

## 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<sup>+</sup> 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<sup>+</sup> T cells due to increased apoptosis of infected cells and a decreased generation of CD4<sup>+</sup> 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 untreated 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%

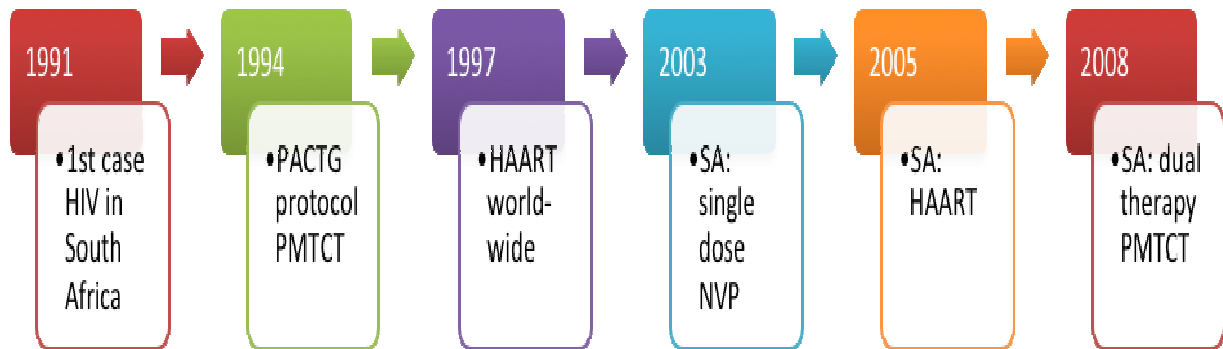
of HIV-infected mothers having no prenatal care [57]. This is in contra-distinction to only 2% non-attendance rates in the general population. In South Africa 56% of expectant mothers attend antenatal clinics, this despite the service being freely available since 1995 in the public health sector [58]. 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 peripartum 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.

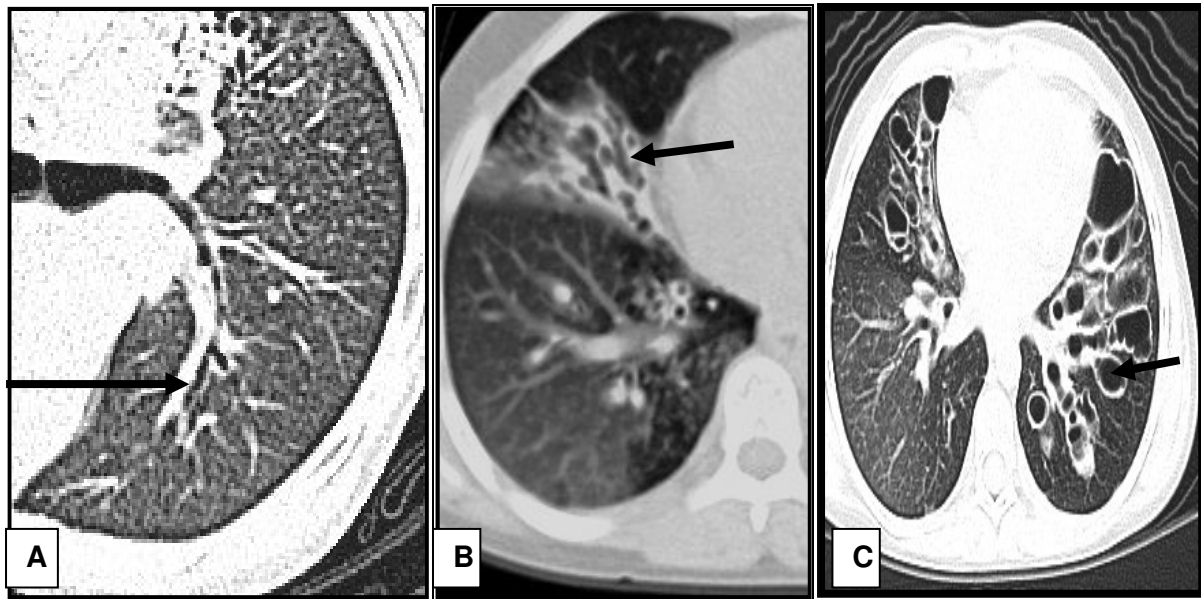
### **2.3 Bronchiectasis**

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 Yukon 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
<b>UK</b>	25%	30%	1%	9%	3%	18%
<b>Australia</b>	23%	13%	3%	13%	3%	40%
<b>Italy</b>	10%	7%	24%	-	4%	55%
<b>Turkey</b>	15%	30%	6%	3%	4%	38%
<b>Taiwan</b>	10%	28%	3%	-	7%	31%
<b>Tunisia</b>	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].

