

HIV-related bronchiectasis in children: an emerging spectre in high tuberculosis burden areas

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SUMMARY

BACKGROUND: Human immunodeficiency virus (HIV) infected children have an eleven-fold risk of acute lower respiratory tract infection. This places HIV-infected children at risk of airway destruction and bronchiectasis.

OBJECTIVE: To study predisposing factors for the development of bronchiectasis in a developing world setting.

METHODS: Children with HIV-related bronchiectasis aged 6–14 years were enrolled. Data were collected on demographics, induced sputum for tuberculosis, respiratory viruses (respiratory syncytial virus), influenza A and B, parainfluenza 1–3, adenovirus and cytomegalovirus), bacteriology and cytokines. Spirometry was performed. Blood samples were obtained for HIV staging, immunoglobulins, immunoCAP®-specific immunoglobulin E (IgE) for common foods and aeroallergens and cytokines.

RESULTS: In all, 35 patients were enrolled in the study.

Of 161 sputum samples, the predominant organisms cultured were *Haemophilus influenzae* and *parainfluenzae* (49%). The median forced expiratory volume in 1 second of all patients was 53%. Interleukin-8 was the predominant cytokine in sputum and serum. The median IgE level was 770 kU/l; however, this did not seem to be related to atopy; 36% were exposed to environmental tobacco smoke, with no correlation between exposure and CD4 count.

CONCLUSION: Children with HIV-related bronchiectasis are diagnosed after the age of 6 years and suffer significant morbidity. Immune stimulation mechanisms in these children are intact despite the level of immunosuppression.

KEY WORDS: human immunodeficiency virus; tuberculosis; bronchiectasis; paediatrics; cytokines

THE INCIDENCE of antenatal human immunodeficiency virus (HIV) infection has increased in South Africa, from 0.4% in 1991 to 29% in 2009.^{1–3} This increase in maternal infection rates, coupled with a delay in the availability of highly active anti-retroviral therapy (HAART) for effective prevention of mother-to-child transmission (PMTCT), has resulted in high vertical infection rates. Universal access to single-dose nevirapine (NVP) was made available in 2003 (South Africa Government Online, <http://www.gov.za>), and a combination of single-dose NVP, together with 6 weeks azidothymidine for PMTCT, in 2008.⁴ Children born prior to 2003 therefore had a higher risk of vertically transmitted HIV and would therefore present with chronic manifestations of HIV.⁵

In a Rwandan study, HIV-infected children were three times more likely to die from respiratory tract infections.⁶ Untreated HIV-infected children have an incidence rate of 11.1 per 100 child-years of acquiring acute lower respiratory tract infections (LRTIs); with HAART this decreases to 2.2/100 child-years.^{7,8}

Recurrent LRTIs place HIV-infected children at risk of airway destruction and subsequent bronchiectasis. The pathogens implicated in LRTIs in HIV-infected children are pneumococcus, *Haemophilus influenzae* and respiratory viruses.⁹

Childhood bronchiectasis has declined in affluent populations due to effective immunisation programmes, less overcrowding, access to medical care, better hygiene and nutrition, with reported rates of 0.49 per 100 000 population in Finland.^{10,11} Certain groups in industrialised countries, such as the Alaskan natives of the Yokun Kuskokwim Delta, the New Zealand Maori and the Aborigines of Australia, have inordinately high bronchiectasis rates, ranging from 3.5 to 16/10 000.^{12–14} Published data on bronchiectasis in developing countries suggest infectious causes, with post adenoviral bronchiolitis obliterans being a common cause of bronchiectasis in Brazil;¹⁵ the high burden of infectious disease and tuberculosis (TB) account for the majority of cases.^{16,17}

South Africa has one of the highest burdens of TB, with rates exceeding 500/100 000.¹⁸ Although HIV

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and TB co-infection has been well documented,¹⁹ the real co-infection rates are unfortunately unclear, as the radiological picture and tuberculin skin test can have a low diagnostic yield in HIV-infected children.^{7,20,21} Lymphocytic interstitial pneumonitis (LIP) can also result in bronchiectasis in HIV-infected children.^{8,22}

Bronchiectasis is an 'orphan' lung disease, as little research funding is devoted to this disease; this is even truer for HIV-related bronchiectasis.²³ Our objective was therefore to investigate possible predisposing and aggravating factors for bronchiectasis, to characterise local and systemic inflammatory markers and to document morbidity related to bronchiectasis in a cohort of HIV-infected children in a high TB burden area.

