilic inflammation related to bronchiectasis may also account for these differences.

The chemokine MIP-1b, which is mainly involved in the host response to bacterial, fungal, viral and parasitic pathogens and selectively attracts $CD4^+$ T lymphocytes, was elevated in the serum and to a lesser extent in the sputum of subjects in this cohort. MIP-1b is known to be a major suppressive factor of HIV produced by $CD8^+$ cells, possibly suggesting that there is continuous immune stimulation by the HI virus in these subjects, mostly systemically, but to a lesser extent, in the lungs [252]. This also suggests that the subjects had effective suppression of HIV replication.

Despite the high levels of the Th-1 driven cytokines, IL-1ra, an anti-inflammatory cytokine, was elevated in the serum and the lungs. This may demonstrate equilibrium, between the pro- and anti-inflammatory cytokines, in HIV-infected persons. IL1-ra was found to be inversely related to $CD4^+$ T cell count by Shebl and colleagues [253]. We could not replicate this finding.

There was a significantly elevated IgE in children with HIV-related bronchiectasis, with no accompanying increase in the Th-2 mediated cytokines. This confirms that IgE elevation is not related to atopy, but probably reflects polyclonal hypergammaglobulinaemia related to T-cell depletion secondary to HIV infection. Contrary to published studies, we found no relationship between IgE and HIV disease staging [136-141,254], although there was a marginally significant difference in IgE levels between the subgroups with and without viral suppression. This may be

related to the small sample size. The other potential explanation for elevated IgE in the presence of bronchiectasis is ABPA, but this was not found in this study group.

No association was documented between atopy and HIV-infection, in children with HIV-infection without bronchiectasis. There was a similar incidence, of 10%, in the HIV-infected bronchiectasis subjects and in the HIV-negative (non-bronchiectasis) control group. The incidence of atopy in HIV-infected children was no greater than that of HIV un-infected children. The presence of atopy in the HIV-infected children without bronchiectasis is probably due to an inherent but independent genetic predisposition to atopy. In a study by Bacot et al, SPTs were positive in 28% of HIV-infected children [137], although adult studies report an incidence of around 9% [255]. It also appears that the stage of HIV disease in HIV-infected children does not influence the development of allergy, nor does it have an impact on IgE values. This may be because the immune mechanisms are truly different. This is consistent with the findings by Bowser et al, in perinatally HIV-infected children [256].

In the International Study of Asthma and Allergy in Childhood (ISAAC), South Africa reported an asthma prevalence of 13.6%, in 13-14-year old children in Cape Town [257]. In the HIV-positive control group, 22% of patients were diagnosed with asthma, suggesting a higher prevalence in HIV-infected children. However, the possibility of chronic lung disease with airway reversibility should be considered, as no pulmonary function testing was performed in these children. A recent review revealed, that in HIV-infected individuals, there is a higher incidence of respiratory complaints, whether this is due to asthma or airway hyper-responsiveness, could not be determined [258].

The pathogenesis of eczema is thought to be related to allergen uptake by the Langerhans cells in the skin via specific IgE bound to the high-affinity IgE receptors on cell surfaces, resulting in an allergen-specific T-cell response in memory $CD4^+$ T cells. It is well known that HIV-infected patients have a higher incidence of dermatitis [259]; including HIV eosinophilic folliculitis, papular urticaria, seborrhoeic dermatitis, psoriasis and pruritus nodularis, which may resemble atopic dermatitis. This makes

the distinction between atopic and non-atopic dermatitis difficult, particularly pruritus nodularis, which has a pruritic component [259]. Patients with HIV have dry skins, and this barrier disruption has been postulated by Rudikoff to favour a Th2-mediated response to exogenous allergens [259]. The presence of dermatitis in our study population was quite striking. Whether or not all these patients had eczema is difficult to delineate. Bacot et al, found no correlation between the presence of atopic dermatitis and the level of immunosuppression in CD4 levels [137]. This was confirmed in the current study.

There is a higher prevalence of rhinosinusitis in HIV infected individuals, related to a decrease in cellular immunity but unrelated to IgE-mediated hypersensitivity. The incidence of allergic rhinitis has been reported to be 20.7% in South Africa [260]. Evidence of causality of rhinitis in patients is complex, as most cases of rhinitis may be the result of infectious agents in HIV-infected individuals [261].

The strengths of the current study are that data on the local and systemic cytokine responses in children with HIV-related bronchiectasis are provided. The relationship between HIV and atopic conditions is addressed, demonstrating no increased incidence, both in the study population, as well as in the control group. There are several limitations in this study. The absence of objective lung function testing in order to assess whether reported asthma was truly present in the control group. Nasal Hansel staining to assess for presence of nasal eosinophils was not performed to confirm the presence of allergic rhinitis. Secondly, there were no blood samples drawn from the HIV-positive non-bronchiectasis control group to assess for any possible differences in the two HIV populations.

6.5 CONCLUSION

In HIV-related bronchiectasis, local and systemic immune stimulation mechanisms appear to remain intact, with possible equilibrium in both inflammatory and anti-inflammatory cytokines, which may be influenced by the use of HAART. It appears that the stage of HIV disease does not influence the development or expression of

allergy. There is a high prevalence of dermatitis and chronic rhinitis in HIV-infected children, probably not atopic in origin.

CHAPTER VII

SOLUBLE TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS-1 IN SPUTUM OF CHILDREN WITH HIV- RELATED BRONCHIECTASIS

7.1 OBJECTIVES

The primary aim of this component of the thesis was to describe the sputum values of soluble triggering receptor expressed on myeloid cell (sTREM)-1 in children with HIV-related bronchiectasis. A secondary aim was to assess whether there is any correlation between sTREM-1 and inflammatory markers in children with HIV-related bronchiectasis. Finally, the study aimed to assess for any differences in sTREM-1 values in children with CF-bronchiectasis when compared to those with HIV-related bronchiectasis.

7.2 SUBJECTS AND METHODS

7.2.1 SUBJECTS

Participants enrolled in this sub-study component of the thesis, included the children already described in Chapter V (Section 5.2.1). Participants were included in the analysis, if sufficient sputum was available from stored samples. Samples were available for sTREM-1 analysis in twenty-four children, 15 (63%) of whom were male. A group of children with stable state CF attending the CF clinic at the Catholic University of Leuven, Belgium, provided induced sputum samples for sTREM-1 determinations and served as a control group for the children with HIV-related bronchiectasis.

7.2.2 METHODS

Clinical investigations

The information collected for the thesis was utilised in this sub-study. The information that was pertinent here included: the age of HIV diagnosis, timing of initiation of HAART and growth parameters (weight, height and BMI) expressed as z-scores [212]. Pulmonary function parameters (FEV_1, FVC, FEV_1/FVC, FEF_{25-75}) were measured using the Viasys SpiroPro Jaeger Spirometer (Hoechberg, Germany). For the control group growth parameters and pulmonary functions (FEV_1 and FVC) were recorded.

Laboratory investigations

Sputum microbiology: Induced sputum samples collected were analysed for bacterial pathogens, including MTB and respiratory viruses (*RSV, Influenza A* and *B*, *Parainfluenza 1-3, Adenovirus* and *Cytomegalovirus*).

Sputum sTREM-1: Induced sputum samples were collected for sTREM-1 determination for the study group and control group were analysed. The sputum sample was weighed; a volume of 0.1% DTT equal to four times the weight of the sputum was added to the tube. The sample was agitated in a vortex mixer with gentle aspiration using a Pasteur pipette, to ensure mixing. This was followed by rocking of the sample with a bench rocker for 15 minutes. A volume of Dulbecco's phosphate buffered saline (D-PBS) equal to the volume of 0.1% DTT was added to and mixed with the liquefied sputum by rocking for 5 minutes. The sample was then centrifuged at 790g (2,250 rpm) for 10 min and the fluid phase contents transferred to a clean tube for determination of the s-TREM-1 concentration with the final value corrected for dilutions carried out during the sputum processing. The results were expressed in picograms/ml (pg/ml).

Serum samples: Blood was collected for total IgG quantification by nephelometry (Siemens Healthcare Diagnostics, BN Prospec Nephelometer, Newark, NJ, USA) using materials and controls supplied by Siemens Healthcare Diagnostics. The measurement of CRP, total white cell count, $CD4^{+}$ T lymphocytes and HIV-1 viral loads were also pertinent for this sub-study.

CT scanning

For diagnostic CT scanning, the following parameters were used: collimation of 24 x 1.2mm gantry rotation time of 500ms, tube voltage of 120kV, effective current of 100mAs online tube current modulation and a table feed of 18m/rotation. Contrast enhancement was achieved by intravenous administration of 100ml of non-ionic contrast material (Ultravist) at a rate of 2ml/sec. Matric size was 512 x 512. Two blinded radiologist were those that carried out the CT scan diagnosis without viewing any clinical data, including morphological testing and special investigations. The scoring system was utilised to score the CT scans [108]. The Bhalla score is a qualitative CT scoring system to assess the severity of bronchiectasis, based on nine morphologic changes such as; peri-bronchial thickening, mucous plugging, abscesses or bronchiectatic sacculations, emphysema, bullae and consolidation or collapse (Appendix D). The Bhalla score is based on subtracting the value of the CT score from 25, with 25 indicating normal lungs and zero being severe bronchiectasis. Images were also analysed for the presence or absence of sites of active or inactive TB.

Statistical analyses

Data analysis was performed using Stata Release 10 (Statacorp LP, College Station, TX, USA) and statistical analyses using the Wilcoxon ranksum test (Mann-Whitney test). The Spearman correlation test was used to test for correlation between sTREM and markers of HIV disease activity and cytokines. The geometric mean values of are reported due to the skewed data of the variables reported. Testing was done at the 0.05 level of significance.

Ethical clearance

The ethical approval obtained for the thesis applied to this study component. Informed consent and assent where applicable was obtained from the parents/ guardians for the participants who served as a control group.

7.3 RESULTS

The baseline characteristics of the study participants are reflected in Table 10. A total of twenty-four children were enrolled in the study. The mean age of the population was 7.0 ± 2.2 years with 15 males. All the children were on HAART at study entry, with a mean number of months on HAART of 17.8 months. The majority of subjects had an HIV staging CDC stage 2 with mean $CD4^+$ T cell percentage count of 21.2 ± 2.2 % (95% CI 16.5 to 25.8) [234]. The majority of subjects did not have complete HIV-viral suppression with a mean HIV-viral load of 14355.8 ± 46449.7 copies/ml (95% CI -5750.6 to 34422.1)

With respect to sputum sTREM-1, the values were highly detectable in the study population as well as the control group, with geometric mean values of (667.2 ± 320.1 and 189.6 ± 89.7), respectively. This difference, between the sTREM-1 for the bronchiectasis participants, when compared that of the CF participants, was statistically significant ($p<0.05$).

There was no correlation between sTREM and $CD4^+$ T cell percentage and HIV viral load ($p=0.95$ and $p=0.84$), respectively. There was also no correlation between sTREM and the degree of bronchiectasis on Bhalla score ($p=0.74$).

The mean IgG was elevated at study entry, with a mean of 29.8 ± 12.5 g/l (95% CI 24.5 to 35.2). For the acute phase reactants, the CRP and the white cell counts were

mildly elevated with levels of 13.4 ± 15.8 g/ml (95% CI 5.6 to 21.2) and 7.8 ± 2.8 x 10^9 (95%CI 6.9 to 9.1), respectively.

With respect to pulmonary function parameters the study group had comparatively lower FEV_1 and FVC when compared to the control group (50.0 ± 3.3 %predicted vs. 60.6 ± 20.3 %predicted and 49.0 ± 3.0 %predicted vs. 59.0 ± 25.8 %predicted), respectively.

For the control group eighteen participants were enrolled with an unbalanced gender distribution, with more females than males (15 vs. 3) enrolled (Table 11). The participants in the control group were also older with a mean age of 14.4 years. The growth parameters of the both the study population and the CF control groups were within normal range according to WHO growth chart for weight, height and BMI z-scores [222].

Table 10. Clinical and laboratory data of children with HIV-related bronchiectasis (N=24)

Parameter	Mean	Standard Deviation
Age (years)	8.0	2.2
Weight (z- score) kg	-1.5	0.92
Height (z-score) m	-1.9	1.5
BMI (z-score) kg/m^2	-0.6	0.9
sTREM-1 (pg/ml)*	677.2	320.1
CRP (mg/ml)	13.4	15.8
WCC ($x10^9/l$)*	7.8	2.8
IgG (g/l)	29.8	12.5
HIV-VL (copies/ml)	14335.8	46449.7
CD4 count (%)	21.2	10.7
HAART (months)	17.8	3.5
FEV_1 (% predicted)	50.0	3.3
FVC (% predicted)	45.0	3.0

*: Geometric mean reported; sTREM: soluble triggering receptor expressed on myeloid cells; CRP: C-reactive protein; WCC: white cell count; HIV-VL: Human immunodeficiency virus viral load; HAART: Highly active antiretroviral therapy; FEV_1: Forced expiratory flow in one second; FVC: Forced vital capacity.