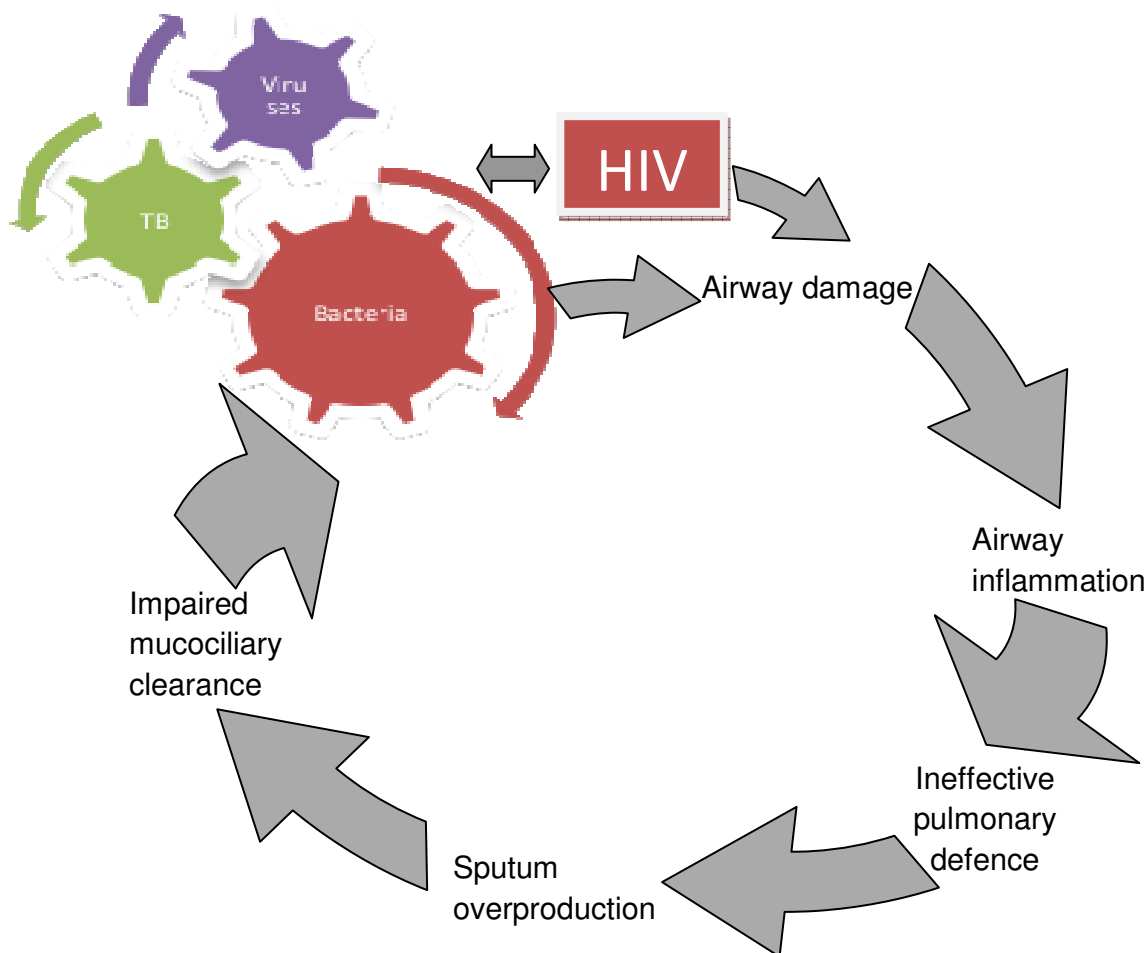
BMF are those fuels that are commonly used in relatively poor communities. They include wood, charcoal, leaves and dung. These substances are used for cooking and heat generation. Most commonly, where these fuels are used for cooking purposes, people particularly women and children, are subjected to 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 *Enterobacteriaceae* 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 hyperproduction. 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> T cells depletion, there is a qualitative defect in CD4<sup>+</sup> T cells, which results in loss of antigen and mitogen-induced IL-2 and interferon gamma (INF- $\gamma$ ) 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]**

	<b>Cytokine</b>	<b>Source</b>	<b>Mechanism</b>
Th-1	IL-1 $\beta$	Macrophages	$\uparrow$ neutrophil production bone marrow, $\uparrow$ TNF- $\alpha$ and IL-6 production, $\uparrow$ MMP production, $\uparrow$ COX-2 production and $\uparrow$ adhesion molecules and chemokines and $\uparrow$ histamine release.
	IL-6	Mononuclear phagocytes, T-cells	$\uparrow$ Liver production of APR, $\uparrow$ 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. $\uparrow$ neutrophil adherence to endothelium by ICAM-1 upregulation.
	TNF- $\alpha$	Activated macrophages and monocytes	PGE2 synthesis, induction of APR production by liver.
	INF- $\gamma$	Activated T cells and NK cells.	$\uparrow$ MHC class I and II expression on nucleated cells, $\uparrow$ 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- $\gamma$ , IL-3, IL-4, IL-5 and GM-CSF.
	IL-4	CD4 <sup>+</sup> T cells, mast cells and basophils	Induces CD4 <sup>+</sup> T cells to differentiate into Th2 cells, promotes immunoglobulin class switching to IgG1 and IgE, stimulates collagen and IL-6 production.
	IL-5	CD4 <sup>+</sup> 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 $\uparrow$ ICAM-1 expression.