PATIENTS AND METHODS

Patients

We screened 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. Patients were enrolled if they were aged 6–18 years, were able to reliably perform lung function tests, and exhibited symptoms suggestive of bronchiectasis, namely chronic productive cough, clubbing or halitosis, and had radiological confirmation of bronchiectasis. Thirteen children aged <6 years were excluded from the study, and 43 subjects (77%) were eligible and screened. Another participant was excluded because the parents refused consent to participate, and seven were lost to follow-up. A final 35 children were included in the analysis.

Signed informed consent was obtained from the parents/guardians of all enrolled subjects. Assent was obtained from all children over the age of 7 years.

Clinical investigations

Information collected included age at HIV diagnosis, timing of initiation of HAART, exposure to environmental tobacco smoke (ETS) and biomass fuels (BMF), prior and current treatment for TB, and growth parameters (weight, height and body mass index [BMI, kg/m²]). Lung function (forced expiratory volume in 1 second [FEV₁], forced vital capacity [FVC], FEV₁/FVC and forced inspiratory flow [FEF_{25–75}]) was measured using the ViasysSpiroPro Jaeger Spirometer (Jaeger, Hoechberg, Germany).

Laboratory investigations

Induced sputum samples were collected. One was analysed for bacterial pathogens, including *Mycobacterium tuberculosis* and respiratory viruses (respiratory syncytial virus, influenza A and B, parainfluenza 1–3, adenovirus and cytomegalovirus). Another sample (0.029–1.53 ml per patient) was assayed for sputum cytokines using the Bio-Plex® system (Bio-Rad Laboratories Inc, Hercules, CA, USA). The fol-

lowing analytes were measured: interleukin (IL) 1β, IL-1Ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17, interferon gamma (IFN-γ), tumour necrosis factor alpha (TNF-α), granulocyte colony stimulating factor (G-CSF) and granulocyte macrophage colony stimulating factor (GM-CSF); results were expressed in pg/ml. Monthly sputum samples were sent for microbiological testing. Of these, 17.8% were collected during an exacerbation, defined as tachypnoea or dyspnoea, change in frequency of cough, increased sputum productivity, fever and chest pain.

Serum samples were collected for the following investigations: CD4+ lymphocytes, HIV viral load, C-reactive protein (CRP) and a panel of immunoglobulins (Ig): IgA, IgE, IgG and IgM. Other serum samples were sent for ImmunoCAP® RAST testing (radioallergosorbent test) for paediatric food mix (FX5), Phadiatop and *Aspergillus fumigatus* (Phadia AB, Uppsala, Sweden.)

Statistical analysis

Data analysis was performed using Stata Release 10 (Stata Corp LP, College Station, TX, USA) and statistical analyses using the Spearman correlation coefficient and the Wilcoxon rank sum test (Mann-Whitney test). Testing was performed at the 0.05 level of significance.

Ethics approval to conduct the study was granted by the Research Ethics Committee of the University of Pretoria, South Africa.

RESULTS

Thirty-five subjects were enrolled, with a male/female ratio of 57:43. Two patients died; both presented with severe bilateral lung disease and oxygen dependence. The diagnosis of HIV was made at a mean age of 6.9 years (range 6–11.1; Table 1). The median total and percentage CD4 count of the subjects was respectively 569 × 10⁹ cells/l and 18.3%. The median HIV viral load was <25 copies/ml: 19 subjects were virologically suppressed, with viral loads <25 copies/ml, and 16 were non-suppressed (Table 2);

Table 1 Study group baseline characteristics

Parameter	Median	Range
FEV ₁ , % predicted	53	5–86
FEF _{25–75} , % predicted	52.0	11–165
CD 4 count, total × 10 ⁹ /l	569	54–1763
CD 4 count, %	18.3	1.68–35.6
HIV viral load, RNA copies/ml	<25*	<25–200 000
IgG, g/l	26	14.6–81.4
IgE, kU/l	770	54–1783
IgA, g/l	2.67	0.47–6.56
IgM, g/l	1.50	0.48–4.33
CRP, mg/l	9.2	0–401

FEV₁ = forced expiratory volume in 1 second; FEF_{25–75} = forced inspiratory flow; HIV = human immunodeficiency virus; Ig = immunoglobulin; CRP = C-reactive protein.

Table 2 Comparison of subjects with and those without viral suppression

Variable, subjects	Suppressed* (n = 19)	Non-suppressed† (n = 15)	P value
C-reactive protein‡	25.4	55.15	0.407
FEV ₁ , l/min‡	54	46	0.195
IgE, kU/l‡	180.8	316.9	0.089
IgG, kU/l‡	27.7	34.9	0.257
Weight, kg‡	21.8	22.5	0.945
Height, cm‡	118.9	118.0	0.945
HAART, months‡	17.5	20.4	0.797
IL-4, pg/ml‡	0.5	0.4	0.242
Sputum IL-8, pg/ml	5 548.0	3 294.2	0.165
Serum IL-8, pg/ml‡	52 113	14 667	0.740
Serum IFN-γ, pg/ml‡	19.1	15.0	0.173

*Viral load >25 copies/ml.

