CHAPTER VI

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

6.1 OBJECTIVES

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

6.2 SUBJECTS AND METHODS

6.2.1 SUBJECTS

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

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

6.2.2 METHODS

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

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

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

Serum and sputum cytokines: These were measured using the Bio-Plex® suspension bead array system (Bio-Rad Laboratories Inc, Hercules, CA, USA), which utilises luminex® Xmap™ multiplex technology to enable simultaneous detection and quantitation of multiple different analytes in a single sample. The system uses an array of microspheres in liquid suspension, conjugated with a monoclonal antibody specific for a target protein. The beads contain different ratios of two spectrally distinct fluorophores, thereby assigning a unique spectral identity. These antibody-coupled, colour-coded beads were then incubated with the serum or sputum samples ($^1/_4$ and $^1/_{10}$ dilutions respectively), and washed, followed by addition of a biotinylated detection antibody, washed again, and finally incubated with

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

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

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

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

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

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

Statistical analysis

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

Ethical Clearance

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

6.3 RESULTS

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

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

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

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

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

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

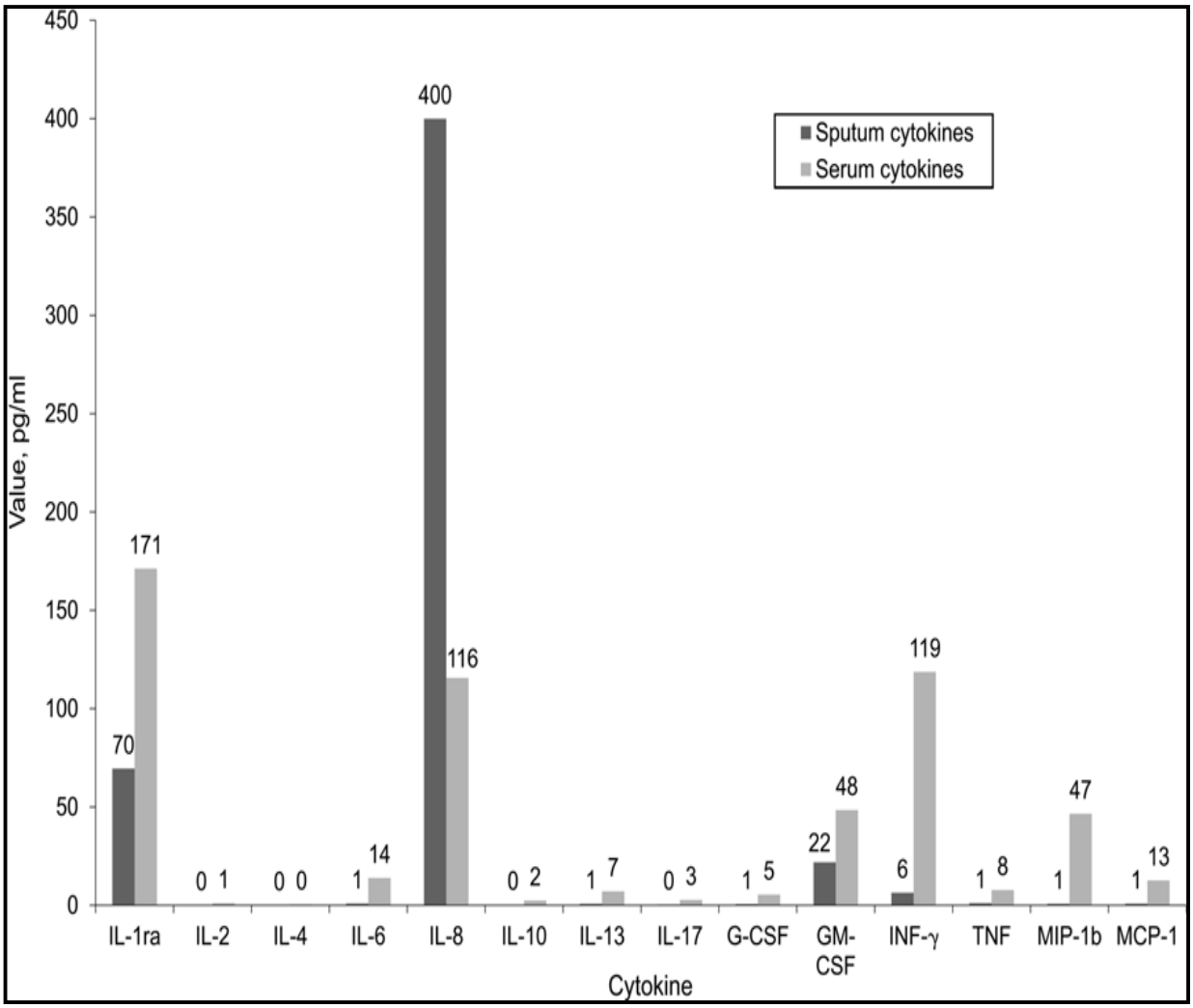

Figure 6. The baseline serum and sputum cytokine values of children with HIV-related bronchiectasis

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

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

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

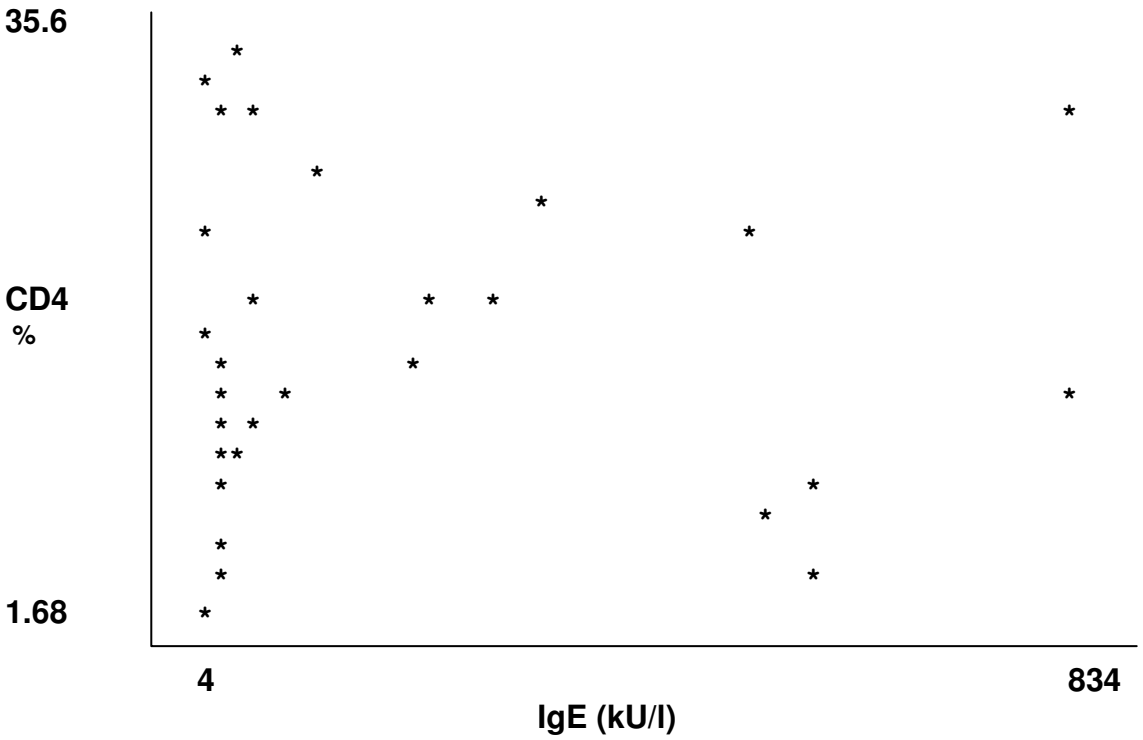

Figure 7. **Plot demonstrating Spearman correlation between Immunoglobulin E (IgE) and $CD4^+$ T percentage cell count of children with HIV-1 related bronchiectasis (r=-0.02; p=0.48)**

