

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-1 β , 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-1 β , IP-10, G-CSF, GM-CSF, sTNFR1, 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/m²) 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 (FEV₁), forced vital capacity (FVC), FEV₁/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, IgG₁, IgG₂, IgG₃ and IgG₄, 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.

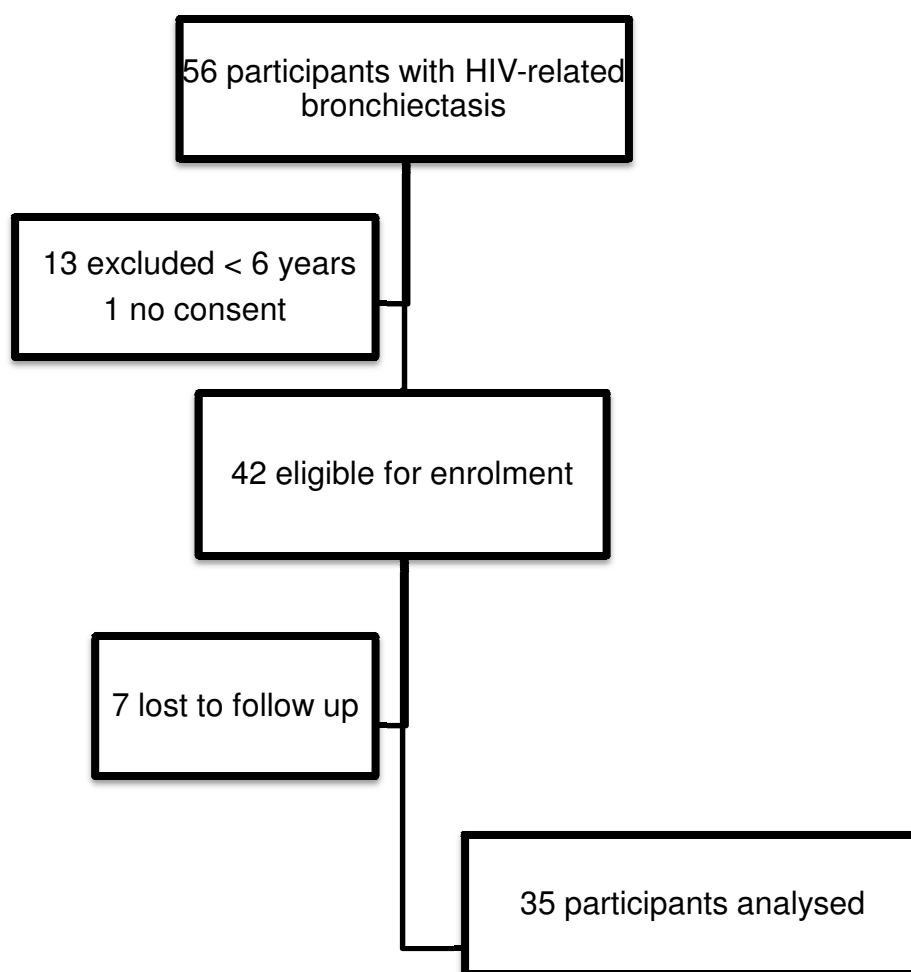


Figure 4. Enrolment and follow-up plan of children with HIV-related bronchiectasis

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 $12.2 - 21.3 \text{ kg/m}^2$). 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 x10⁹ 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 X10⁶)	569.0	456.2
CD4 count (%)	18.3	9.1
FEV₁ (% predicted)	53.0	18.9
FEF₂₅₋₇₅ (% predicted)	52.0	35.3
FVC (% predicted)	46.4	14.3

FEV₁: Forced expiratory volume in 1 second; FEF₂₅₋₇₅: 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
FEV₁‡ (% 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; FEV₁: 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 FEV₁ and FVC were 53.0 %predicted (range 5 - 86) and 46.4 %predicted (range 15-71), respectively. The mean FEF₂₅₋₇₅ was 52 %predicted (range 11 - 165). Only eight (22.8%) children had a positive bronchodilator response, defined as a 15% increase in FEV₁ after administration of a bronchodilator. When comparing the FEV₁ 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 FEV₁ or FEF₂₅₋₇₅ ($r = -0.13$ and $r = 0.04$), respectively. There was also no statistically significant difference between participants with HIV suppression and non-suppression with respect to FEV₁ or FEF₂₅₋₇₅ (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.