†Viral load <25 copies/ml.

‡Mean values.

FEV₁ = forced expiratory volume in 1 second; Ig = immunoglobulin; HAART = highly active antiretroviral therapy; IL = interleukin; IFN-γ = interferon gamma.

all but one had received HAART at enrolment. The median number of months on HAART was 18 months (range 0–60). There were no statistically significant differences between the suppressed and non-suppressed individuals with respect to FEV₁, anthropometric parameters, months on HAART, IL-4, IL-8, IFN-γ or IgG. There was, however, a marginally significant difference between virologically suppressed and non-suppressed subjects with respect to IgE ($P = 0.089$). The mean BMI for the cohort was 15.3 kg/m² (range 12.1–23.2).

A total of 161 sputum cultures were performed over the 1-year follow-up period (multiple samples were collected from all 35 patients; Figure 1). At presentation, 42.8% of the subjects had a positive culture for a bacterial pathogen. The most common organisms were *H. influenzae* and *parainfluenzae*, which

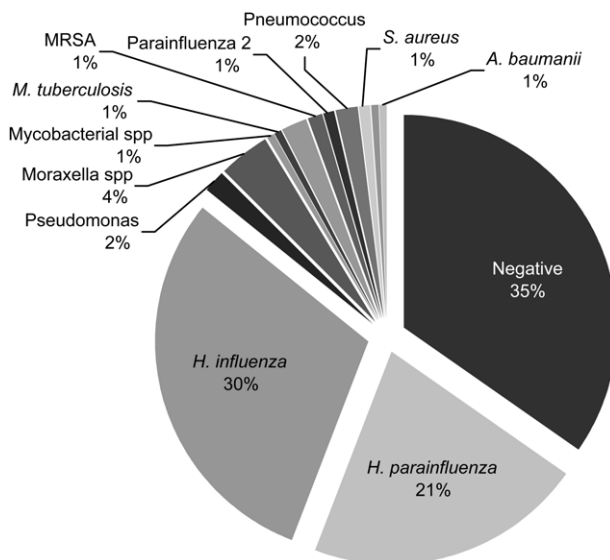
accounted for 49% of all cultures; 2% of cultures were identified as *Pseudomonas aeruginosa* and 1% as *Staphylococcus aureus*. Two subjects had mycobacteria other than tuberculosis (MOTT), namely *M. fortuitum* and *M. avium intracellulare*. Of the study population, 48.5% had previously received one course of anti-tuberculosis treatment, 21.2% two courses and 6% three courses. Only one subject had a positive viral identification on sputum (parainfluenza type 2).

With respect to lung function, the median FEV₁ was 53% predicted (range 5–86), while the median FEF_{25–75} was 52% predicted (range 11–165). Only eight children had a positive bronchodilator response, defined as a 15% increase in FEV₁ post-bronchodilator. When comparing the FEV₁ of those with positive or negative sputum culture at enrolment, the groups did not differ significantly ($P = 0.524$). There was also a lack of correlation between IgG and FEV₁ or FEF_{25–75} (respectively $r = -0.049$ and $r = 0.02$).

Thirty-six per cent had been exposed to ETS, with at least one smoker among household contacts. The mean CD4 count for children exposed and non-exposed to ETS did not differ significantly ($P = 0.327$). With respect to FEV₁, there was also no statistically significant difference between ETS-exposed and non-exposed children ($P = 0.64$, 95% confidence interval [CI] 40.598–55.506). The two children who died were both exposed to ETS. BMF exposure to paraffin oil, coal stoves and other indoor coal fire heat sources was present in 40% of children.

The mean total IgE for the group was 770 kU/l, with only 10% of all children having a positive specific IgE on RAST testing for inhalants or foods. Total IgE and CD4 count were not correlated ($r = -0.02$, $P = 0.482$). IgG was the most significantly elevated immunoglobulin (median 26 g/dl). CRP levels were low, with a median value of 9.2 mg/l. There was a lack of correlation between CRP and serum cytokines IL-6 and IL-8 ($r = 0.259$ and $r = 0.324$, respectively).