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

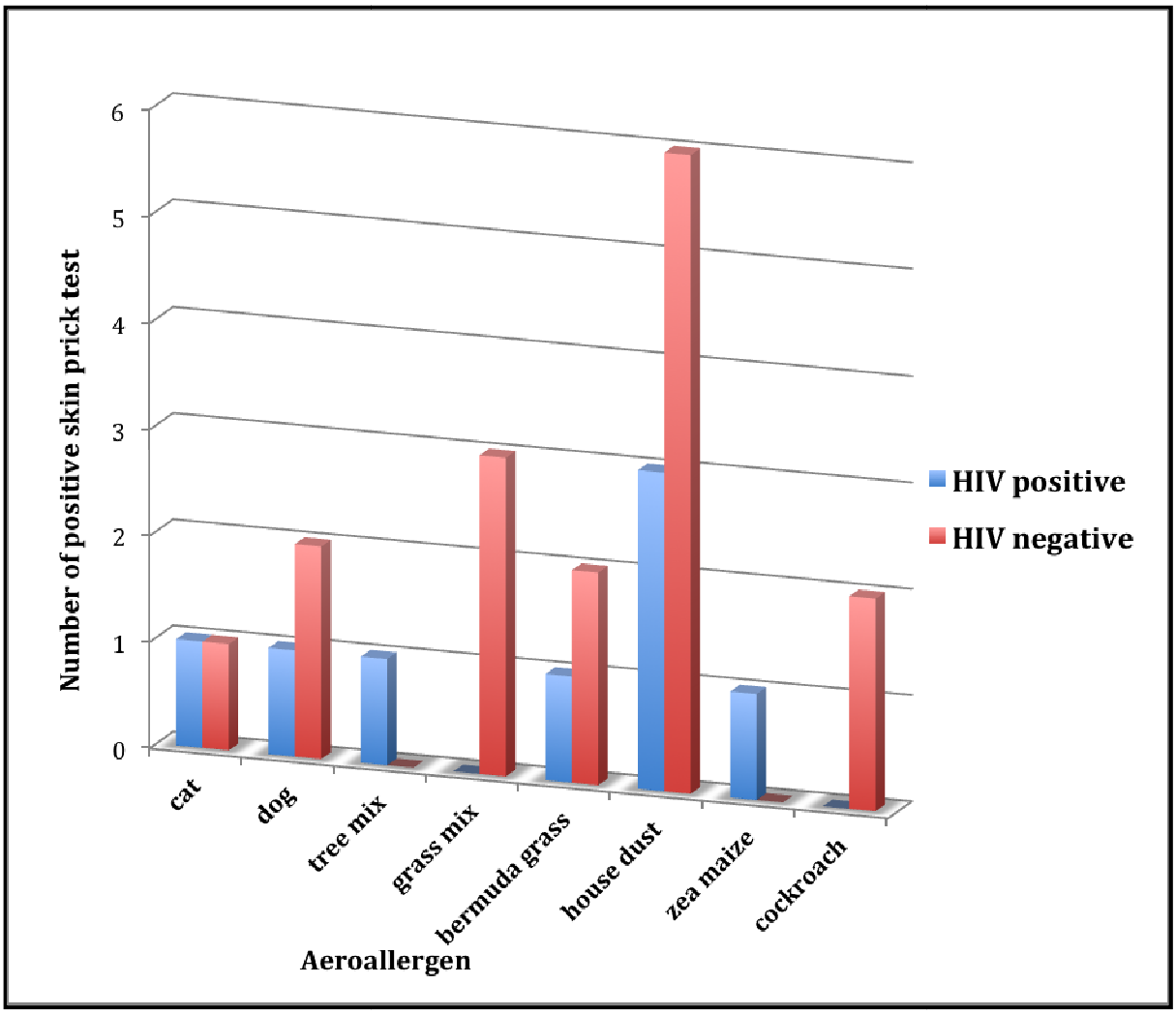

Figure 8. **Graphic presentation of specific positive skin prick tests in HIV-infected without bronchiectasis and HIV-negative children, some patients with more than one positive aeroallergen**

