Lung Infiltrates in Antiretroviral-naive HIV-infected Children with Chronic Lung Disease: Value of Non-bronchoscopic Bronchoalveolar Lavage in the Detection of *Candida albicans*

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Summary

Objective: To describe the etiology of lung infiltrates in HIV-infected antiretroviral-naive children with chronic persistent/recurrent lung disease in whom routine cultures were negative and were non-responders to World Health Organization standard antimicrobial therapy.

Method: Non-bronchoscopic bronchoalveolar lavage (NBBAL) was performed on these non-responders.

Results: Fifty children were enrolled. Single isolates on NBBAL were seen in 28 cases, dual pathogens in 5 cases and no growth in 14 cases. *Haemophilus influenzae* (*n* = 12), *Candida albicans* (*n* = 5) and *Mycobacterium* spp. other than tuberculosis (*n* = 4) were the commonest pathogens seen. Eight cases with no growth had segmental or lobar collapse: in five cases, NBBAL was therapeutic and in two cases, a diagnosis of lymphoma was made on open lung biopsy. Thirty-two of the 38 cases (84%) had favorable outcomes on follow-up.

Conclusion: *Haemophilus influenzae*, *C. albicans* and *Mycobacterium* spp. other than tuberculosis are important pathogens in children with HIV and HIV-associated chronic lung disease.

Key words: lung infiltrates, HIV, chronic lung disease, non-bronchoscopic bronchoalveolar lavage.

Introduction

Of the approximately 1.8 million children aged <15 years with HIV, 90% experience some respiratory illness during their lifetime [1]. Many of these children die of respiratory failure. In 2008, AIDS accounted for 280,000 deaths in children aged <15 years [1]. AIDS is now the leading cause of death in sub-Saharan Africa and the fourth biggest killer worldwide. An estimated 600,000 new pediatric HIV infections occur each year, of which >90% (1500/day) occur in sub-Saharan Africa [1].

Pneumonia is the commonest cause for hospitalization in HIV-infected children from Africa [2]. Common bacterial and viral etiologies of acute lower respiratory tract infection include *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, non-typhoidal *Salmonellae*, *Mycobacterium tuberculosis*, *respiratory syncytial virus*, *Cytomegalovirus* spp. and *adenovirus* [2–4]. Chronic lung diseases (CLDs), defined as lung infiltrates persisting for >3 months with clinical features of chest deformity, clubbing, suffused conjunctiva, stunting and halitosis, are common in HIV-infected children and occur more frequently in the absence of, or with delay in, instituting highly active antiretroviral therapy (HAART). Common causes of HIV-related CLDs include lymphoid interstitial pneumonitis, bronchiectasis, tuberculosis and lymphoma [5]. Acute persistent (clinical features present for >3 weeks) or recurrent (>1 episode/annum or >2 episodes during life) lung infiltrates are common in HIV-infected children who have advancing age but remain HAART naive [6]. The role of fungal infections, mycobacteria other than tuberculosis (MOTT), has not yet been defined in these children. The institution of HAART reduces the frequency of acquisition of these lung infections [7].

The etiology of the lower respiratory tract infiltrates in children with CLD and persistent/recurrent...
infiltrates is unknown. HIV-infected children with CLD and non-responsive World Health Organization (WHO)-defined pneumonia and negative blood cultures were subjected to non-bronchoscopic bronchoalveolar lavage (NBBAL), and samples obtained were sent for microbiological analysis.

**Method**

This was a descriptive analysis of data from the electronic charts of HIV-infected children with CLD who underwent NBBAL at Inkosi Albert Luthuli Central Hospital, Durban, South Africa from August 2002 to April 2005. The study received ethical approval from the biomedical ethical research committee from the University of KwaZulu Natal. HIV infection was confirmed by positive results on two separate HIV enzyme-linked immunosorbent assay tests from two different kits (Roche and AMPLICOR) taken 1 month apart as per standard protocol. All children were prescribed cotrimoxazole prophylaxis before and after the procedure. Clinical and radiological features of persistent, recurrent, destructive, interstitial, cystic lung disease or collapse were used to describe specific clinical diagnostic entities. Blood, sputum and urine tests were sent routinely. All children had received at least 1 week of standard WHO empiric antimicrobial therapy (amoxicillin for severe pneumonia and penicillin with gentamicin for very severe pneumonia), and >50% of enrolled subjects had received or were currently on a course of anti-tuberculosis (TB) therapy. None of the subjects was prescribed HAART. Standard supportive therapy including physiotherapy was performed for at least 1 month where clinically indicated. HIV-infected children with CLD and lung infiltrates not responding to this therapy, and in whom culture results were negative, were offered NBBAL. Informed consent was taken before performance of NBBAL. Samples obtained were sent for microscopy, culture and sensitivity for bacteria, including mycobacteria, as well as viruses and fungi, including immunofluorescence staining for Pneumocystis jiroveci.