Table 11. Baseline parameters of children with cystic fibrosis-related bronchiectasis (N=18)

Characteristic	Median (SD)
Age (years)	14.4 ± 3.5
Weight z-score (kg)	-1.2 ± 1.6
Ht z-score (cm)	-0.9 ± 1.5
BMI z-score (kg/m^2)	-1.1 ± 1.2
sTREM-1 (pg/ml)*	189.6 ± 89.7
FEV_1 (%predicted)	60.6 ± 20.3
FVC (%predicted)	59.0 ± 25.8

*: Geometric mean reported; BMI: Body mass index; sTREM: Soluble triggering receptor expressed on myeloid cells; FEV_1: Forced expiratory volume in one second; FVC: Forced vital capacity; z-scores: Expressed according to the World Health Organization growth charts

7.4 DISCUSSION

In the current study of children with HIV-related bronchiectasis, significantly elevated sTREM-1 levels in the study participants was found. Moreover, the level of sTREM-1 was significantly higher in children with bronchiectasis secondary to HIV-infection when compared to those with CF-related bronchiectasis. Despite being younger, the children with HIV had significantly more respiratory morbidity, with lower pulmonary function parameters and overall lower anthropometric measurements, when compared to the CF control group. Whether, this accounts for the higher sTREM-1 values, possibly related to a more aggressive inflammatory process in the lungs, needs further exploration. There was also no correlation between the level and sTREM and the degree of bronchiectasis or the level of immunosuppression.

sTREM-1 is a marker of innate immune function and is expressed on blood neutrophils, monocytes and alveolar macrophages [161,262,263]. The function of sTREM-1 is to upregulate the immune system in response to antigenic challenge. This has been previously reported to be of value particularly in the acute phase response to antigens, and therefore has been suggested by some authors to be a potential biomarker to aid in the diagnosis of inflammatory lung diseases [162,164,262].

CF lung disease is characterized by chronic endobronchial infection. The susceptibility for this protracted lung infection is multifactorial but is linked to abnormal chloride channel function leading to airway dehydration and thick mucus. One of the causes may be an abnormal innate immunity. In CF increased colonisation of the airways points to abnormal innate immune mechanisms; which also affect CXC chemokine receptor (CXCR) clearing of neutrophils, with resultant decreased neutrophil activity [161]. In vitro evidence in CF cultured monocytes has shown a reduced production of sTREM-1 upon lipopolysaccharide stimulation, suggesting that in CF, monocytes have been “locked in” on endotoxin tolerance, which results in down regulation of sTREM-1 levels upon challenge [171]. The current study has been able to demonstrate in-vivo evidence of elevated sputum sTREM-1 in CF participants, but the level was comparatively lower than subjects with HIV-related bronchiectasis. This may suggest that the inflammatory milieu in HIV-bronchiectasis may be conducive to more severe lung tissue destruction, and subsequently more severe morbidity.

A recent study in COPD adults by Rohde et al, found no difference in sTREM-1 levels between subjects with stable state COPD and those with COPD exacerbations whose levels of serum sTREM were 97.5 pg/ml and 110.9 pg/ml respectively although this difference was not statistically significant [168]. In the current study the sputum sTREM levels were higher that those described by Rohde, although this may be due to the differences in site of collection. This suggests that in conditions of persistent inflammation sTREM may not be reliable in differentiating acute exacerbations and quiescent periods [168,263]. The present study also found no

association between exacerbations and increased levels of sTREM-1, although the number of participants with exacerbations was too small to draw any meaningful conclusions. Rasdak et al, found a negative correlation between pulmonary function impairment in adults with COPD and sTREM-1 [264].

Previous studies of sTREM-1 in immunocompromised patients with febrile neutropenia have shown a correlation of sTREM-1 with pulmonary disease severity [265]. Tintinger et al, also found a correlation of sTREM-1 with disease severity in adults with CAP, half of whom were HIV-infected [169]. As in this study, the HIV-infected participants in their study had higher sTREM-1 levels reflecting an overactive innate immune system.

Recent evidence from adults has shown sTREM-1 to be beneficial in the discrimination between colonisation and active disease of NTM infections; this may play a potential role in those subjects with chronic lung disease who are at risk of NTM [170]. This requires further study especially in HIV-infected patients where the diagnosis of TB and NTM is problematic.

This study demonstrates that the immune mechanisms in HIV-bronchiectasis may be different to those of CF-bronchiectasis, suggesting that future therapeutic interventions that target innate immune mechanisms may be useful.

The strength this study is that it provides pilot data on sTREM-1 two chronic inflammatory lung conditions with different pathophysiological mechanisms. The limitations of this study are the small sample size. There were moreover, insufficient study participants with exacerbations in order to draw any conclusions about sTREM-1values in patients with or without exacerbations.

7.5 CONCLUSION

The pulmonary innate immune functions are over-active in HIV-related bronchiectasis, with elevated sTREM values, which are higher than those in cystic fibrosis.sTREM-1 does not correlate with any markers of HIV-disease activity, pulmonary function parameters and is not useful to diagnosis of pulmonary exacerbations in HIV-related bronchiectasis.

CHAPTER VIII

POSITRON EMISSION TOMOGRAPHY IN THE PREDICTION OF INFLAMMATION IN CHILDREN WITH HUMAN IMMUNODEFICIENCY VIRUS-RELATED BRONCHIECTASIS

8.1 OBJECTIVES

The primary aim of this component of the thesis was to evaluate the ability of 2-[F-18]-fluoro-2-deoxy-D-glucose positron emission tomography (^{18}F-FDG-PET) to detect sites of active inflammation in children with HIV-related bronchiectasis, with or without exacerbations. A secondary end-point was to assess whether ^{18}F-FDG-PET findings could agree with local and systemic inflammatory biomarkers or HIV disease activity markers.

8.2 SUBJECTS AND METHODS

8.2.1 SUBJECTS

The participants enrolled are those already described in Chapter V, with a total of forty-one included in the study (Section 5.2.1).

8.2.2 METHODS

Clinical evaluations

The information that was applicable for this sub-study of the thesis included: the presence of a respiratory exacerbation as per criteria defined in Chapter V (5.2.2). Pneumonia was diagnosed by the presence of symptoms suggestive of an exacerbation together with a new area of consolidation and air bronchograms on HRCT chest or CXR.

Sputum samples: The sputum samples germane to this sub-study were the samples for microbiology (Section 5.2.1) as well as for cytokine determination for; IL-8, INF-γ and TNF-α (Section 6.2.2).

Blood samples: Serum for the following was relevant for this sub-study: CRP, $CD4^{+}$ T lymphocytes; HIV-1 viral load; and the cytokines IL-8, INF-α and TNF-γ.

^{18}F-FDG PET/CT scanning

Whole body ^{18}F-FDG PET scans were acquired on a PET-CT scanner (Biograph, Siemens) from the skull top to the pelvis after fasting for a minimum of 4hours. Patients received a dose of ^{18}F-FDG based on their body weight using the following formula: ((body weight/10) +1))*37MBqwith a minimum activity of 74MBq and a maximum of 370MBq. PET/CT images were acquired at 60 minutes after intravenous injection of ^{18}F-FDG. Images were acquired in a 3-dimensional mode and reconstructed with and without attenuation correction (CT-based) using ordered subset expectation maximisation (OSEM) yielding axial, sagittal and coronal slices. This study measured the maximum standardised uptake value (SUVmax) in four zones of the lungs using whole body ^{18}F-FDG-PET. ^{18}F-FDG-PET images were analysed for the presence or absence of active 'lesion' sites by two experienced and blinded nuclear medicine physicians by consensus.

All children underwent high resolution CT scanning in combination with the PET scan, the methodology of which is described in Section 7.2.2

Ethical clearance

The ethical approval obtained for the thesis applied to this study component.

Statistical analysis

Statistical analysis was performed using Stata Release 10 (Statacorp LP, College Station, TX, USA). The Fisher exact test was used for analysis of categorical variables and logistic regression for the relationship between PET uptake and i) consolidation, ii) exacerbations, iii) bacterial colonisation and iv) previous TB. The Wilcoxon rank-sum (Mann-Whitney) test was used to compare the differences in the cytokines and CRP between participants with and without positive uptake on ^{18}F-FDG PET. Statistical significance was defined as $p<0.05$.

8.3 RESULTS

There was positive tracer uptake on PET in 18 (46.9%) participants. Twelve patients (29.2%) had a clinical exacerbation at the time ^{18}FDG-PET was performed. Of these twelve only six (50%) had positive uptake on ^{18}F-FDG-PET. Twelve of the eighteen participants with positive FDG uptake were not regarded as having a clinical exacerbation (Figure 9). There was no statistically significant difference in the ^{18}F-FDG uptake in participants, with or without an exacerbation, at the time of PET (odds ratio 1.4 (95% CI 0.4 to 5.5; p=0.61). The sensitivity and specificity of PET to detect exacerbations was 50% and 59% respectively.

Of the patients with tracer uptake, 9 had bilateral uptake, which involved segments in both the right and left lung. There was uptake involving the left lower lobe in 7 of 18 subjects, with the left lower lobe being the only area of uptake in 6 and in one subject there was uptake in both the left and right lower lobes. The left lower lobe was the lobe with the most significant uptake of all the lobes involved.

In the total study population there was consolidation on the CT scan in twelve participants. Of participants with consolidation, three had a clinical exacerbation at the time of PET. Nine participants had positive ^{18}F-FDG uptake and consolidation at the time of ^{18}F-FDG-PET. This was statistically significant odds ratio 6.67 (95% CI 1.5 to 30.6; p=0.01) [Figure 10].

There was no statistically significant difference in mean SUVmax between participants, with or without the presence of a bacterial organism on culture (p=0.73). There was microbiological confirmation of mycobacterial infection in three patients; with two having *Mycobacterium tuberculosis* complex and another *Mycobacterium avium intracellulare* infection at the time of the PET scan. All participants with active TB or MOTTs had positive uptake on ^{18}F-FDG-PET. The mean SUV was higher for the participants with consolidation when compared to those with TB (4.4 vs. 2.5).It should be noted that the TB positive participants had received two and three months of anti-TB treatment, respectively. There was no statistically significant difference, in ^{18}F-FDG uptake, between participants with previous TB and those without previous TB odds ratio 0.72 (p=0.65).

All the participants included were on HAART. The mean number of months on HAART was 17.6 ± 17.7 months for the study population, with the majority of subjects 22 (54%) having HIV viral suppression; with viral loads of <25 copies/ml. The median HIV viral load was 61.7 ± 254243.0 copies/ml (95% CI 13.5 to 281.8). There was no statistically significant difference between subjects with positive ^{18}F-FDG uptake as compared to those with no uptake, with respect to number of months on HAART (19.8 ± 19.0 months and 14.8 ± 16.3 months, 95% CI -6.1 to 16.2; p=0.37), CD4 % (19.0 ± 8.3% and 19.8 ± 11.8%, 95% CI -7.6 to 5.8; p=0.99) or HIV viral load (85210.0 ± 333895.0 copies/ml and 15997.7 ± 36601.5 copies/ml, 95% CI -96056.0 to 214480.7; p=0.24).

The mean Bhalla score for all the subjects was 13.9 ± 4.3. There was no statistically significant difference in the Bhalla scores when comparing participants with and without ^{18}F-FDG uptake (13.2 ± 1.1 and 14.9 ± 0.8, 95% CI -4.6 to 1.0; p=0.20). There was presence of bronchiectasis in 116 lobes of the participants. The most affected lobes were the left lobe and right lower lobes in 42 (36%) and 26 (22.4%), respectively. There was also no statistically significant difference, with respect to the presence of exacerbations and Bhalla scores, in these individuals (p=0.19).

The mean CRP was significantly higher in the subjects with ^{18}F-FDG uptake when compared to those without uptake (15.0 ± 95.1 mg/ml and 4.2 ± 19.9 mg/ml), respectively. This difference was not statistically significant (95% CI -107.0 to 29.6; p=0.09) (Table 2). The CRP was similar in subjects with presence or absence of a bacterial or viral organism cultured from the sputum. There was no difference in the serum neutrophil value in the subjects, with and without, ^{18}FDG uptake (4.2 ± 6.3 $x10^9$ and 3.9 ± 2.9 $x10^9$, 95% CI -2.9 to 3.5; p=0.87), respectively.

Table 12. Baseline characteristics of children with HIV-related bronchiectasis undergoing ^{18}F-FDG-PET (N=41)

Characteristic	Mean ± SD	95% CI
Age (years)	8.2 ± 2.2	7.3 - 8.6
Exacerbation	12 (29.2)	
HAART (months)	17.6 ± 17.9	12.0 - 23.5
$CD4^+$ T cell (%)	19.3 ± 9.9	16.2 - 22.5
HIV-VL (copies/ml)¶	61.7 ± 254243.5	13.5 - 281.8
Bhalla score	13.9 ± 4.3	11.6 - 14.9
CRP (mg/ml)	8.8 ± 63.2	4.8 - 15.9
Serum		
IL-8¶ (pg/ml)	218.3 ± 178560.2	91.4 - 520.9
TNF-α (pg/ml)	2.3 ± 0.9	1.9 - 2.6
INF-γ (pg/ml)	204.9 ± 349.8	78.8 - 331.0
Sputum		
IL-8¶ (pg/ml)	785.0 ± 9352.1	349.5 - 1766.3
TNF-α (pg/ml	1.1 ± 0.7	0.8 - 1.3
INF-γ (pg/ml)	16.0 ± 21.5	7.8 - 24.2

¶: Geometric means reported ;(): Percentages in parenthesis; CRP: C reactive protein; IL-8: Interleukin 8; HIV-VL: HIV viral load; $CD4^+$ T cell %: Percentage of cluster differentiation 4; TNF- α: Tumour necrosis factor alpha; INF-γ: Interferon gamma; SD; Standard deviation.