Of the cytokines analysed in serum and sputum (Figure 2), IL-8 was the most significantly elevated, with median values of respectively 400 and 116 pg/ml. IFN-γ, a T-helper 1 (Th-1) cytokine, was also elevated. IL-1ra, an anti-inflammatory cytokine, was elevated in serum and sputum. There was no correlation between CD4% and HIV viral load and IL-8 ($r = -0.071$ and $r = -0.213$ respectively), or between Th2 cytokines IL-2, IL-4, IL-13 and IgE ($r = -0.22$, $r = -0.21$ and $r = 0.06$, respectively). There was, however, a positive correlation between IL-4 and HIV viral load ($r = 0.42$). There was no correlation between IL-1, IL-6, IL-8 and number of months on HAART ($r = 0.27$, $r = 0.287$, $r = 0.128$, respectively). The chemokine macrophage inflammatory protein-1 beta (MIP-1b) was elevated in serum as compared to sputum (47 vs. 1 pg/ml). Monocyte chemoattractant protein-1 (MCP-1) was also elevated in serum, but to a lesser extent than MIP-1b (13 pg/ml).

**Figure 1** Cumulative data for patients (n = 35): infecting pathogens (n = 161). MRSA = methicillin-resistant *S. aureus*.

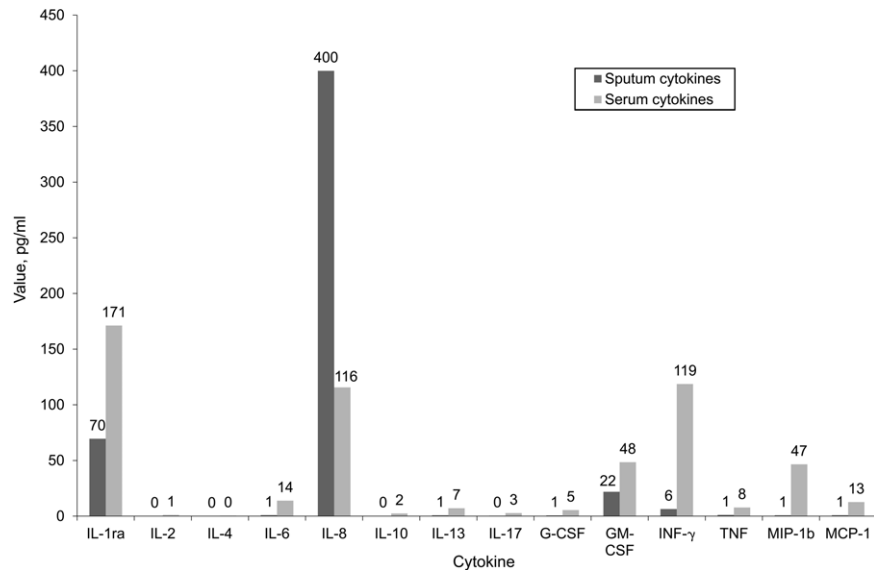


Figure 2 Sputum and serum cytokine values and ranges. IL = interleukin; G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte macrophage colony stimulating factor; IFN-γ = interferon gamma; TNF = tumour necrosis factor; MIP-1b = macrophage inflammatory protein-1 beta; MCP-1 = monocyte chemotactic protein-1.

GM-CSF was elevated in both serum and sputum (48 and 22 pg/ml, respectively). Very low levels of IL-2, 4, 10, 13, G-CSF and TNF were present in both sputum and serum.

DISCUSSION

In our cohort of children with HIV-related bronchiectasis, the diagnosis of HIV infection is delayed, with the majority being diagnosed after the age of 6 years. It is presumed that the majority of these children had vertically transmitted HIV. This may demonstrate a failure of the PMTCT programme, as HIV-infected women and their newborn children are not offered HIV testing and subsequent follow-up. These children with HIV-related bronchiectasis were possibly of the 'slow-progressor' phenotype.

H. influenzae and *parainfluenzae* were the predominant organisms cultured. In South Africa, *H. influenzae* Type B (Hib) vaccination has been universally available for all children since July 1999, with absolute cases of Hib decreasing by 65% in children aged <1 year from 1999–2000 to 2003–2004, while rates of non-typeable *H. influenzae* have increased, especially in HIV-infected children.²⁴ Although the Hib vaccine is less effective in HIV-infected children than in non-infected children; Madhi et al. found that the Hib vaccine reduced overall invasive Hib disease by 83% in all children.²⁵

S. aureus was also not a major pathogen in our population. McNally et al. found that the risk of *S. aureus* nasal carriage (and therefore predicted sepsis) was 2.86 times higher in HIV-infected children presenting with acute pneumonia.²⁶ The presence of

bacterial organisms did not seem to affect disease severity.