NBBALs were performed according to the following protocol [8]: Patients were prepared by fasting, sedated with midazolam 0.1–0.5 mg/kg and preoxygenated with 100% inspired oxygen for approximately 1 min before initiation of the procedure. Children were monitored by electrocardiography and pulse oximetry. Resuscitation equipment was readily available. The patient was intubated orally with an appropriate-sized endotracheal tube, and a closed suction circuit was connected to the endotracheal tube. The suction catheter was wedged as far distally as possible and the first sample discarded to exclude contamination. The catheter was then reintroduced, and 1 ml/kg 0.9% saline at room temperature was injected through the side port while the child was tilted to the affected side. Secretions mixed with the instilled saline were suctioned until no more fluid could be withdrawn. The child was then tilted to the opposite side and the procedure repeated. Chest physiotherapy and postural drainage were performed during suctioning. The procedure was abandoned at any point during the procedure if the oxygen saturation dropped to <85% and temporarily halted if there was a transient drop in saturation to between 85 and 90%.

**Results**

Fifty patients underwent NBBAL during the study period. The mean age of the enrolled subjects was 5.9 years (range 1–14 years); 60% were male. All children had received routine *Haemophilus influenzae* type B immunization.

**Quality of lung aspirates and the microbiological yield from NBBAL**

The quality of aspirates obtained through NBBAL was excellent. The correlation between the number of pus cells, epithelial cells and organisms cultured suggested contamination in just two cases (Table 1). Fourteen cases had no growth; four had bronchiectasis with collapse and four had isolated collapse. Two cases with isolated collapse and persistent and deteriorating lung lesions were subjected to an open lung biopsy, and a diagnosis of B-cell lymphoma was confirmed on histology. Positive cultures were identified in 34 (68%) cases. Single pathogens were identified in 28 cases; dual pathogens with pus cells and few/no epithelial cells were seen in five cases and one child had multiple pathogens with many pus cells and few epithelial cells.

**Pathogens identified on NBBAL**

The commonest pathogen identified was *H. influenzae*, seen as a single isolate in eight cases and as a dual pathogen in four cases (Table 2). *Candida albicans* was identified as a single isolate in five cases and as a dual or multiple pathogen in a single case each. In this cohort, 12% of the patients had a positive growth for *C. albicans*, whereas one patient had *Aspergillus niger* cultured. MOTT was diagnosed in three cases as a single isolate and in one case with multiple infections. *Mycobacterium tuberculosis* was identified in three cases as single isolates and in one case with multiple infections. *Methicillin-resistant S. aureus* (MRSA), and *Pseudomonas aeruginosa*, *Enterobacter cloacae* and *Klebsiella pneumoniae* were rarely seen. *Moraxella catarrhalis* was seen in three cases as a
dual pathogen. Only a single case with a virus was identified.

Sensitivity of pathogens
Single isolates of *H. influenzae* were all sensitive to ampicillin, while dual isolates of *H. influenzae* with other bacteria were sensitive to co-amoxyclavunate. All cases of *S. pneumoniae* were sensitive to penicillin. Two of the three isolates with *S. aureus* were methicillin-resistant, while all gram-negative pathogens were extended spectrum beta-lactamase producing. All cases of *C. albicans* were sensitive to fluconazole, while those with MOTTS were sensitive to clarithromycin, quinolones and rifampicin. *Mycobacterium tuberculosis* cases were sensitive to first-line anti-TB therapy.