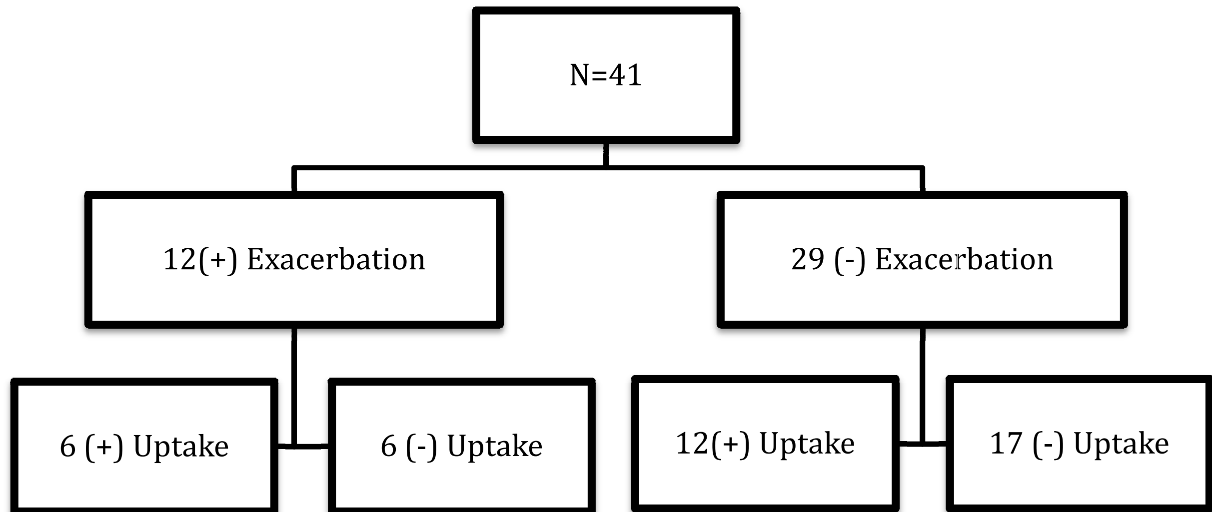

Figure 9. **Flow diagram of ^{18}F-FDG PET results of children with human immunodeficiency virus-related bronchiectasis**

(+): Presence of exacerbation or FDG uptake; (-): No exacerbation or FDG uptake

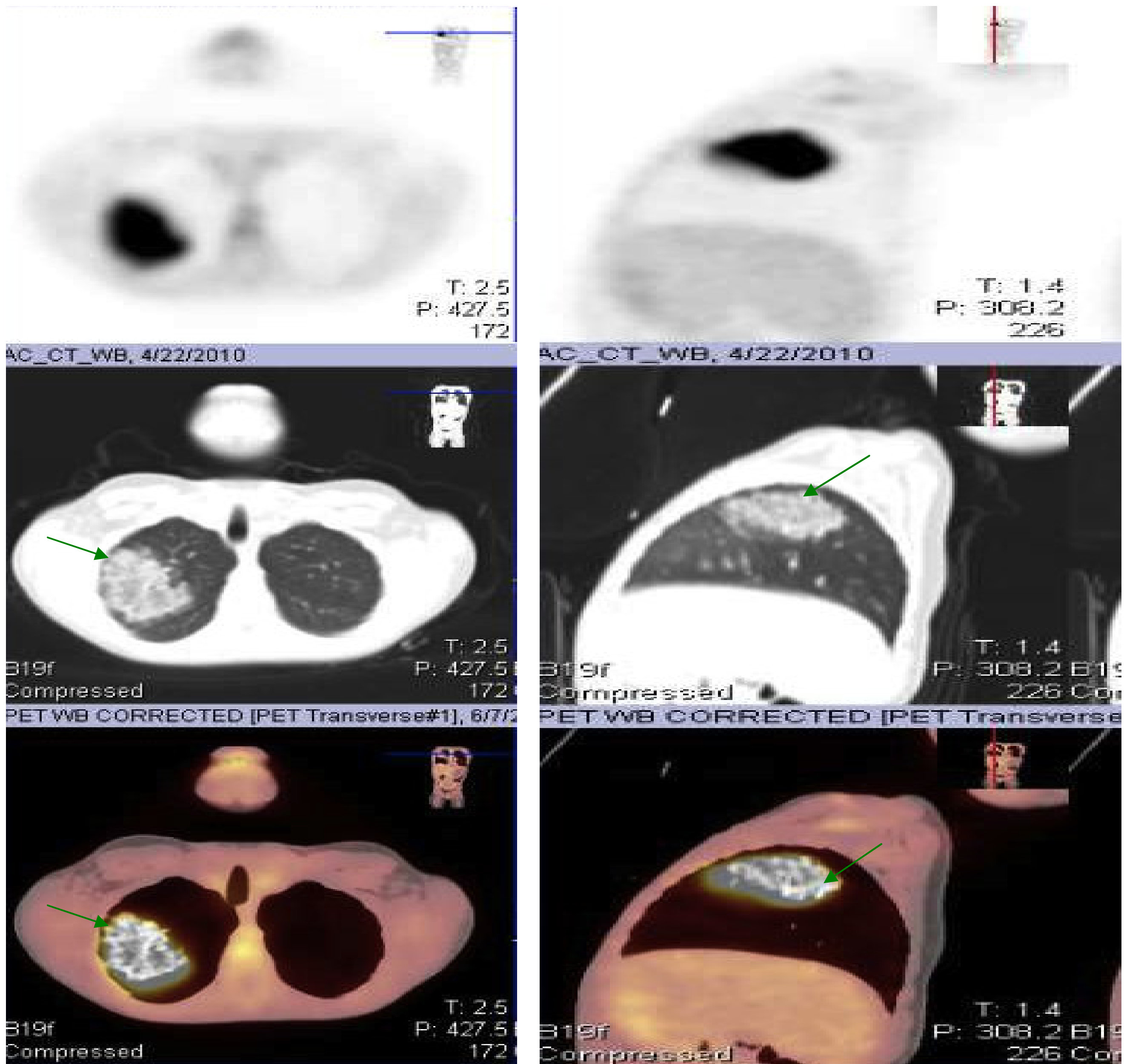

Figure 10. **Transverse and axial views ^{18}F-FDG PET/CT of patient with consolidation and positive ^{18}F-FDG uptake in the right upper lobe (indicated with arrows)**

With respect to the cytokines, IL-8 was elevated in both serum and sputum (median 115.6 ± 178560.2 pg/ml and 332.8 ± 9352.1 pg/ml), respectively. There was no correlation between serum and sputum IL-8 and the Bhalla score (p=0.32 and p=0.37), respectively. There was also no difference in serum IL-8 and sputum IL-8 between patients, with and without, ^{18F}-FDG uptake on PET (95% CI -10278.6 to

6319.6; $p=0.62$ and 95% CI -58157.3 to 167156.8; $p=0.32$), respectively. There was no statistically significant difference with respect to the sputum TNF-α and serum TNF-α between subjects, with and without, ^{18}FDG uptake (1.9 ± 3.4 pg/ml and 1.0 ± 2.3 pg/ml; p= 0.67 versus 5.9 ± 18.3 pg/ml and 12.8 ± 10.6 pg/ml; $p=0.68$), respectively. The INF-γ was elevated, more significantly, in the serum than the sputum. Median levels of INF-γ in sputum and blood did not differ with respect to ^{18}FDG uptake on PET (2.1 ± 23.1 pg/ml and 18.4 ± 19.3 pg/ml, 95% CI -23.1 to 9.33; $p=0.39$) and (118.7 ± 431.7 pg/ml and 150.0 ± 181.0 pg/ml, 95% CI -152.6 to 303.4; $p=0.50$), respectively. All comparisons are reflected in Table 13.

Table 13. Inflammatory markers for children with HIV-related bronchiectasis with and without ^{18}F-FDG uptake

Inflammatory marker	No ^{18}FDG uptake N=23	^{18}FDG uptake N=18	P value (95% CI)
CRP (mg/ml)	4.2 ± 19.9	15.0 ± 95.1	0.09 (-107 to 29.6)
Neutrophil (x10^9/l)*	4.2 ± 6.3	3.9 ± 2.9	0.87 (-2.9 to 3.5)
Sputum cytokines			
IL-8 (pg/ml)	222.5 ± 9203.0	1799.0 ± 10341.0	0.62 (-10278.6 to 6319.6)
TNF-α (pg/ml)	1.9 ± 3.4	1.0 ± 2.3	0.67 (-2.6 to 4.9)
INF-γ (pg/ml)	2.1 ± 23.1	18.4 ± 19.3	0.39 (-23.1 to 9.33)
Serum cytokines			
IL-8 (pg/ml)	113.3 ± 4194. 0	1205.3 ± 549.0	0.32 (-58157.3 to 167156.8)
TNF-α (pg/ml)	5.9 ± 18.0	12.8 ± 10.6	0.68 (-8.2 to 12.5)
INF-γ (pg/ml)	118.7 ± 431.6	150 ± 181.7	0.50 (-152.6 to 303.4)

*: Neutrophils measured in serum; FDG: Fluorodeoxyglucose; CRP: C reactive protein; IL-8: Interleukin 8; TNF-α: Tumour necrosis alpha; INF- γ: Interferon gamma; Wilcoxon ranksum test done for comparing subjects with and without ^{18}F-FDG uptake.

8.4 DISCUSSION

No differences, in SUV-max values, in relation to sites of lung involvement, were found between those individuals with clinical signs of an exacerbation and those without an exacerbation. Hypothetically, this may relate, to the plethora of variables and their inter-individual contribution to ^{18}F-FDG uptake in such patients, to the lack of a gold standard definition for exacerbations or to an anamnestic effect by the caregiver or participants. Under inflammatory conditions, neutrophils and activated macrophages display a high ^{18}F-FDG uptake, which is in part due to the up-regulated glucose transporter system and to an increase of affinity for deoxyglucose increased by various cytokines and growth factors [266,267]. This mechanism might explain

the positive correlation between the rate of ^{18}F-FDG uptake in the lung field and the number of neutrophils present in bronchoalveolar lavage fluid [221].

Other researchers have demonstrated, using cell autoradiography, that neutrophils are the predominant cells that take up ^{18}F-FDG in bronchoalveolar lavage fluid of CF participants [222]. A recent study, with 20 CF participants, found that using a cut-off of SUV > 3, the authors could characterize foci being of low or high intensity and this could be used as a working threshold. In addition, scans showing high tracer uptake, supported the clinical definition of an exacerbation [221]. This lack of correlation between SUV and serum neutrophils has been confirmed for sputum neutrophils in the current study. This finding has been confirmed in previous studies where a lack of uptake was noted in subjects with CF bronchiectasis, despite elevated sputum neutrophils [219]. Although cells are continually migrating to the inflammatory site**,** mucociliary clearance and cough are responsible for their removal from the lungs. The current study did not assess sputum neutrophils, therefore the lack of correlation could be due to the fact that neutrophils distant from the “inflammatory site” were measured and not local neutrophil populations. The implication of this finding is that in HIV-related bronchiectasis, systemic neutrophils may not be highly activated, despite seemingly adequate immune restoration by antiretroviral therapy and HIV virological suppression.

Previous studies have however shown a correlation between FDG uptake and the presence of consolidation on CT namely in acute lobar pneumonia and bronchiectasis [218]. This suggests that a PET study may be more reliable in acute lobar pneumonia, where there are sufficient numbers of neutrophils at the inflammatory site. In this series, the majority of participants with positive ^{18}F-FDG uptake and consolidation did not fulfil the clinical criteria of an exacerbation. This may suggest that ^{18}F-FDG PET is more sensitive in assessing inflammation and thus superior to the clinical assessment for the detection of bronchiectasis inflammation and the presence of exacerbations. As with previous studies of non-CF bronchiectasis the anatomical localisation of bronchiectasis was mostly in the lower lobes, in over half of the lobes affected [268].

In the current study systemic and pulmonary cytokines IL-8, TNF-γ and INF-α were elevated. IL-8, a cytokine produced by neutrophils, was the cytokine most significantly elevated [125,126]. Despite the presence of these cytokines in serum and sputum, there was no demonstrable uptake on the ^{18}F-FDG-PET scan. This may be explained by the fact that the majority of participants in this study population had a positive culture of pathogens in their airways. The presence of colonising organisms has been postulated to produce factors that suppress the respiratory burst of neutrophils, by affecting surface receptors or through the presence of substances capable of affecting neutrophil activity in mucus [269].

Other authors have demonstrated a correlation between IL-8 and a modified Bhalla score [270]. This study was not able to confirm this finding. Two explanations for this difference may be a difference in sample size; this study having forty-one versus their smaller study population of 27 subjects, and their inclusion of children with heterogeneous causes of bronchiectasis.

Importantly, in the series presented, the CRP significantly higher and the intensity of ^{18}F-FDG uptake although this was no statistically significant suggesting the presence of an acute inflammation. This finding concurs with other studies where ^{18}F-FDG associated with a high CRP level has been found to quantitatively delineate infection and inflammation in a diverse group of disorders including CF, pneumonia, pulmonary fibrosis and interstitial pneumonitis [271, 272].