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

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

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

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

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

6.4 DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

6.5 CONCLUSION

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

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

CHAPTER VII

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

7.1 OBJECTIVES

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

7.2 SUBJECTS AND METHODS

7.2.1 SUBJECTS

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

7.2.2 METHODS

Clinical investigations

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

Laboratory investigations

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

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

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

CT scanning

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

Statistical analyses

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

Ethical clearance

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

7.3 RESULTS

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

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

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

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

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

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

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

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

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

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

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

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

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

7.4 DISCUSSION

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

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

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

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

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

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

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

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

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

7.5 CONCLUSION

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

CHAPTER VIII

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

8.1 OBJECTIVES

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

8.2 SUBJECTS AND METHODS

8.2.1 SUBJECTS

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

8.2.2 METHODS

Clinical evaluations

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

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

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

^{18}F-FDG PET/CT scanning

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

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

Ethical clearance

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

Statistical analysis

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

8.3 RESULTS

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

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

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

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

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

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

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

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

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

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

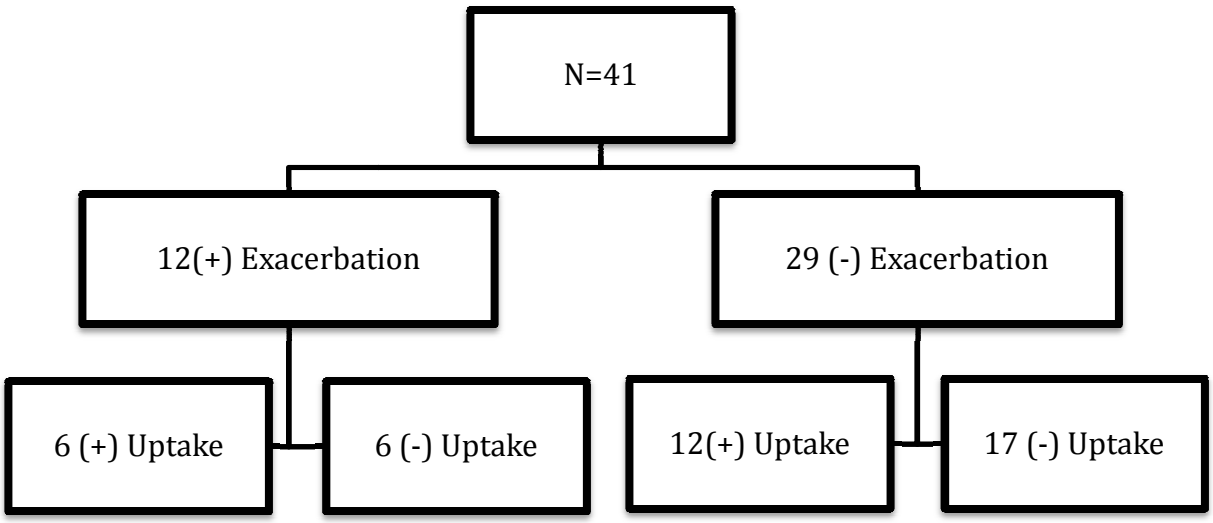

Figure 9. Flow diagram of ^{18}F-FDG PET results of children with human immunodeficiency virus-related bronchiectasis