Outcome
Thirty-eight of the 50 cases returned for follow-up following institution of appropriate management post-NBBAL; 11 cases were lost to follow-up and 1 child died 2 months after enrollment with meningitis. Thirty-two (84%) patients, 27 with positive growth and 5 with no growth, improved on clinical, laboratory and radiological investigations at 3-month follow-up. Of the six cases who failed to improve post-NBBAL, two had bronchiectasis with MRSA co-infection, one had dual infection with *S. aureus* and *E. coli* and three had no growth. A diagnosis of lymphoma was confirmed in two of the latter three cases. Repeat NBBALs were not performed.

Discussion
The major finding of this study was the identification of non-routine pathogens from NBBAL in a group of HIV-infected antiretroviral-naive patients with CLD and persistent or recurrent lung infiltrates after non-response to standardized WHO-recommended antibiotic therapy and co-trimoxazole prophylaxis. The high prevalence of *C. albicans* isolates in these patients is of concern as clinicians rarely prescribe antifungal therapy for such cases. Chronic *Candida* infection has been associated with oropharyngeal, laryngeal or oesophageal disease in HIV-infected children [9]. Our findings suggest that *C. albicans* may play an important role in chronic infections of the lower respiratory tract. Empiric therapy with fluconazole should be considered for these patients. The identification of MOTTS in these cases supports the early search for these pathogens in non-responsive cases. Empiric therapy with fluconazole should be considered in these patients. In TB-endemic areas, TB is a common cause of chronic respiratory infection in HIV-infected children [5–7, 9, 10]. These cases are poorly responsive to standard TB therapy and require specific therapy. An active consideration of this diagnosis should be made in non-responsive cases.

A second major finding of this study was the frequent isolation of pathogens to which immunization should prevent and standard antimicrobial therapy should eradicate. Despite receiving *H. influenzae* type B immunization, amoxicillin or penicillin and gentamicin and anti-TB therapy, significant yields of *H. influenzae* and *M. tuberculosis* were seen. None of the *H. influenzae* isolates was typed, but possible explanations for this high yield could be repeated reinfections from sanctuary sites, ineffectiveness of the immunization campaigns and development of *H. influenzae* and *M. tuberculosis* with non-vaccine serotypes or the pathogens developing an innate resistance mechanism to prevent clearance. The latter was supported by variations in the sensitivity patterns seen when *H. influenzae* was isolated as a single or dual/multiple pathogen.

Regarding the isolation of *M. tuberculosis*, several explanations could be considered. Firstly, reactivation and reinfection have been reported previously among similarly ill HIV-infected children. Secondly, drug-resistant disease could be possible, although this was excluded. Thirdly, immune reconstitution inflammation syndrome may result in reactivation TB at initiation of anti-TB therapy or HAART, but all the patients were HAART naive and none had...
### Table 2
Clinical diagnoses and microbiological yield from NBBAL in HIV-infected children with chronic lung disease and persistent/recurrent lung infiltrates