A limitation of this study is the small sample size, which may explain the lack of correlation of ^{18}F-FDG uptake with the inflammatory markers. In addition bronchoalveolar lavage or induced sputum specimens, to obtain neutrophils, may have provided better fit with ^{18}F-FDG and inflammatory markers.

This study provides pilot data for a larger trial sufficiently powered to investigate the association of ^{18}F-FDG PET and inflammatory biomarkers. The lack of availability if PET scanning, as well as the high cost of this diagnostic modality may also preclude it's widespread use, considering the findings in the current study.

8.5 CONCLUSION

There is a lack of a significant correlation of ^{18}F-FDG uptake and clinical analysis of an exacerbation, although the presence of ^{18}F-FDG uptake in subjects without an exacerbation suggests that ^{18}F-FDG-PET may be more sensitive in assessing inflammation than currently available tools such as systemic and sputum cytokines or acute phase reactants. ^{18}F-FDG-PET has demonstrated no significant correlation with markers of HIV disease activity.

CHAPTER IX

THE EFFICACY OF LOW DOSE ERYTHROMYCIN IN IMPROVING THE OUTCOME OF HUMAN IMMUNODEFICIENCY VIRUS-INFECTED CHILDREN WITH BRONCHIECTASIS

9.1 OBJECTIVES

This sub-study of the thesis was conducted as a randomised, double-blind, placebo-controlled trial to assess the efficacy of erythromycin when compared to placebo, in reducing the number of pulmonary exacerbations in children with HIV-related bronchiectasis over a period of 52 weeks. Secondary end-points were to assess whether or not erythromycin had an impact on pulmonary function parameters and pro- and anti-inflammatory chemokines/cytokines both systemically and locally, in the lungs.

9.2 SUBJECTS

9.2.1 SUBJECTS

The baseline characteristics of some of the participants have been previously described as they form part of a larger study of children described in Chapter V (Section 5.2.1). The inclusion criteria for enrolment have been described in Chapter IV and are summarised below.

Inclusion criteria

Children aged 6 to 18 years with confirmed HIV infection confirmed by positive HIV Elisa if diagnosis age ≥ 18 months or a positive HIV PCR if diagnosis age was ≤ 18 months). The presence of bronchiectasis was confirmed on high resolution CT scanning with exclusion of other causes of bronchiectasis including a sweat test. All

children had tube able to perform reliable pulmonary function tests and be able to present for monthly follow up visits for a period of 52 weeks.

Exclusion criteria

Children were excluded there was presence of the following at presentation: abnormal liver function tests (ALT/AST > 2.5 times normal) and abnormal urea and creatinine. Other exclusion criteria included the use of one of the following medications: carbamazepine, anti-coagulants (warfarin), ciclosporin or long-term midazolam therapy.

All the participants were randomised to receive either erythromycin (Adco erythromycin estolate) at a dose of 125 mg per os daily if ≤15kg body weight or 250mg per os daily if > 15kg body weight, or a matching placebo daily. This erythromycin dose was chosen as a quarter of the expected daily dose in line with previous studies [188,191]. Participants were followed up monthly for a period of 52 weeks

9.2.2 METHODS

Randomisation and Blinding

Participants were randomly assigned (1:1) according to a randomisation plan, generated by the statistician, to the erythromycin arm or the placebo arm. All the study personnel performing the clinical evaluations and study procedures were blinded to the treatment assignment. The participants were followed up by the blinded clinicians and treated with usual care therapy if they experienced an exacerbation. An exacerbation was defined as per protocol criteria defined in Chapter V (5.2.2). Compliance was assessed with the use of a medication diary, as well as verbal interviews of the caregivers at every study visit.

Clinical investigations

Information relevant to this sub-study of the thesis included: the age at HIV diagnosis, timing of initiation of HAART and growth parameters at study entry and study end (Section 5.2.2). Pulmonary function measurements; $FEV_{1,}$FVC and $FEF_{25/75}$ were measured at each study visit. All participants underwent PET/CT scan at study entry and study end. The Bhalla score was performed on all the scans by two blinded radiologists who independently scored the CT scans, they were blinded to the clinical data, morphological testing and special investigations of the participants, with any disagreements resolved by consensus [108]. The methodology of the Bhalla scores is described in Section 7.2.2.

Laboratory investigations

The pre-treatment cytokine data for this group of participants was described in Section 5.2.2. In this sub-study, the pre- and post treatment serum and sputum cytokine specimens were analysed simultaneously using a modified, improved version of the original assay. Analysis of the cytokines present in the supernatants was performed using the Bio-Plex Suspension Array System (Bio-Rad Laboratories, Inc. Hercules, Canada) and a Bio-Plex Pro^{TM} assay kit (Bio-Rad Laboratories, Inc). The Bio-Plex Pro^{TM} assay kit is a magnetic bead-based multiplex assay designed to measure multiple cytokines in different matrices. The assay kit used in this sub-study included the following cytokines: IL-1β, IL-6, IL-8, IL-10, TNF-α, IP-10 and TNF-R1. The results are expressed as pg/ml.

Blood and serum samples: Samples relevant for this sub-study of the thesis were drawn at study entry and end of study for; CRP, IgG, total white cell count, $CD4^{+}$ T lymphocytes and HIV-1 viral load (detailed methodology in Section 5.2.2)

Sputum elastase: Concentrations of the phagocyte-derived, primary granule protease, elastase, in sputum specimens were measured using a commercial,

capture, sandwich ELISA procedure (Hycult Biotechnology, Uden, The Netherlands), and the results expressed as nanograms elastase/ml (ng/ml) sputum.

Sputum sTREM: Sputum samples were collected for sTREM analysis at study entry and study end via the methodology described in Section 7.2.2 with the results expressed as pg/ml.

Sputum samples: Sputum samples were collected at monthly intervals in all the participants for microbiological testing including MTB where applicable.

Statistical analyses

The sample size calculation was based on the number of pulmonary exacerbations requiring antibiotic therapy, which was estimated to be 3 per year. A sample size of 25 patients per study arm was determined to have a 90% power to detect a clinically relevant reduction in exacerbations of 30%, where a mean of 2 and a standard deviation of 1 exacerbation were assumed; with a presumed drop out rate of 10%, when testing was one-sided at the 0.05 level of significance. Analysis of variance (ANOVA) was used to compare medication groups with respect the mean number of exacerbations, as there was no baseline value. For the study variables, treatment arms were compared with respect to change from baseline to end of study using ANCOVA, with baseline values as covariates. Wilcoxon test was used to assess the pooled data for IL-8, TNF-α and lung function tests. The Spearman correlation test was used to assess correlations between the cytokines and markers of HIV disease activity ($CD4^+$ T cell counts and HIV viral load). Data analysis was performed using Stata Release 10 (Statacorp LP, College Station, TX, USA).

Ethical clearance

The ethical approval obtained for the thesis applied to this study component.

9.3 RESULTS

As demonstrated in Figure 11, a total of fifty-six children were screened with forty-three meeting all inclusion criteria. Two children died prior to randomisation. Ten (23%) participants (four in placebo arm and six in erythromycin arm) were lost to follow up during the 52-week follow up period. A total of thirty-one participants of whom 58% were male, completed all study-related procedures and were included in the final analysis. The baseline characteristics of the two treatment arms are reflected in Table 14. The characteristics of the two study arms were generally balanced, with the exception of gender distribution with more males (55%) in the erythromycin arm and more females in the placebo arm. This was not significant.

All children were on HAART prior to enrolment. HIV virological suppression was achieved in the majority of participants with a geometric mean of (0.0 ± 22514.3 copies/ml and 80 ± 9635.2 copies/ml, p=0,97) in the erythromycin and placebo arms, respectively. The total circulating $CD4^{+}$ T cell counts and percentage counts in the erythromycin arm were lower than in the placebo arm (650.9 ± 446.7 and 881.6 ± 505.8; p<0.01 versus 16.3 ± 6.7% and 22.6 ± 11.9%; p=0.01), respectively and this was statistically significant. The lower significant $CD4^{+}$ T cell counts were reflective of a shorter period on HAART when comparing the two study arms with the number of months on HAART being (12.0 ± 12.8 months and 17.0 ± 22.0 months), in the erythromycin arm when compared to the placebo arm, although this was not statistically significant.

At study entry the growth parameters of children in both study arms were within the normal range. The compliance in both study arms was excellent, with more than 90% patients taking study medication- confirmed with the use of a recorded diary card and pill count of returned medication.

There was no statistically significant change when comparing the Bhalla scores at baseline and study end in both treatment arms, indicating stability in the degree of bronchiectasis over the study period.

Of the microbiological cultures over the year only 2% of organisms cultured were PA and 2% mycobacteria other than tuberculosis- *M. fortuitum* and *M. avium intracellulare* as well as one MTB.

There was no statistically significant difference in the mean number of exacerbations in the treatment versus the placebo arm (2.14 ± 2.28 per year and 2.18 ± 1.59 per year; $p=0.17$), respectively. However, 18% (erythromycin) vs. 0% (placebo) of study participants had no exacerbations during the study duration.

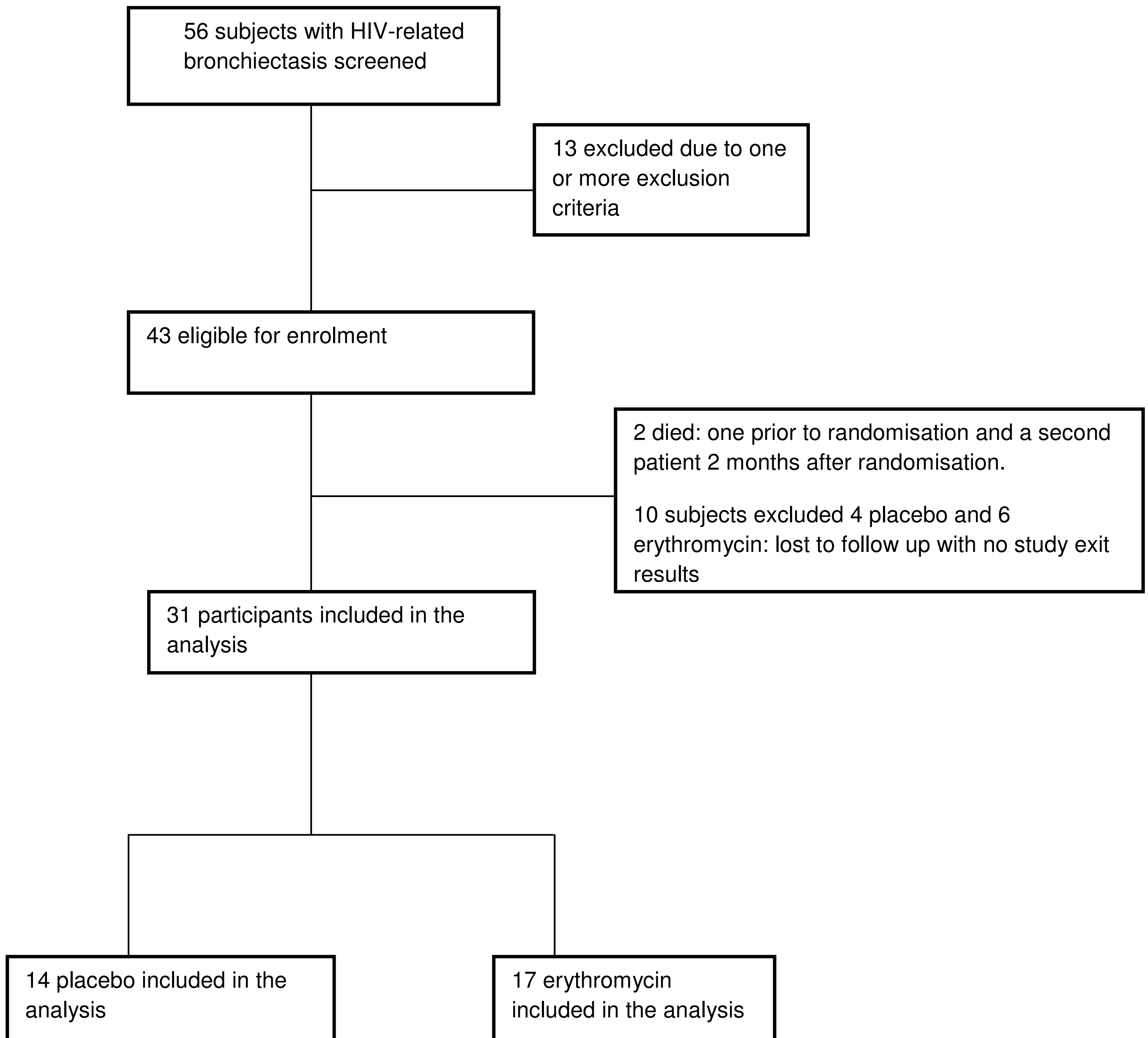

Figure 11. **Enrolment and randomisation of participants included in the study**

Table 14. Baseline characteristics of children with HIV-related bronchiectasis treated with erythromycin or placebo

Characteristic	Placebo (mean± SD)	Erythromycin (mean± SD)	P value
Age (years)	9.1 ± 2.1	8.4 ± 2.4	0.15
Exacerbations	2.1 ± 2.3	2.2 ± 1.6	0.47
Months on HAART	17.0 ± 22.0	12.0 ± 12.8	0.57
Weight z -score (kg)	-1.8 ± 0.9	-1.6 ± 1.6	0.77
Height z-score (cm)	-1.7 ± 1.4	-1.7 ± 1.5	0.50
BMI z-score (kg/m^2)	-0.6 ± 0.9	-0.5 ± 1.3	0.91
$CD4^+$ T cell count (%)	22.6 ± 11.9	16.3 ± 6.7	**0.01**
$CD4^+$ T cell (total $x10^6$)	881.6 ± 505.8	650.9 ± 446.7	**<0.01**
HIV viral-load (copies/ml)*	80.0 ± 22514.3	0.0 ± 9635,2	0.97
FEV1 (% predicted)	53.5 ± 13.6	56.0 ± 15.1	0.54
FVC (% predicted)	45.0 ± 14.3	49.0 ± 14.4	0.94
$FEF_{25/75}$ (% predicted)	55.1 ± 25.3	56.0± 25.7	0.89
IgG (g/ml)	24.8 ± 15.4	26.2 ± 8.4	0.54
CRP (mg/l)	3.6 ± 16.1	9.4 ± 18.8	0.08
Bhalla score[¶]	11.5 ± 4.3	15.0 ± 4.0	0.02
Compliance (% medication)	91.0 ± 9.9	92 ± 9.9	0.87

SD: Standard deviation; BMI: Body mass index; HAART: Highly active antiretroviral therapy; CD4: Cluster differentiation cell; HIV: Human immunodeficiency virus; FEV1: Forced expiratory volume in one second; FVC: Forced vital capacity; IgG: Immunoglobulin G; CRP: C reactive protein; WCC: White cell count; [¶]Bhalla score: Appendix D; * Geometric means reported.