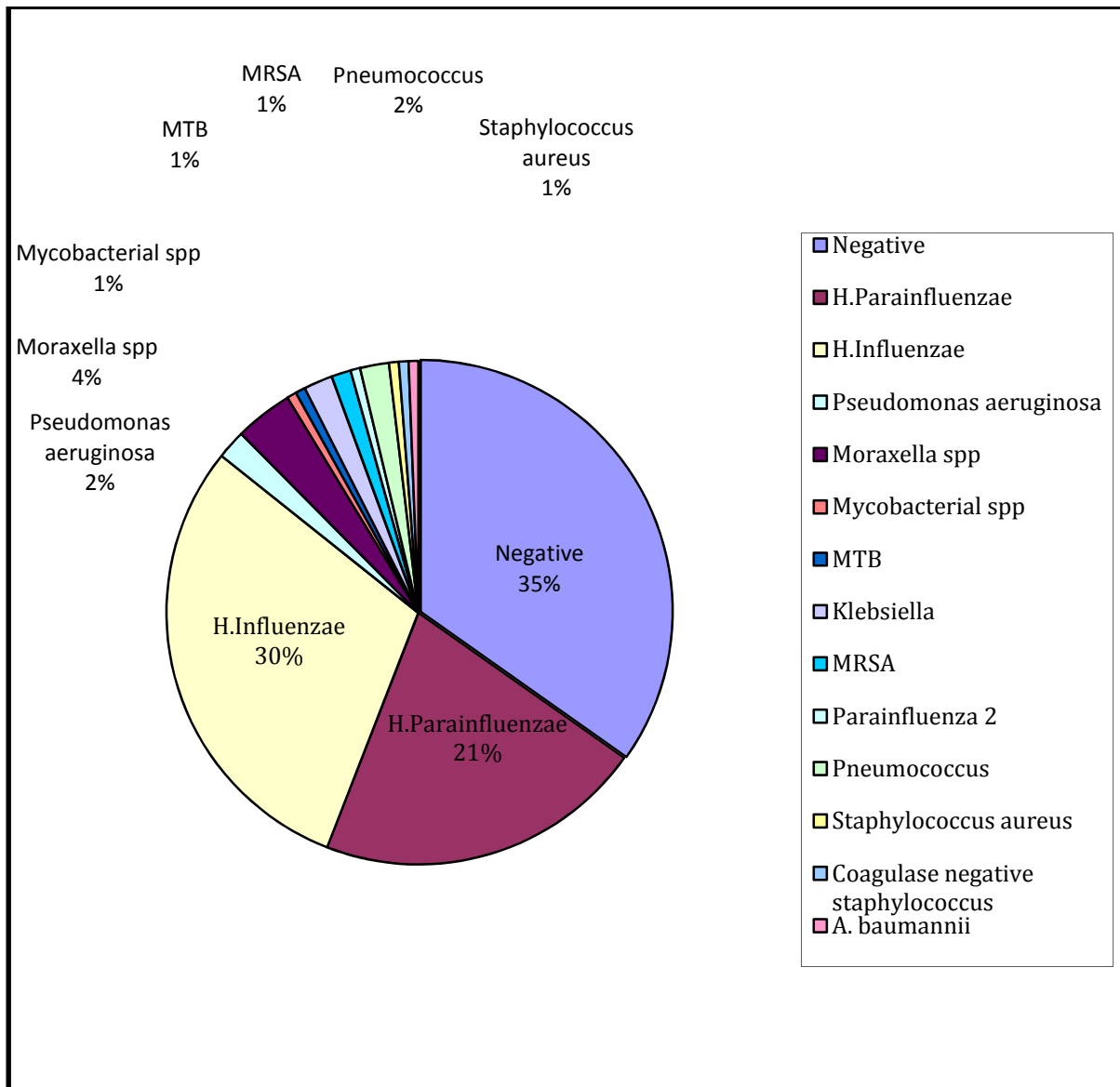


Figure 5. Sputum microbiology results of children with HIV-related bronchiectasis - cumulative data for 161 samples (N= 35).

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 \pm SD) [57.0 ± 223.5 kU/l and 159.0 ± 316.9 kU/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 FEV₁, 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% versus 97% 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, it 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 than 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 FEV₁ 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 FEV₁ of 66% of predicted [234]. Haidopoulou et al, also reported FEV₁ 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 hyperstimulation 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 FEV₁ 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 its 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.