<table>
<thead>
<tr>
<th>Indication for NBBAL</th>
<th>Growth with many pus cells &gt;10/hpf</th>
<th>Growth with moderate pus cells 5–10/hpf</th>
<th>Growth with few pus cells &lt;5/hpf</th>
<th>Growth with no pus cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent pneumonia (n = 25) Isolated (n = 19)</td>
<td>Haemophilus influenzae (4) MOTT (1)</td>
<td>Enterobacter (1) Candida (2)</td>
<td>Candida albicans (2) Escherichia coli + Staphylococcus aureus (1)</td>
<td>Adenovirus (1)</td>
</tr>
<tr>
<td>With pneumatocele (n = 3)</td>
<td>MOTT + Streptococcus pneumoniae + Candida albicans (1)</td>
<td>H. influenzae + Moraxella catarrhalis (1)</td>
<td>Contaminant (1) No growth (2)</td>
<td>Contaminant (1) No growth (1)</td>
</tr>
<tr>
<td>With collapse (n = 3)</td>
<td>MOTT (1) Mycobacterium tuberculosis (1)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Recurrent pneumonia (n = 3) Isolated (n = 2)</td>
<td>H. influenzae (1) S. pneumoniae (1) H. influenzae + M. catarrhalis (1)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pneumatocele (n = 1)</td>
<td>S. pneumoniae (1) No growth (1)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lymphoid interstitial pneumonitis (n = 6)</td>
<td>MOTT (1)</td>
<td>Mycobacterium tuberculosis (1)</td>
<td>Mycobacterium tuberculosis (1)</td>
<td>–</td>
</tr>
<tr>
<td>Persistent pneumonia (n = 2)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bronchiectasis + persistent pneumonia (n = 2)</td>
<td>H. influenzae (1)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Recurrent pneumonia (n = 2)</td>
<td>Klebsiella pneumoniae (n = 1)</td>
<td>H. influenzae + C. albicans (1)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Bronchiectasis (n = 12) Isolated (n = 1)</td>
<td>Pseudomonas aeruginosa (n = 1)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>With persistent Pneumonia (n = 6)</td>
<td>Methicillin-resistant S. aureus (2) H. influenzae (1) S. pneumoniae (1)</td>
<td>–</td>
<td>No growth (1) No growth (1)</td>
<td>–</td>
</tr>
<tr>
<td>With collapse (n = 4) Recurrent pneumonia (n = 1) Collapse (n = 4)</td>
<td>H. influenzae (1) No growth (1)</td>
<td>No growth (1)</td>
<td>No growth (1)</td>
<td>No growth (1)</td>
</tr>
<tr>
<td>Total (50)</td>
<td>Single, 18 Dual, 1 Multiple, 1 No growth, 2</td>
<td>Single, 4 Dual, 2 Contaminant, 0 No growth, 3</td>
<td>Single, 4 Dual, 2 Contaminant, 1 No growth, 6</td>
<td>Single, 2 Dual, 0 Contaminant, 1 No growth, 3</td>
</tr>
</tbody>
</table>
recently commenced anti-TB therapy. The relatively high prevalence of MOTTs in this cohort suggests that an active consideration of this diagnosis should be made in non-responsive cases.

The prevalence of nosocomial or hospital-based microbes was low in this cohort. This may be due to lack of hospitalization of these cases—a policy that is practiced at our institutions. The routine use of broad-spectrum antimicrobial cover that treats nosocomial pathogens should be avoided. Furthermore, NBBAL was therapeutic in five children with lung collapse. In two children with persistent infiltrates with collapse a diagnosis of lymphoma was confirmed on lung biopsy. Children with HIV infection have an increased risk of malignancy, especially non-Hodgkin’s lymphoma, Kaposi’s sarcoma, leiomyosarcoma and Hodgkin’s lymphoma [11]. A possible malignancy should be considered in cases with non-response to empiric antimicrobial therapy and negative cultures on NBBAL.

Several limitations of this study exist. A major concern with the findings of this study is the correlation of the microbiological findings with the clinical disease entity. Although the study classifies the cases into clinical entities, it does not attempt to address the diversity in terms of the pathological process. The relevance of the clinical microbiological findings could be questioned in terms of being asymptomatic colonization but given that these isolates occurred in symptomatic patients with radiological and laboratory abnormalities and there was response to therapy, we believe that they are truly pathogenic. Another major limitation to the findings of this study was the lack of routine administration of HAART to these children, as this would significantly impact on the findings and should be standard of care, although at the time of the study access to HAART was limited. In a recently published study of adolescents admitted to a hospital in Zimbabwe, Ferrand, et al. [12] found that one-quarter of the HIV-infected cohort who had chronic disease had their HIV status only diagnosed during the study and consequently were not taking HAART. It would be prudent to repeat this study on similar cases prescribed HAART. Another concern was lack of a comprehensive evaluation of NBBAL samples. Specimens were not sent for lipid-laden macrophages (aspiration syndrome) or hemosiderin-laden macrophages (pulmonary hemorrhage). Furthermore, full analysis of the antimicrobial sensitivity pattern was not provided by the microbiologist. Only results of antimicrobials to which the organism was sensitive were available. Nevertheless, the high yield of quality samples obtained in 48 of the 50 cases and the high treatment response rate post-NBBAL make findings from this study extremely useful.

In conclusion, antiretroviral-naive HIV-infected patients with CLD and persistent/recurrent lung infiltrates not responding to standardized antimicrobial therapy should have a search for unusual microbiological pathogens. In situations where this is not possible for these patients, empiric cover with antibiotics and an antifungal agent should be initiated early. Failure to respond to this regimen should initiate an active search for Mycobacterium tuberculosis and MOTTs. Tissue biopsies should be considered for persistent changes where no diagnosis is confirmed. Further studies to confirm these findings are essential.

References