For the characteristics of the participants at the end of the study period (summarised in Table 15), there was an improvement in weight, which was more pronounced in the placebo versus the erythromycin arm. This difference was not statistically significant (p=0.45). There was a significant improvement in the BMI z-scores when comparing the two study arms although this was not statistically significant; this was

more so for the participants in the placebo arm when compared to the erythromycin arm (-0.6 ± 0.9 and -0.2 ± 1.0 versus -0.5 ± 1.3 and -0.4 ± 1.6; p=0.08), respectively. The immunological status of the subjects improved in both study arms with increases in the $CD4^{+}$ T cell counts and decrease in the HIV viral load, although these differences were not statistically significant between the study arms (p=0.88 and p=0.43), respectively.

For the pulmonary function parameters, there was an improvement (although not statistically significant) in FEV_1 (56.0 ± 15.1 %predicted and 68.0 ± 21.0 %predicted versus 53.5 ± 13.6 %predicted and 62.5 ± 13.6 %predicted; p=0.31) pre-and post therapy, for the erythromycin and placebo groups, respectively and FVC (49.0 ± 14.4 %predicted and 63.0 ± 17.9 %predicted versus 45.0 ± 14.3 %predicted and 58.0 ± 12.1 %predicted; p=0.46). After pooling the data for the pulmonary functions, increases in both the FEV_1 and FVC, from baseline to end of study, were statistically significant (52.7 to 61.5 %predicted; p=0.005 and 46.0 to 59.9 %predicted; p<0.001), respectively. There was no change in the pooled data for $FEF_{25/75}$ % predicted at study entry compared to study end (53.4 ± 28.1 %predicted and 52.5 ± 25.2 %predicted), respectively.

Table 15. Characteristics of children with human immunodeficiency virus related bronchiectasis pre- and post- treatment with erythromycin and placebo

Characteristic	Placebo (SD)		Erythromycin (SD)		
	Entry	End	Entry	End	P Value*
Weight z -score (kg)	-1.8 ± 0.9	-0.9 ± 0.8	-1.6 ± 1.6	-1.7 ± 1.7	0.45
Height z-score (cm)	-1.7 ± 1.4	-1.6 ± 1.4	-1.7 ±1.5	-1.9 ± 1.4	0.97
BMI z-score (kg/m^2)	-0.6 ± 0.9	-0.2 ± 1.0	-0.5 ± 1.3	-0.4 ± 1.6	0.08
CD4$^+$ T cell count (%)	22.6 ± 11.9	29.3 ± 11.4	16.3 ± 6.7	21.7 ± 7.8	0.88
CD4$^+$ T cell (total x 10^6)	881.6 ± 505.8	939.3 ± 530.6	650.9 ± 446.7	1036.7 ± 461.8	0.47
HIV viral load (copies/ml)	80.0 ± 22514.3	0.0 ± 26685.9	0.0 ± 9635.2	0.0 ± 19231.2	0.34
FEV$_1$ (% predicted)	53.5 ± 13.6	62.5 ± 13.6	56.0 ± 15.1	68.0 ± 21.0	0.31
FVC (% predicted)	45.0 ± 14.3	58.0 ± 12.1	49.0 ± 14.4	63.0 ± 17.9	0.46
IgG (g/ml)	24.8 ± 15.4	22.7 ± 6.9	26.2 ± 8.4	19.0 ± 5.4	0.24
CRP (mg/l)	3.6 ± 16.1	2.4 ± 21.0	9.4 ± 18.8	4.0 ± 73.9	0.98
Bhalla score	11.5 ± 4.3	12.5 ± 4.1	15.0 ± 4.0	15.0 ± 3.3	0.62

Z-scores according to WHO growth charts [225]; CD4: cluster differentiation 4 cells; HIV: Human immunodeficiency virus; FEV$_1$: Forced expiratory flow in 1 second; FVC: Forced vital capacity; IgG: Immunoglobulin G; CRP: C-reactive protein; *ANCOVA test used for analysis of data.

Table 16. Summary of serum and sputum cytokines in children with human immunodeficiency virus related bronchiectasis before and after treatment with erythromycin or placebo

Cytokine	Erythromycin Median (95% CI)		Placebo Median (95% CI)		P value
	Entry	End	Entry	End	
Serum					
IL-1β (pg/ml)	3.3 (1.1-9.7)	4.0 (2.3-7.0)	4.1 (2.1-8.0)	5.3 (2.1-13.3)	0.31
IL-6 (pg/ml)	6.9 (3.0-15.9)	6.1 (3.0-12.5)	18.4 (4.9-69.2)	14.9 (4.6-47.8)	0.31
IL-8 (pg/ml)	18.9 (9.0-39.6)	18.1 (7.4-44.2)	24.2 (7.0-83.7)	39.4 (12.9-119.8)	0.26
IL-10 (pg/ml)	3.9 (3.0-5.2)	3.9 (2.5-6.1)	4.8 (3.3-6.9)	4.3 (3.0-6.0)	0.51
IP-10 (pg/ml)	4667.9 (2620.9-8613.5)	3636.9 (2420.0-5465.8)	2734.6 (2341.7-5956.0)	3235.4 (2311.7-4528.3)	0.24
TNF-α *(pg/ml)	101.9(-70.3-274.1)	78.2 (-63.5-219.8)	55.0 (-27.2-137.2)	51.7 (3.8-99.7)	0.74
TNF-R1 (pg/ml)	111.8 (94.7-132.0)	106.9 (92.5-123.6)	119.5 (103.8-137.5)	115.4 (100.8-132.1)	0.95
Sputum					
IL-1β (pg/ml)	544.8 (198.0-1499.1)	575.3 (177.1-1869.1)	870.3 (366.8-2064.9)	823.2 (434.5-1559.6)	0.99
IL-6 (pg/ml)	5.6 (2.5-12.6)	2.9 (1.4-6.2)	5.6 (2.4-13.1)	4.6 (92.2-9.9)	0.39
IL-8 (pg/ml)	932.7 (341.1-2550.2)	268.4 (81.0-888.9)	1476.6 (537.5-4056.6)	808.3 (274.7-2378.3)	0.99
IL-10 (pg/ml)	0.6 (0.5-0.9)	0.6 (0.4-1.0)	0.8 (0.4-1.33)	0.7 (0.5-0.9)	0.93
IP-10 (pg/ml)	16.7 (4.1-68.5)	7.8 (5.9-10.5)	9.1 (6.9-11.9)	11.2 (6.3-19.7)	0.32
TNF-α *(pg/ml)	15.0 (8.2-21.8)	10.5 (5.7-19.4)	17.0 (9.9-24.2)	10.9 (6.2-19.0)	0.97
Elastase(ng/ml)	17.6 (13.2-23.4)	18.9 (13.0-66.6)	17.9 (13.1-24.6)	20.3 (16.5-25.0)	0.92
sTREM ((pg/ml)	635.2 (196.5-2053.2)	261.0 (92.9-733.0)	722.0 (237.3-2197.3)	633.8 (244.5-1642.8)	0.22

All mean reported as geometric means unless indicated; * Arithmetic means reported; sTREM: Soluble triggering receptor expressed on myeloid cells; IL- Interleukin: TNF-α: Tumour necrosis factor alpha, IP-10: Interferon gamma induced protein -10; Units of all the cytokines in picograms per millimetre except elastase in nanograms per litre. ANCOVA test used to obtain p-values for mean change from post treatment to mean change pre-treatment.

After intervention in both erythromycin and placebo study groups, there was a decrease in IgG. The change in IgG from baseline to study end was not attributed to the use of erythromycin as the change in both treatment groups was no statistically significant (p=0.24). There was no correlation between IgG and FEV_1 at study entry and study end (p=0.75 and p=0.73) for the pooled data for the study population. CRP also decreased from study entry when compared to the end of the study, although this decrease was not statistically significant when comparing the two treatment arms (p=0.98).

With respect to the pro-inflammatory cytokines, the chemokine IL-8 was most significantly elevated in the sputum, with a moderate decrease post-intervention in both the erythromycin and placebo arms. The changes in both the treatment arms were not statistically significant (p=0.99) (Table 16).

After pooling the data for sputum IL-8 for the whole study population, there was a statistically significant decrease of log values of sputum IL-8 from baseline to study end (geometric means 1234.5 and 434.5 pg/ml; p=0.04), respectively.

IL-1β was also elevated in the sputum. There was a modest decline in this cytokine in the erythromycin arm and a moderate elevation in the placebo arm. The change from baseline to study end of IL-1β in both the treatment arms, was not statistically significant (p=0.99). Although TNF-α levels declined in both treatment arms, the decline could not be attributed to the use of erythromycin. The pre- and post treatment serum TNF-α levels were independent of $CD4^+$ T cell percentage counts (p=0.74 and p=0.62) and HIV viral load (p=0.48 and p=0.90), respectively. There was also no statistically significant difference with respect to median IL-6 in the erythromycin and placebo arm with respect to the change pre-and -post treatment (p= 0.31 and p=0.39), respectively.

The chemokine IP-10 was elevated in serum and less so in sputum at baseline. There was a modest decline in serum IP-10 in the erythromycin arm and an increase in the placebo arm, although the difference in the change from baseline was not statistically significant (p=0.24). There was no correlation between IP-10 and $CD4^+$ T cell percentage count (p=0.34) and HIV viral load (p=0.11). IP-10 was also not correlated with the pulmonary function parameters FEV_1 (p=0.55) and FVC (p=0.15).

For the anti-inflammatory cytokine IL-10, the values were not elevated in both serum and sputum, with no statistically significant change in the levels after intervention with erythromycin or placebo (p=051 and p=0.93), respectively.

Sputum elastase, a protease and marker of neutrophilic activation, did not change at baseline or study end in the two treatment arms. sTREM levels were elevated in both treatment arms at baseline (635.2 ± 1535.5 pg/ml and 722± 1738 pg/ml) with a decline at study end in both treatment arms, although the change in the decline was not statistically significant when comparing the two treatment arms (p=0.22). There was no correlation between sTREM and IL-10 at study entry as well as study end (p=0.11 and p=0.25), respectively. After adjusting for the $CD4^+$ T cell count and HIV viral load there was not statistically significant difference in the sTREM pre- and post-intervention.

9.4 DISCUSSION

The use of macrolides for their immunomodulatory properties in CF-bronchiectasis is currently regarded as standard of care in those with PA colonisation. In paediatric non-CF bronchiectasis, the evidence base is tenuous, with a need for more robust data on the role of macrolides in a form of bronchiectasis where PA is rarely cultured.

The current study showed no additional benefit with low dose erythromycin relative to placebo on the reduction of exacerbations in a cohort of HAART treated children with HIV-related bronchiectasis. We also found no effect of erythromycin on local and systemic pro-inflammatory cytokines and pro-inflammatory mediators IL-1β, IL-6, IL-8, TNF-α and TNF-R1. The anti-inflammatory cytokine IL-10 was not elevated in either the local or systemic circulation samples. This is consistent with findings in a group of HIV-positive women on HAART [145]. Pulmonary function parameters and sputum IL-8 improved significantly in the study population, although this cannot be attributed to the use of erythromycin.

Medical interventions to treat HIV-related bronchiectasis should incorporate immune system restoration with HAART, promotion of mucociliary clearance and normal growth. Although secretion clearance techniques form a fundamental part of current guidelines for bronchiectasis treatment, they have not been shown to impact on pulmonary function, effecting mainly a reduction in cough frequency and improved quality of life [173,174].