Seventy-five per cent of our study population had a prior diagnosis of TB, three of which were microbiologically confirmed. The difficulties of diagnosing TB in HIV-infected children are well documented, and in a high TB burden area there may be over-reliance on radiological diagnosis.^{7,20,21} The limitations of this approach are that TB may have a similar radiological picture to bronchiectasis, and this may therefore explain how bronchiectasis may be missed. MOTT infections occur with bronchiectasis, which may be mislabelled as TB. Almost a quarter of children in our study received two courses of anti-tuberculosis treatment. This is not surprising, as current guidelines depend heavily on chest X-ray interpretations for TB diagnosis at the primary health care level.²⁷ LIP rates in our study population were low and therefore do not explain the bronchiectasis in our group.

The median FEV₁ in our study was 53% of predicted (range 5–86); this is in comparison to New Zealand children with non-cystic fibrosis (CF) related bronchiectasis, where Munro et al. reported a baseline predicted FEV₁ of 66%.²⁸ HIV-related bronchiectasis seems to cause accelerated lung function decline compared to other causes of non-CF-related bronchiectasis. Our cohort was undernourished, with a low BMI. The impact of nutrition on lung morbidity is well described in CF, where the lower the BMI, the higher is the morbidity from lung disease.²⁹ Whether this was due to increased metabolic demands from chronic lung disease, HIV infection or a surrogate marker for socio-economic status of the children is unclear.

There was a significantly elevated IgE in our study.

Previous studies in adults and children infected with HIV have shown a relationship between IgE and HIV stage.^{30–35} We could not replicate this finding, and found no increase in the Th-2 mediated cytokines in relation to the elevated IgE. This confirms that IgE elevation is not related to atopy but probably reflects polyclonal hypergammaglobulinaemia related to T-cell depletion. There was a marginally significant difference in IgE levels between the subgroups with and without viral suppression; this was, however, not statistically significant, and may be related to the small sample size. In a previous study, we documented no increase in skin prick test positivity in HIV-infected children, confirming that atopy was not responsible for an elevated IgE.³⁶ The other potential explanation for elevated IgE is the presence of allergic bronchopulmonary aspergillosis, but this was ruled out. As with HIV-infected children with acute pneumonia,³⁷ we found elevated IgG levels, probably reflecting immune hyperstimulation related to HIV infection.

The predominant cytokine in our cohort was IL-8. This is similar to CF-related bronchiectasis, where oxidative stress results in increased IL-8 levels.^{38,39} IL-8 is a marker of neutrophil-driven inflammation, where elevation may suggest that the disease process in HIV-related bronchiectasis is neutrophil-dependent. Whether the neutrophil driven inflammatory process in HIV-bronchiectasis is dependent on the innate or adaptive immune mechanisms requires further exploration. All potentially relevant cytokines related to inflammatory disease of this nature that represent Th 1-driven inflammation, including IL-1, IL-6, GM-CSF and IFN- γ , were elevated, reflecting an ability to mount immune responses against pathogens, although the levels did not correlate with HIV staging or use of HAART. We therefore postulate that the presence of an aggressive immune response against pathogens may trigger airway inflammation and subsequent bronchiectasis. Although in HIV infection the dominant abnormality is immunosuppression, the local and systemic immunological responses seem to be exaggerated. MIP-1b, which is mainly involved in the host response to bacterial, fungal, viral and parasitic pathogens and selectively attracts CD4 lymphocytes, was elevated in the serum and, to a lesser extent, in the sputum of our subjects. MIP-1b is also known to be a major suppressive factor of HIV produced by CD8+ cells, possibly suggesting that there is continuous immune stimulation systemically, and, to a lesser extent, in the lungs.

ETS exposure does not explain FEV₁ or CD4 count variability. An adult study by Feldman et al. reported a statistically significant difference in morbidity and mortality of smokers with HIV infection.⁴⁰ A previous study in our population of 121 HIV-infected children showed no difference in HIV staging in ETS exposed and non-exposed children, consistent with our current finding.⁴¹ Kabali et al. also found no as-

sociation between cigarette smoking and HIV disease progression.⁴²

The limitations of our study were the small sample size and lack of objective measurements to quantify ETS exposure. Larger trials are needed to confirm these findings.

CONCLUSION

Children with HIV-related bronchiectasis have the diagnosis of HIV infection made at a median age of 6 years. In a high TB burden area, the differential diagnosis of an abnormal chest X-ray in children with chronic cough or previously treated TB should include bronchiectasis. Even in a setting of HIV-related bronchiectasis, local and systemic immune stimulation mechanisms appear to remain intact.

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