CHAPTER IX

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

9.1 OBJECTIVES

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

9.2 SUBJECTS

9.2.1 SUBJECTS

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

Inclusion criteria

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

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

Exclusion criteria

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

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

9.2.2 METHODS

Randomisation and Blinding

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

Clinical investigations

Information relevant to this sub-study of the thesis included: the age at HIV diagnosis, timing of initiation of HAART and growth parameters at study entry and study end (Section 5.2.2). Pulmonary function measurements; FEV_1,FVC and $FEF_{25/75}$ were measured at each study visit. All participants underwent PET/CT scan at study entry and study end. The Bhalla score was performed on all the scans by two blinded radiologists who independently scored the CT scans, they were blinded to the clinical data, morphological testing and special investigations of the participants, with any disagreements resolved by consensus [108]. The methodology of the Bhalla scores is described in Section 7.2.2.

Laboratory investigations

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

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

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

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

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

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

Statistical analyses

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

Ethical clearance

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

9.3 RESULTS

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

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

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

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

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

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

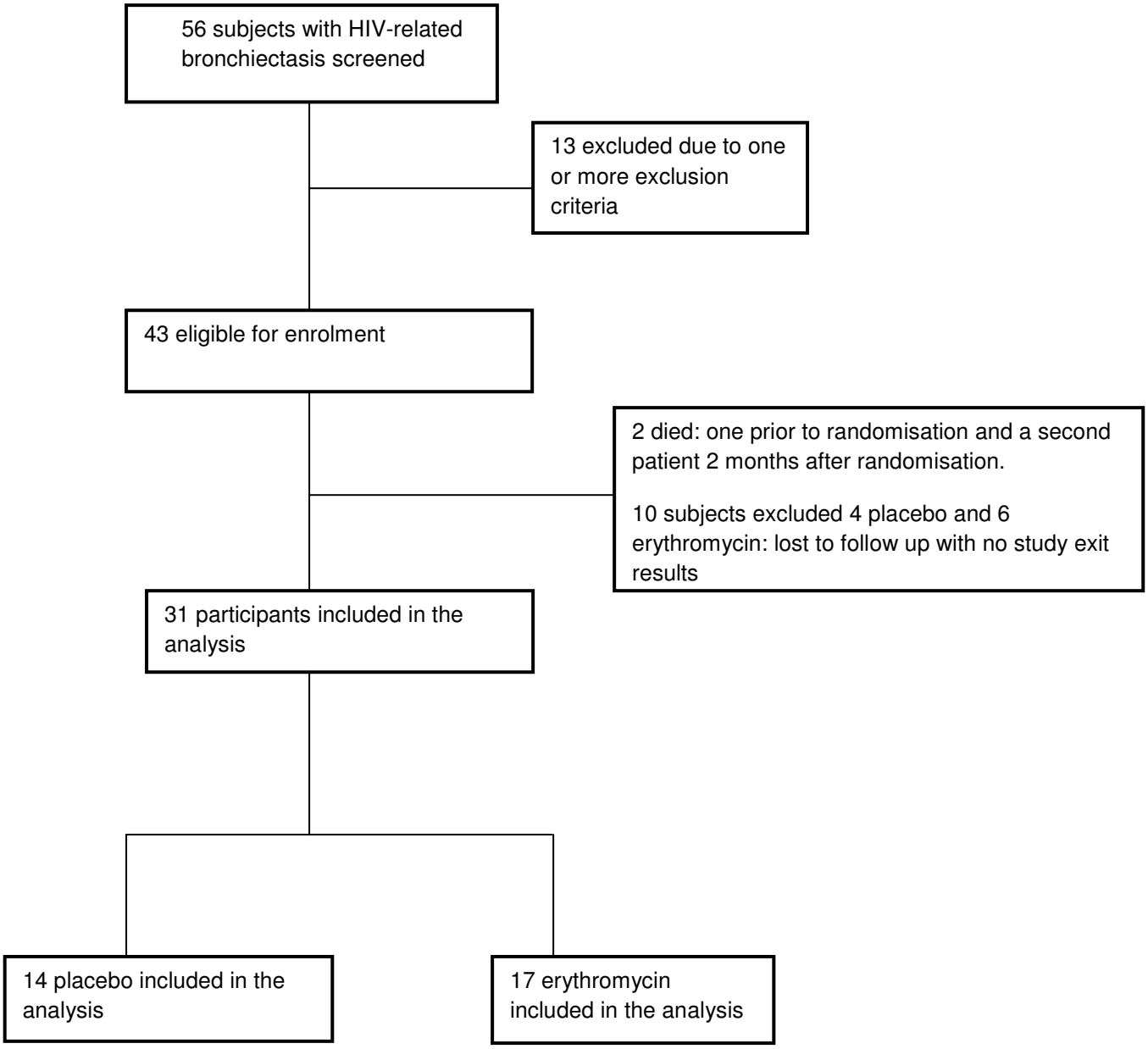

Figure 11. Enrolment and randomisation of participants included in the study

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

9.4 DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

9.5 CONCLUSION

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

CHAPTER X

SUMMARY AND CONCLUSION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

CHAPTER XI

STUDY LIMITATIONS AND RECOMMENDATIONS

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

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

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

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

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

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

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

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

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

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

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

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

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

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