There is currently no data on the effect of HAART on the progression of lung disease in HIV-related bronchiectasis. One study in adults, has suggested possible decline in pulmonary functions in patients on HAART, although this study was confounded by the fact that more than half of the subjects were smokers [274]. The restoration of the immune system with the use of HAART is accompanied by a reduction of pro-inflammatory cytokines [274]. The effect of HAART on the $CD4^{+}$ T cell population is known to continue for the first three to five years on HAART and to taper off thereafter [274,275].

Erythromycin is a 14-member ring macrolide and like other macrolides has been found to have immunomodulatory (anti-inflammatory) effects, with clinical benefit first described in diffuse panbronchiolitis, a chronic inflammatory lung disease characterised by intense neutrophilic inflammation, by Kudoh and colleagues [193]. Unlike the newer macrolides, erythromycin is cheap and freely available even in

cost-restricted environments. Ensuing studies in both adults and children with non-CF bronchiectasis have shown a reduction in sputum volume, inhibition of virulence factor production by bacteria, diminished neutrophil influx and down-regulation of IL-8 production, a reduction in pulmonary exacerbations and modest improvements in lung function with the use of erythromycin [29,30,195]. Data on the effect of macrolides on non-CF bronchiectasis exacerbations is limited by lack of long-term randomised controlled trials. A small, uncontrolled study by Serisier et al, demonstrated a reduction in the number of exacerbations observed in 24 adults over 12 months (from four to two per year) [29]. A one-year retrospective review showed a reduction in exacerbations with the use of azithromycin [24]. In this study, 32% of participants had a previous culture or were colonized with PA.

The lack of efficacy in the current study may be attributed to the fact that there were no participants colonized with PA*,* or that in children the numbers of exacerbations are fewer. The follow up period may, therefore, have needed to be longer.

There is conflicting evidence on the effect of macrolides on pulmonary function parameters in children with non-CF bronchiectasis. Tsang et al, found a significant improvement in FEV_1 and FVC, over 8 weeks in 11 patients treated with erythromycin, whilst Yalcin et al, found no effect of clarithromycin on 17 children [28,30]. We found no effect of erythromycin on either FEV_1 or FVC in this study.

However, on pooling the data from both study arms a significant increase in both pulmonary function parameters was evident at the end of the one-year period of the trial. This is an unusual finding and could be postulated to be attributable to either “continued” sub-clinical immune restoration from HAART, or possibly as a result of improved overall care of subjects, which includes airway clearance techniques and early treatment of exacerbations.

In vitro data has shown declines in cytokines with the use of macrolides in bronchiectasis [27]. There is only one randomised study, which assessed cytokines

as an end-point after the use of clarithromycin. Bronchoscopic samples obtained after three months of clarithromycin, revealed a decrease in IL-8, but not TNF-α [28]. This study did not replicate this finding, possibly due to the superior tissue penetration of clarithromycin when compared to erythromycin or the waning effect of the beneficial effect of the macrolides over the one year period when compared to the three month study period.

Serum IP-10- a cytokine, involved in the trafficking of monocytes and activated T helper cells to sites of inflammation, was significantly elevated in the serum of participants. Elevated levels of this chemokine were previously found to be associated with HAART failure or TB [276-279]. These associations were, however, excluded in our cohort of children who were screened for TB and found to be uninfected and there was actually improvement in HIV disease activity markers in our participants. IP-10 levels did not change significantly over the one-year follow up period.

Prior studies in CF reveal a correlation between elevated IgG levels and disease severity [142,143]. In the current study there was a decrease in the IgG levels in both the active and placebo groups. These improvements though could not be attributable to erythromycin. IgG was also not correlated to pulmonary function parameters in this study. This marker still holds promise as a marker for disease severity in HIV-related bronchiectasis, and requires further investigation.

Elastase, a protease released by disrupted neutrophils, has been found in CF to be responsible for 90% of the protease activity resulting in damage to the extracellular components such as elastin, collagen and proteoglycans with subsequent pulmonary destruction [154, 280-282]. Values in CF, greater than 500ng/ml, have been found in adults in stable state CF [283]. In the current study, the levels were significantly lower than those previously described in CF. One explanation for this may be the low prevalence of PA, which can be an independent source of proteases. Downey et al, demonstrated no change in soluble and free elastase levels in a group of CF participants after a course of antibiotic therapy [284]. There was also no change in

the levels of elastase in the current study over a period of one year. The low levels of elastase in the current study suggest that, perhaps in HIV-bronchiectasis, elastase may not be a major role player in the pathogenesis of lung destruction and hence erythromycin may not function on this process.

Previous studies of sTREM in immunocompromised HIV-infected and febrile neutropenic patients have shown a correlation of sTREM with pulmonary disease severity [166,265]. In the current study of children with HIV-related bronchiectasis, significantly elevated sTREM levels were found and they declined over the duration of the study, although this was independent of erythromycin use. The role of this marker needs further exploration as a potential marker of disease severity in bronchiectasis.

The strengths of this study are that preliminary evidence of the effect of HAART and adjunctive care, which includes lung clearance techniques and treatment of exacerbations; on improvement in pulmonary function parameters and sputum IL-8 in children with HIV-related bronchiectasis is provided. This study also demonstrated the lack of effect of erythromycin on both the number of exacerbations, pulmonary function dynamics and cytokines/chemokines in children with HIV-related bronchiectasis.

The limitation of this study is that the number of patients was small, as only children referred to the Centre were included. In addition, quality of life assessments were not conducted. It is, however, very unlikely that even a larger study, should it be possible, would find benefit from erythromycin on exacerbations in this disease. It seems likely that with no numerical difference in exacerbations over one year, patients would have to be followed up for many years to detect the slightest benefit, if any, and this would then obviate the major reason for using this cost-effective macrolide.

9.5 CONCLUSION

Administration of HAART and adjunctive care, which includes airway clearance and treatment of exacerbations, in children with HIV-related bronchiectasis is associated with significant improvement in pulmonary function tests and IL-8, with no additional benefit from the use of erythromycin.

CHAPTER X

SUMMARY AND CONCLUSION

The findings of this thesis have added to the current body of literature on HIV-related bronchiectasis in children. There are new findings in all aspects of HIV-related bronchiectasis, including epidemiological considerations, risk factors, diagnostic procedures, co-morbid and infection status and finally therapeutic strategies. A significant amount of new data is presented and some of the data requires consideration for inclusion in guidelines of the management of children with HIV-infection in South Africa. Most South African guidelines for HIV-infection in children are silent on bronchiectasis and this could be corrected based on these new suggestions.

With regards to the epidemiology of children with HIV-related bronchiectasis, of importance is that the children affected have the diagnosis of HIV-infection made late, with a mean age of over 7 years. Another important finding is despite the diagnosis of HIV, there is a further delay of one year before the diagnosis of bronchiectasis is established. The late diagnosis of HIV is probably related to the fact that these children are of the “late-progressor” phenotype, with manifestation of HIV-infection beyond the first year of life. There is also no evidence presented that the route of HIV-infection acquisition is anything other than vertical transmission. This therefore demonstrates a failure of the PMTCT program in the study population.

An interesting observation is that the children in this cohort had bronchiectasis related to HIV status. HAART was being taken by almost all subjects. However, despite the use of anti-retroviral therapy, only 54% of subjects had virological suppression at study entry, with the majority having received over 18 months of HAART. This in its own right is unfavourable to an improved outcome from lung disease.

The anthropometric measurements of children with HIV-related bronchiectasis in this study were within normal limits, with the majority of children having acceptable weight, height and BMI z-scores. This despite them having both a chronic inflammatory lung disease and HIV-infection, both of which can increasemetabolic demands. This finding has important implications because it has always been assumed that chronic diseases related to HIV-infection results in failure to thrive. This may not be true or may be modified by antiretroviral therapy.

CRP is not a useful marker of lung infection in the context of bronchiectasis. Despite seeming colonisation by bacteria in many patients, CRP cannot be used as an indiscriminate measure of infection status in these children. CRP may have some value as a biomarker of exacerbations if used serially, but this was not tested in this study. Importantly, in the series presented, the elevated CRP and the intensity of ^{18}F-FDG uptake were marginally statistically significant. There also seemed to be some differences in the CRP levels with subjects with incomplete HIV viral suppression having higher levels although, this did not reach statistical significance. A future research question would be to assess the role of this inflammatory marker and its relationship to the HIV disease activity.

In these children, almost half had a previous diagnosis of TB. Additionally, over three-quarters had previously received two or more courses of anti-TB therapy. This confirms to the potential key role of TB as a principal initiator and risk factor for bronchiectasis in this cohort of children [15,16]. The current TB diagnostic guidelines in South Africa rely heavily on the use of chest radiography in conjunction with presenting clinical symptoms for the diagnosis of TB in children. In the context of HIV-infected children then, this study highlights that health-care providers should consider the diagnosis of bronchiectasis in the differential diagnosis, particularly in the child over the age of 6, who presents with a chronic productive cough. There should be a high index of suspicion, if there is additional historical evidence of prior TB or unsuccessful TB therapy.

The pathogens identified in the airways of children with HIV-related bronchiectasis are similar to those previously described in other forms of bronchiectasis [285]. *H. influenza*e is a common pathogens identified, with *S. aureus* playing a minor role as compared to findings in children with HIV-infection presenting with CAP. However, the significance of *H. parainfluenzae* has not been previously recognised. This conclusion is significant for three reasons. Firstly, even if a vaccine to protect against *H. influenzae* becomes widely used in South Africa it does not protect against this organism. Secondly, in children with bronchiectasis; empiric therapy should not include the use of anti-staphylococcal therapy, which forms part of guideline treatment in HIV-infected children with CAP. And finally therapy for Haemophilus, and potentially resistant species of this organism, should form the basis of treatment of pulmonary exacerbations. The routine use of amoxicillin together with clavulanic acid should be considered as a routine in pulmonary exacerbations of bronchiectasis. PA also has a minor role to play in HIV-related bronchiectasis in children similar to other forms of non-CF bronchiectasis [286]. Mycobacteria other than TB are also infrequently identified as pathogens.

When obtaining a history from children with HIV-related bronchiectasis identification of environmental exposures is critical. As demonstrated in the current thesis the majority of children were from a low socioeconomic background; with almost all children receiving some form of social support (welfare) grant. Alternative sources of heating and cooking with biomass fuels are therefore still commonly used. The impact of biomass fuels is well described as having a negative impact respiratory health [241-243]. Over half of the children in the current study were exposed to BMF. This may therefore be additional risk factor for accelerated pulmonary function decline as well as increased respiratory morbidity. Previous studies in HIV-infected adults have shown an association between smoking worsening in HIV-morbidity and death [121]. Over a third of children in this study were exposed to ETS, with no correlation demonstrated between ETS exposure and markers of HIV disease activity. Despite this finding, ETS exposure is known to increase lower respiratory tract infection in children and should be avoided. Health care workers should therefore educate caregivers on the risk posed by these environmental pollutants and suggest possible ways to reduce their impact.

Previous studies have revealed the association of immune-depletion in HIV-infected individuals with progressive increases serum Ig levels, particularly IgE, and this has been linked to the development of atopy in some adult studies [137-141]. In children with HIV-infection with and without the presence of bronchiectasis, there was no relationship between IgE and either the progression HIV disease or the cytokines that could be considered in related to allergy (namely the Th2 cytokines). Elevated IgE could also not be ascribed to ABPA. As with previous studies, HIV-infected children demonstrated a higher prevalence of nasal symptoms as well as dermatitis, which was unrelated to atopy [259,261].

HIV-related bronchiectasis is associated with accelerated pulmonary function decline when compared other forms of non-CF related bronchiectasis [234,235]. One prior study has shown accelerated decline in lung function in HAART treated patients [274]. The reason for the lower pulmonary function parameters in this cohort of children is unclear, but may be related to the “initiating” pathogen of the bronchiectasis, or to the delay in the diagnosis.

The other Ig that was elevated was IgG. IgG could not correlated either with the degree of immunosuppression or pulmonary function parameters. This is in contra-distinction to CF-bronchiectasis, where IgG is inversely related to pulmonary function parameters [142,143]. These findings suggest, therefore, that the increase in serum Igs in this cohort of patients is probably related to immune activity as a consequence of HI- virus related compensatory B cell stimulation and is therefore less reliable than in other forms of bronchiectasis. The IgG level also decreased in the whole study population over time, this may be related to immune restoration with decreased B cell stimulation over time.

In HIV-infection, the primary pathology involves not only depletion of T helper cells, but also other subtle abnormalities in all the pathways involved in both the innate and adaptive immune system. In this thesis we explored the innate immune system markers involved in the early responses to antigenic stimulation in the lung, these

included the cytokines and chemokines responsible for alveolar macrophage and neutrophil activity.

A novel finding in this thesis is that in children with HIV-related bronchiectasis, sTREM a mediator involved in the innate immune system seems to be overactive, more so that when compared to children with CF-related bronchiectasis, which is independent of the presence of an exacerbation. The children with HIV-related bronchiectasis, although a younger cohort when compared to the CF group, exhibited more severe pulmonary disease. This difference could perhaps explain the differences in the sTREM levels. sTREM may therefore be a potential marker of progressive pulmonary disease.