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

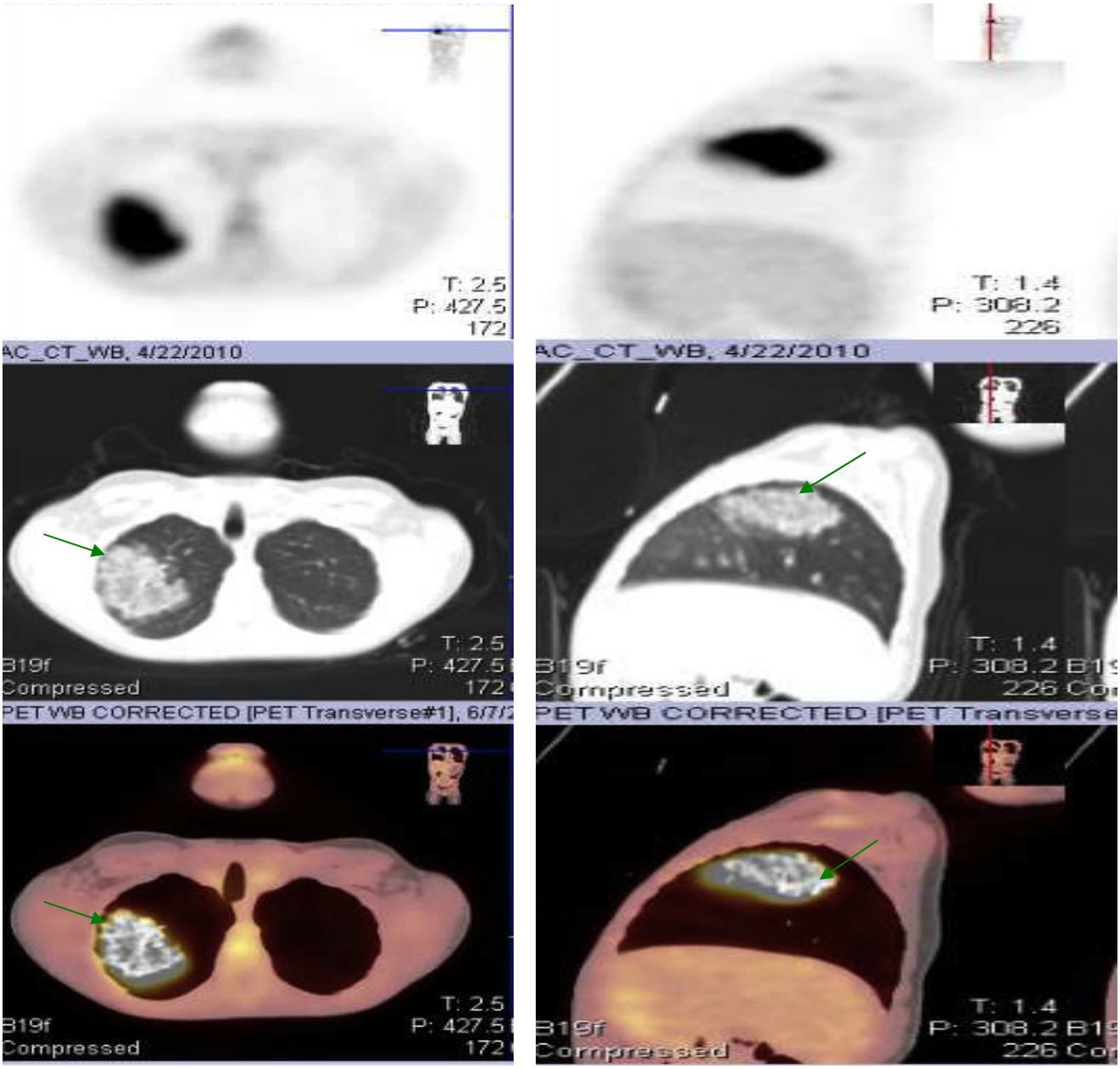

Figure 10. **Transverse and axial views ^{18}F-FDG PET/CT of patient with consolidation and positive ^{18}F-FDG uptake in the right upper lobe (indicated with arrows)**

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

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

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

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

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

8.4 DISCUSSION

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

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

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

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

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

Other authors have demonstrated a correlation between IL-8 and a modified Bhalla score [270]. This study was not able to confirm this finding. Two explanations for this difference may be a difference in sample size; this study having forty-one versus their smaller study population of 27 subjects, and their inclusion of children with heterogeneous causes of bronchiectasis.
Importantly, in the series presented, the CRP significantly higher and the intensity of ^{18}F-FDG uptake although this was no statistically significant suggesting the presence of an acute inflammation. This finding concurs with other studies where ^{18}F-FDG associated with a high CRP level has been found to quantitatively delineate infection and inflammation in a diverse group of disorders including CF, pneumonia, pulmonary fibrosis and interstitial pneumonitis [271, 272].

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

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

8.5 CONCLUSION

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