	<b>Cytokine</b>	<b>Source</b>	<b>Mechanism</b>
Chemokines	MIP-1 $\beta$	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- $\alpha$ .
	IL-6	Phagocytes and T cells	Inhibits TNF- $\alpha$ and IL-1 and increases IL-1ra.
	IL-10	CD4 <sup>+</sup> T cells, activated CD8 <sup>+</sup> T cells, and activated B cells	Inhibits IFN- $\gamma$ production by NK cells, inhibition of IL-4 and IFN- $\gamma$ 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-1 $\beta$ , IL-6, IL-8 and TNF- $\alpha$ .

↑; Increase; Th: T helper; IL: Interleukin; MHC: major histocompatibility complex; MMP metalloproteinase; TNF- $\alpha$ : tumour necrosis factor alpha; MIP-1 $\beta$ : 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 ( $F_{\epsilon}R1$ ) [141]. With declining  $CD4^+$  T cells, 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  $F_{\epsilon}R1$  positive cells and may also upregulate CCR3 expression [144]. Some pro-inflammatory cytokines INF- $\alpha$ , INF- $\gamma$  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- $\alpha$ ) 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-1 $\beta$ , IL-6, IL-8, IL-12, IL-17, GM-CSF and TNF- $\alpha$  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  $\text{NF}\kappa\beta$ .  $\text{NF}\kappa\beta$  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  $\kappa\beta$  ( $\text{NF}\kappa\beta$ ) with subsequent release of pro-inflammatory cytokines IL1 $\beta$ , IL-8 and TNF- $\alpha$  [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- $\alpha$  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- $\alpha$ . 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- $\alpha$  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 macrocyclic 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**

<b>14 member ring macrolide</b>	Erythromycin
	Troleandomycin
	Clarithromycin
	Roxithromycin
<b>15 member ring macrolide</b>	Azithromycin
<b>16 member ring macrolide</b>	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 troleandomycin 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 NF $\kappa$ B production, 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<sub>1</sub>, 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-1 $\beta$ , IL-8, TNF- $\alpha$ , and leukotriene B<sub>4</sub>). 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 <sup>30</sup>	1999	Erythromycin	RDBPCT	Adult	↑FEV <sub>1</sub> , ↑FVC ↓sputum volume
Yalcin <sup>28</sup>	2006	Clarithromycin	RPCT	Paediatric	↓sputum volume, ↓sputum cytokines
Koh <sup>31</sup>	1997	Roxithromycin	RDBPCT	Adult	↓airway reactivity to methacholine
Davies <sup>194</sup>	2004	Azithromycin	Prospective open-label	Adult	↓symptoms and ↑D <sub>LCO</sub>
Cymbala <sup>198</sup>	2005	Clarithromycin	Randomised open-label, crossover	Adult	↓sputum volume
Serisier <sup>29</sup>	2011	Erythromycin	Retrospective RCT	Adult	↓exacerbations ↓antibiotic use
Coeman <sup>18</sup>	2011	Erythromycin	Retrospective observational	Adult	Improved symptom scores
Anwar <sup>24</sup>	2008	Azithromycin	Retrospective observational	Adult	↑FEV <sub>1</sub> ↓exacerbations

*Abbreviations:* ↑, increased, ↓, decreased, D<sub>LCO</sub>, pulmonary diffusion capacity for carbon monoxide; FVC, forced vital capacity; FEV<sub>1</sub>, 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 ( $^{18}\text{F}$ -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].  $^{18}\text{F}$ -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,  $^{18}\text{F}$ -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  $^{18}\text{F}$ -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  $^{18}\text{F}$ -FDG accumulation in inflamed tissues is not only related to increased glucose metabolism in inflammatory cells, but also by macrophage proliferation and recruitment.  $^{18}\text{F}$ -FDG, a glucose analogue in which the oxygen molecule in position 2 is replaced by a positron-emitting  $^{18}\text{F}$ 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  $^{18}\text{F}$ -FDG accumulation in inflammatory tissues is related to increased glucose metabolism that is produced by stimulated inflammatory cells, macrophage proliferation, and healing [210,211].  $^{18}\text{F}$ -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  $^{18}\text{F}$ -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  $^{18}\text{F}$ -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  $^{18}\text{F}$ -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  $^{18}\text{F}$ -FDG that is related to the high glycolytic rate of inflammatory cells. There are also case reports of the value of  $^{18}\text{F}$ -FDG PET to detect opportunistic infections such as *Mycobacterium avium intracellulare* lesions in HIV- uninfected individuals [217].

$^{18}\text{F}$ -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  $^{18}\text{F}$ -FDG uptake in the lung field, and the number of neutrophils present on bronchoalveolar lavage fluid [222].  $^{18}\text{F}$ -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  $^{18}\text{F}$ -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  $^{18}\text{F}$ -FDG PET is a highly sensitive tool for assessing inflammation and infection, although it still lacks specificity for the accurate diagnosis of lung disease.  $^{18}\text{F}$ -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  $^{18}\text{F}$ -FDG PET for chronic pulmonary diseases**

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

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