In HIV-bronchiectasis neutrophil driven inflammation seems to be the predominant, with IL-8 being the predominant cytokine produced both locally and systemically. The increased levels of serum GM-CSF and sputum elastase levels also reflect the presence of “active” neutrophil driven inflammation.

TNF-α was elevated systemically and not in the sputum. This cytokine has been previously been found to be correlated with quantitative HIV viral load [286]. The current data did not show any association between TNF-α and HIV disease progression; although there were modest declines over the one-year follow up period.

With regards to anti-inflammatory cytokines, IL-ra was elevated in the systemic circulation and this was independent of the HIV-immune status. There was no significant increase in the other anti-inflammatory cytokines, IL-10 and IP-10, and these low levels persisted even after a one-year follow up period. Previous investigators have shown a relationship between IP-10 and HAART failure [278,279]. The current study could not replicate this finding. It therefore seems that in HIV infection there is a marked elevation of selected pro-inflammatory and anti-inflammatory cytokines. The reason for the concomitant increase in these counter-

active cytokines is still unclear. The role of the HI-virus on continuous immune stimulation systemically and the secondary immune-modulatory activity of HAART require further exploration. These findings indicate that even in the face of HIV-infection, where there is potential depletion of the immune system, aspects of the local and systemic immune system may still function, and even function in excess, in bronchiectasis.

A “gold standard” objective test for the diagnosis of exacerbation in bronchiectasis is missing from the literature. This thesis established that the use of metabolic imaging (PET/CT) in order to aid in the diagnosis of active inflammation in HIV-bronchiectasis, has limited diagnostic value. Its value is limited to the confirmation of pneumonia where a pneumonic consolidation is present. There was, in addition, no correlation between the presence of ^{18}FDG uptake and inflammatory markers including local and serum cytokines the confirmation of pneumonia with the presence of consolidation. Despite the limited numbers, the study has suggested that ^{18}FDG-PET may be more useful in contributing to the diagnosis of TB in HIV-infected children. Future studies, which focus specifically on the role of PET in TB diagnostics, with a particular emphasis on the differentiation of MTB and NTM are necessary.

From the CT findings, the anatomical localisation of bronchiectasis in HIV-infected children is similar to those in other forms of non-CF bronchiectasis, being mainly in the lower lobes [268]. From the data presented, the limited role of PET/CT in bronchiectasis is therefore not necessary or feasible for routine use, particularly in cost-restricted environments and in young children where routine scanning is contra-indicated because of the high radiation burden attached to CT scanning.

There is a resurgence of research on the use of macrolides for their anti-inflammatory properties in bronchiectasis. In the randomised, double-blind, controlled trial of patients with HIV-related bronchiectasis, a lack of efficacy of erythromycin in reducing the number of pulmonary exacerbations was documented.

In addition, erythromycin did not appear to have an effect on both the inflammatory or anti-inflammatory cytokines and chemokines. The lack of efficacy of erythromycin does not preclude the potential benefit that other macrolides may confer, specifically the newer macrolides that have a superior tissue penetration and a better side effect profile. The only potential problem that could be identified with use of the newer macrolides in this role is resistance that may be induced in MOTT bacteria [287]. MOTT organisms occur more commonly in HIV-infected individuals. In addition, they may be more expensive limiting their availability in cost-restricted environments.

Over the one-year follow up of the cohort of children studied, there was a significant improvement in pulmonary function parameters, growth parameters, as well as a reduction in the pro-inflammatory cytokine IL-8. This finding could only be attributed to the use of both HAART, the use of airway clearance techniques and active management of exacerbations. These improvements were also reflected by the stabilisation of the degree of bronchiectasis on the Bhalla scores over the follow up period. This suggests therefore that, early identification of HIV-infection and diagnosis of bronchiectasis as well as early initiation of anti-retroviral therapy significantly reduces morbidity and improves outcome. This is novel and promising, as in HIV-related bronchiectasis, improved care and use of basic treatment strategies contribute to retarding disease progression.

In conclusion, the early identification of both HIV-infection and bronchiectasis, can improve the outcome of children if a therapeutic program that includes HAART, airway clearance therapy, aggressive treatment of exacerbations and avoidance of environmental pollutants is instituted.

CHAPTER XI

STUDY LIMITATIONS AND RECOMMENDATIONS

A number of study limitations have been identified. The most important of these include:

The small sample size makes dogmatic conclusion difficult. However, this study enrolled all the patients with this condition that were available to study. It is unlikely that a larger study with more subjects will be possible. Only children over 6 years of age who were able to perform reliable spirometry were included. Some understanding of the disease in young children should be sought.

Despite an attempt to define exacerbations more clearly, the study was unable to prospectively measure changes in symptoms or biomarkers that may suggest a better working definition. If this had been possible it would have contributed to finding a definition of an exacerbation in the context of HIV-associated bronchiectasis.

The small sample size may be contributing to the lack of significant findings for changes over time and between intervention groups. For example the lack of positive findings for changes on PET scan, cytokine values, spirometry and erythromycin use may be masked by the sample characteristics.

Finally the loss of 10 subjects to follow up is disappointing but does reflect on the nature of a chronic disease that has a social impact. HIV-infection in children occurs frequently in the context of poverty and loss of family members. The children in this study lived throughout northern South Africa and many were unable to afford transport costs. Many children lived without primary care givers.

In light of the findings from this thesis, the recommendations that need to be made on a policy level are that all HIV-infected children who present with recurrent chest symptoms with a chronic productive cough, clubbing and halitosis, particularly if they have previously been treated for TB, should have bronchiectasis excluded.

The possible risk factors for HIV-related bronchiectasis include untreated or poorly managed recurrent chest infections. Additional risk factors also include biomass fuels and environmental tobacco smoke.

When assessing HIV-infected children anthropometry may not be useful in categorising the cause of chronic cough in HIV-infected children already initiated on HAART.

In terms of special investigations, CRP is not a useful screening test for either exacerbations of bronchiectasis or colonisation by bacterial organisms in HIV-infected children with bronchiectasis. Serial, rather than time point defined measurement of CRP, for example, may have been useful in suggesting an exacerbation or impending exacerbation.

An increased IgE does not warrant an allergy diagnosis unless specific symptoms suggest a need for further investigation.

The empiric treatment for an exacerbation of HIV-related bronchiectasis, in children, should include antibiotics that will cover *H. Influenzae* and *H. parainfluenzae.* This may necessitate a revision of guidelines to suggest empiric use of amoxicillin together with clavulanic acid as therapy for pulmonary exacerbations of this condition. There should also be special attention paid to relatively inexpensive airway clearance techniques and nutrition and these benefits are magnified if

children with this condition are co-habited into special clinics. All children should be on HAART as its benefits may be beyond viral suppressive ability.

There is no proven benefit from the use of low dose erythromycin in children with HIV-related bronchiectasis. Prompt antibiotic therapy needs to be instituted when an exacerbation is present as this improves the outcome. Caregivers need to be well-versed on the manifestations of an exacerbation. All children should be referred to specialist paediatric pulmonology centres for further management as this improves outcome.

Despite the disappointing lack of benefit form the use of the macrolide (erythromycin), it may be prudent to continue to explore the benefits of additional immunomodulatory agents and anti-inflammatory drugs. It may be possible that one of the newer macrolides may confer benefit. It may also be logical to test the benefits of a host of other agents that have similar effects such as statins, leukotriene receptor antagonist and future “tailor made” antibiotics which might have only immunomodulatory effects without anti-bacterial effects.

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APPENDIX A

SUBJECT DATA COLLECTION SHEET

Study: Low dose erythromycin in improving the outcome of HIV-infected children

DATA COLLECTION SHEET: Visit 1

1. PATIENT INFORMATION

Name		
Hospital number		
Study number		
Date of admission	DD/MM/YY	
Gender	male	female
Age	Months	
Date of birth	DD/MM/YY	

2. PREVIOUS MEDICAL HISTORY

a. HIV			
	Patient previously tested	Y	N
	If Yes, previous result	positive	negative
	Currently: 1. Consent for ELISA	Y	N
	2. CD4 count	%	total
	3. Viral load		
	Antiretroviral treatment	Y	N
	Specify: 1. Drugs	1. 2. 3. 4.	

	2. Start date ARV		
b. Previous admissions	Previously admitted to hospital	Y	N
c. Treatment	Did the patient receive antibiotics? If yes specify:	Y	N
c. Anthropometric Findings			
Length	cm		
Weight	kg		

d. Examination

General	Temperature on admission		°C
	Generalized lymphadenopathy (0.5 cm present in at least 2 sites, bilateral lymph nodes counting as one site)	Y	N
	Clinically pale	Y	N
	Oedema	Y	N
	Jaundice	Y	N
	Hepatomegaly	Y	N
	Splenomegaly	Y	N
	Oral thrush	Y	N
	Parotomegaly	Y	N
	Eczema	Y	N
	HIV encephalopathy	Y	N

	Neurodevelopmentally normal		Y	N
Respiratory system	Respiratory rate		/ min	
	Heart rate		/ min	
	Peripheral saturation	without oxygen	%	
		with oxygen	%	
	Recession		Y	N
	intercostal	Subcostal	suprasternal	
	Flaring of alae nasi		Y	N
	Clinically cyanosed		Y	N
	Grunting		Y	N
	AUSCULTATORY FINDINGS			
	Focal abnormality		Y	N
	Diffuse abnormality		Y	N
	Clear chest		Y	N
	Hyperinflation		Y	N
	Crepitations		Y	N
	Bronchial breathing		Y	N
	Wheezing		Y	N
	Comments:			

OTHER SYSTEMS	
CVS	
GIT	
CNS	
ENT	

STUDY VISIT COMPLETION FORMS

Study: Low dose erythromycin in improving the outcome of HIV-infected children with bronchiectasis
Pt #: ___ ___ ___Init**:** ___ ___ ___

Visit	CD4 count	Viral load	Sputum MCS	Cytokine assays (blood/ sputum)	Sputum resp virus/TB	Liver function test
Visit 1	X	X	X	X	X	X
Visit 2			X			
Visit 3			X			
Visit 4			X			
Visit 5			X			
Visit 6			X			
Visit 7			X			
Visit 8			X			
Visit 9			X			
Visit 10			X			
Visit 11			X			
Visit 12	X	X	X	X	X	X

STUDY VISIT COMPLETION FORMS

Study: Low dose erythromycin in improving the outcome of HIV-infected children with bronchiectasis

Pt #: Init:

	CXR	PET CT	Lung function test	Nitric oxide	Sweat test	Clinical exam
Visit 1	X	X	X	X	X	X
Visit 2			X			X
Visit 3			X			X
Visit 4			X			X
Visit 5			X			X
Visit 6			X			X
Visit 7			X			X
Visit 8			X			X
Visit 9			X			X
Visit 10			X			X
Visit 11			X			X
Visit 12	X	X	X	X		X

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APPENDIX B

ETHICAL APPROVAL

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federalwide Assurance. FWA 00002567, Approved dd 22 May 2002 and Expires 24 Jan 2009.
RB 0000 2235 IORG0001762 Approved dd Jan 2006 and Expires 21 Nov 2008.

Date: 3/06/2008

PROTOCOL NO.	100/2008~A
NEW TITLE	Low dose erythromycin in improving outcome of HIV-positive children with bronchiectasis
STUDY DE.G.REE	PhD
SPONSORS POSTAL ADDRESS	Level D3 New Steve Biko Academic Hospital, Malherbe Street, Capital park.
MEETING DATE OF THIS STUDY	28/05/2008

This **Protocol** and **Informed Consent** and **all the attachments** have been considered by the Faculty of Health Sciences Research Ethics Committee, University of Pretoria on 28/05/2008 and found to be acceptable.

Advocate AG Nienaber	(female) BA (Hons) (Wits); LLB; LLM (UP); Dipl.Datametrics (UNISA)
*Prof V.O.L. Karusseit	MBChB; MFGP (SA); MMed (Chir); FCS (SA): Surgeon
*Prof M Kruger	(female) MB.ChB. (Pta); MMed. Pead. (Pret); PhD. (Leuven)
*Dr N K Likibi	MB.BCh; Med.Adviser (Gauteng Dept.of Health)
*Snr Sr J. Phatoli	(female) BCur (Et.Al) Senior Nursing-Sister
*Dr L Schoeman	(female) BP harm, BA Hons (Psy), PhD
*Dr R Sommers	(female) MBChB; MMed (Int); MPhar.Med;
Mr Y Sikweyiya	MPH; Master Level Fellowship in Research Ethics; BSC (Health Promotions) Postgraduate Dip in Health Promotion
*Prof TJP Swart	BChD, MSc (Odont), MChD (Oral Path) Senior Specialist; Oral Pathology
*Dr A P van Der Walt	BChD, DGA (Pret) Director: Clinical Services of the Pretoria Academic Hospital
*Prof C W van Staden	MBChB; MMed (Psych); MD; FTCL; UPLM; Dept of Psychiatry

DR R SOMMERS; MBChB; MMed (Int); MPhar.Med.
SECRETARIAT of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria, Pretoria Academic Hospital

*Members attending the meeting.

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The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

* FWA 00002567, Approved dd 22 May 2002 and Expires 24 Jan 2009.
* IRB 0000 2235 IORG0001762 Approved dd Jan 2006 and Expires 13 Aug 2011.

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Faculty of Health Sciences Research Ethics Committee
Fakulteit van Gesondheidswetenskappe Navorsingsetiekkomitee

Date: 20/11/2008

PROTOCOL NO.	100/2008~B
Informed Consent Document	(Give reason for █
PROTOCOL TITLE	Chronic inflammatory lung disease in human immunodeficiency virus (HIV) infected children. Epidemiological considerations, aetiological determinants and the efficacy of low dose erythromycin in bronchiectasis.
INVESTIGATOR	Principal Investigator: Refiloe Masekela
SUPERVISOR	R.J Green
DEPARTMENT	Dept: Paediatrics Phone: 012 354 5271 Fax: 012 354 5275 E-Mail: Refiloe.masekela@up.ac.za Cell: 079 489 0936
MEETING DATE OF THIS STUDY	19/11/2008

This **Amendment** has been considered by the Faculty of Health Sciences Research Ethics Committee, University of Pretoria on 19/11/2008 and found to be acceptable

* *Members attended & Feedback at the meeting .*

*Dr A Nienaber	(female) BA (Hons) (Wits); LLB; LLM (UP); Dipl.Datametrics (UNISA)
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*Dr N K Likibi	MB.BCh; Med.Adviser (Gauteng Dept.of Health)
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*Dr L Schoeman	(female) BP harm, BA Hons (PSy), PhD
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*Mr Y Sikweyiya	MPH; Master Level Fellowship in Research Ethics; BSC (Health Promotion) Postgraduate Dip in Health Promotion
*Prof TJP Swart	BChD, MSc (Odont), MChD (Oral Path), PGCHE
*Dr A P van Der Walt	BChD, DGA (Pret) Director: Clinical Services of the Pretoria Academic Hospital
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APPENDIX C

PATIENT INFORMATION LEAFLET, CONSENT FORM AND ASSENT FORM

A. CONSENT FORM AND PATIENT INFORMATION SHEET

Study title: Low dose erythromycin therapy in improving outcome of HIV-positive children with bronchiectasis

Dear Parent / Guardian

Your child ________________________ is currently suffering from a problem of the chest and lungs that has come about because of HIV infection. This letter serves to request your permission to enrol your child in a study to investigate treatment options for children with this condition.

What is the purpose of the study?

This condition is usually treated with antibiotics when your child gets sick. We would like to test the effect of using a small quantity of a common antibiotic known as erythromycin on the disease process. Erythromycin is a medicine that reduces inflammation, and is often used in a number of other conditions and is generally tolerated well. It is possible that erythromycin may reduce the chances of your child getting sick and may have a good effect on improving his general condition and prevention infections in the lung. Your child may or may not get the antibiotic as we would like to see if will have any effect in the improvement of his/her lung status.

What is the duration of the study?

The duration of this study will be 12 months. Your child will need to give one tablet every evening for this whole time.

Procedures to be followed

Participating in this study would imply that your child would be treated with usual antibiotics in a standard way and the erythromycin as well. Your child’s condition and response to the treatment will be monitored monthly at the usual clinic you attend.

Simultaneously, a small volume of the blood, urine and sputum, that are routinely collected, will also be tested for specific cytokine responses to infection.

Cytokines are specific substances released by the fighting cells of the body during stress situations like infection. Some of these cytokines are pro-inflammatory, or causing inflammation, while others are anti-inflammatory, or regulating this immune response by opposing the inflammatory response. These two groups of agents are usually working in a balanced way, and should something like specific chest infections impair this balance, damage to the cells may occur. We will be following your child up very closely at monthly intervals. We will then collect the normal sputum samples and monitoring his/her progress as well as looking for complications that the medication can cause. After 12 months we will repeat the sputum, urine and blood testing for the cytokine levels as well as the chest x-rays and CT scans. This information will guide us in better understanding of the lung damage caused by chest infections in your child.

What will be done at each visit will be as follows:

Visit 1:

- Sputum samples
- Blood tests
- Lung function tests
- Nitric oxide measurements
- Chest x-ray
- PET CT scan
- Sweat test
- Clinical examination

Visit 2-11

- Lung function test
- Sputum sample
- Clinical examination

Visit 12 (study ends)

- Lung function test
- Nitric oxide measurement
- Chest x-ray
- PET CT chest

- Sputum sample
- Blood sample
- Clinical examination

Risks and discomfort involved

It is important to note that no additional discomfort will be caused to the usual blood tests and investigations performed on a child with this condition.

We do not expect side effects from short-term erythromycin use and the risk is very small. Erythromycin used for a long time may cause some nausea, vomiting or diarrhoea. It is also possible that this antibiotic may make the other bugs in your child's lung resistant (stop responding) to some of the antibiotics we may need to use for pneumonia. In rare cases erythromycin can cause an allergic reaction and it may also cause damage to the liver which results in swelling of the liver and abdominal pains.

Drug interactions

Erythromycin can also interact with other medicines your child may be taking for example midazolam (Dormicum) by decreasing the level in the blood. Erythromycin can also increase the level of the following drugs in the blood: ebastine, carbamazepine (Tegretol), ciclosporin, ergotamine and warfarin. Should your child be taking any of these drugs the doctor will monitor the levels of these drugs closely and may not enrol your child in the study.

As mentioned previously, erythromycin is used in a number of other conditions and generally tolerated well. Should your child's condition deteriorate or an adverse (bad) reaction happen with the medication you are to contact Dr Masekela immediately at any time of day or night on the number 079 489 0936/ 012 354 5271. The medication will be stopped in case of a severe reaction to the medication; that is an allergic reaction or evidence of liver damage form the medication.

HIV testing

This letter then further serves to ask your permission to do a HIV test on your child if it was not done before. A specific consent form in the ward will also be used. It is important that the doctor who presents this form to you explain the following to you:

- The reasons we want to test your child
- That HIV is virus or bug that attacks the fighting cells of your body and make the body weak so that it can't fight infections as well as before
- How HIV is transmitted: through sexual contact, blood transfusions or dirty needles e.g. drug users or from mother to child. Transmission from mother to child can happen either during the pregnancy, the birth process or breastfeeding
- The stages of HIV in an adult and how it differs in children
- Currently there is no cure for HIV. We can however treat the infections the child gets because the body is weak. The doctor should also explain to you how anti-retroviral drugs can improve the quality of life and where they are available
- If your child's test is positive, the probability that you are also positive is high, and you should yourself be tested as well. Advice regarding future pregnancies and the availability of any treatment should also be given.

It is important to know that the results may only be given to you and that post-test counselling will also be done. You may decline the HIV test and the treatment of your child will not be influenced by that decision.

Has the study received ethical approval

The study protocol was submitted to the Faculty of Health Sciences Research Ethics Committee, University of Pretoria, and that committee has granted written approval.

The study has been structured in accordance with the Declaration of Helsinki, which deals with the recommendations guiding doctors in biomedical research involving humans.

Confidentiality

All information obtained during the course of this trial is strictly confidential, and personal information will remain confidential at all times. Data that may be reported

in scientific journals will not include any information, which identifies your child as a patient in this study.

Source of additional information

During your child's stay in hospital she will be under the care of Dr Masekela on 012 354 5271/079 489 0936, If you have any questions, which were not fully explained by the doctor obtaining consent, please do not hesitate to ask him/her.

This letter serves to request your permission to enrol your child in this study group. You may refuse such permission and your child's management will not be affected in any way.

<u>INFORMED CONSENT</u>

I, ____

the parent/guardian of

____ *(Name of patient)*

_________________________________ *(Hospital Number)*

Have been informed about and understand the nature, benefits, risks and purpose of the trial, and all my questions have been answered. I hereby give permission that my child may participate in the above study.

I further have been fully informed regarding HIV and all my questions have been answered. I therefore give / do not give consent to perform an HIV test on my child

____

Signature of the parent/guardian Date

I hereby confirm that the above parent / guardian have been fully informed about the nature, conduct and risks of the above trial.

____

Name of the investigator

__________________________________ ____________________

Signature of the investigator   Date

____

Name of witness

_______________________________   ________________________

Signature of witness   Date

B. ASSENT FORM

Name of study: Low dose erythromycin in improving outcome of HIV-positive children with bronchiectasis

I understand that I have been asked to participate in a study about my chest problem (bronchiectasis). I understand that I will use erythromycin (the new medicine), in addition to my normal medicines that I take for my chest.

I will be asked to have a check up of my chest by the doctor and I will be asked to blow in the two machines to check the size and the swelling of my lungs.

I will be asked to come for visits to the doctor regularly in order that the doctor can check how my chest is doing and a photograph will be taken of my chest to see how far my chest problem is. I will have to ask questions about my illness with every visit. I will also give my mucus to the doctor to check with every visit. I will be blowing into the machine with every visit so that the doctor can check my lungs. I will also have blood taken from me at the beginning of the study and after 12 visits (one year). A new photo of my chest will also be done after one year.

I understand that the medicine that the doctor wants to give me can make me feel sick. I can vomit or my stomach can work a lot from taking the medicine. This medicine may also give me a rash or bad reaction. The doctor will check me to see if it's very bad in which case she will decide to stop it if I get too sick.

I understand that I do not have to participate. If I do participate, I can quit at any time. I also understand that I do not have to answer any questions I don't want to answer or do anything I don't want to do.

My parents, teachers or anyone else will not know what I have said or done in the study. No one but the researchers will know.

This study is being done by Dr Refiloe Masekela of Pretoria Academic hospital. Her phone number is 012-354 5271 or 079 4890936.

If I have any questions or concerns about the study, I can call and ask her about them. When I sign my name, this means that I agree to participate in the study and

that all of my questions have been answered. I have also been given a copy of this form.

Name: ______________________ Signature____________________

Name of Witness__________________

Witness signature_____________________ Date______________

APPENDIX D

BHALLA SCORE

Category	0	1	2	3
Severity of bronchiectasis	Absent	Mild (luminal diameter slightly greater than accompanying vessel)	Moderate (lumen 2-3 times the diameter of vessel)	Severe (lumen >3 times diameter of vessel)
Peribronchial thickening	Absent	Mild (wall thickness equal to diameter of adjacent vessel)	Moderate (wall thickness greater than and up to twice the diameter of adjacent vessel)	Severe (wall thickness >2 times the diameter of adjacent vessel)
Extent of bronchiectasis*	Absent	1-5	6-9	>9
Extent of mucous plugging*	Absent	1-5	6-9	>9
Sacculations or abscesses*	Absent	1-5	6-9	>9
Generations of bronchial divisions involved (bronchiectasis/plugging)	Absent	Up to 4th generation	Up to the 5th generation	Up to 6th generation and distal
No of bullae	Absent	Unilateral (not >4)	Bilateral (not >4)	>4
Emphysema*	Absent	1-5	>5	
Collapse/consolidation	Absent	Subsegmental	Segmental/lobar	

No of bronchopulmonary segments affected: for the calculation of the CT score is subtracted from 25 [108]

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TERMINOLOGY AND ABBREVIATIONS

ABPA	Allergic bronchopulmonary aspergillosis
AIDS	Acquired immunodeficiency syndrome
CAP	Community acquired pneumonia
CCR3	CC chemokine receptor-3
CCR5	CC chemokine receptor-5
CDC	Centre for Disease Control
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane regulator
COPD	Chronic obstructive pulmonary disease
CRF	Circulating recombinant forms
CXR	Chest x ray
D_{LCO}	Pulmonary diffusion capacity for carbon monoxide
FEV_1	Forced expiratory volume in one second
FEF_{25-75}	Forced expiratory flow over 25-75% of expiration
$F_{c\varepsilon}R1$	Human F epsilon R positive cells
FVC	Forced vital capacity
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte macrophage colony stimulating factor
HAART	Highly active anti-retroviral therapy
H. influenzae	*Haemophilus influenzae*
HIV	Human immunodeficiency virus
H. parainfluenzae	*Haemophilus parainfluenzae*

HRCT	High resolution chest tomography
ICAM-1	Intracellular adhesion molecule-1
Ig	Immunoglobulin
IL	Interleukin
INF-γ	Interferon gamma
IP-10	Interferon gamma inducible protein-10
IPT	Isoniazid prophylaxis treatment
LRTI	Lower respiratory tract infection
MCP-1	Monocyte chemotactic protein-1
MIP-1	Macrophage inflammatory protein-1
MMP	Metalloproteinase
MRSA	Methicillin resistant staphylococcus aureus
NE	Neutrophil elastase
NFκβ	Nuclear factor kappa-beta
NTM	Non-tuberculous mycobacteria
PA	Pseudomonas aeruginosa
PAMPs	Pathogen associated molecular patterns
PcP	Pneumocystis jerovicii pneumonia
PET	Positron emission tomography
PMTCT	Prevention of mother to child transmission
PRP	Pattern recognition proteins
RAST	Radio Allergo Sorbent Test
rhDNAse	Recombinant DNAse

RSV	Respiratory syncytial virus
S. aureus	Staphylococcus aureus
sTNFR1	Soluble tumour necrosis factor receptor-1
sTREM	Soluble triggering receptor expressed on myeloid cells
Tat	HIV-trans-activating protein
TB	Tuberculosis
Th1	T helper-1
Th2	T helper-2
TLR	Toll-like receptors
TNF-α	Tumour necrosis factor alpha
URF	Unique recombinant forms
VCAM-1	Vascular cell adhesion molecule -1
WHO	World Health Organisation