ORIGINAL ARTICLE

Pulmonary disease in HIV-infected Patients at the University Teaching Hospital, Lusaka, Zambia

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INTRODUCTION

Sub-Saharan Africa carries two-thirds of the world's HIV/AIDS burden, and accounted for nearly three-quarters of AIDS-related deaths in 2008.[1] Zambia has an adult HIV prevalence estimated at 14.3%,[2] and AIDS and AIDS-related illnesses have contributed to the high mortality among patients.[3] This burden of disease necessitated a rapid scale-up of provision of free anti-retroviral therapy in the urban primary health care setting of the capital city, Lusaka, from 2004, resulting in a decline in overall mortality.[4]

Analysis of data of patients initiating antiretroviral treatment in Lusaka between January 2006 and January 2007 shows that mortality was highest in the first 90 days of treatment initiation, a trend noted in other developing countries. Among the strongest predictors of early mortality was a CD4+ count of less than 200/μL.[4]

Due to a lack of extensive diagnostic facilities, the specific causes of mortality remain largely unknown in Zambia. Potential major causes of death are nutritional deficiencies, metabolic derangements, endocrinopathies, malignancies, and opportunistic infections, affecting different organ systems, particularly the lungs.[5]

The lungs are an important target of HIV-associated complications. Patients with HIV are at increased risk of having pneumonias, cancers and other pulmonary conditions.[5] Furthermore, pulmonary infections are the leading causes of morbidity and mortality in HIV-infected individuals.[6,7]

In the developed world, rates of pneumonia are much higher in the HIV-infected population[8] and particularly so in those with a CD4+ count less than 200/μL,[9,10] a fact echoed in African studies.[11,12] *Pneumocystis jirovecii* pneumonia (PJP) has remained the most common pulmonary infection in Western settings; in contrast to Africa where reported rates of PJP are varied but generally low.[13] On this continent tuberculosis and pneumococcal pneumonia have been the most-commonly reported respiratory infections.[14]

Considering the variations in the epidemiology of lung infections in HIV, the use of treatment algorithms by the World Health Organization needs to take into account local prevalence of these infections, but such information is scarce and variable.[15]

Rapid and accurate aetiological diagnosis of pneumonias in HIV-infected patients is thus essential to establishing the local prevalence patterns of disease. However this remains a challenge in developing countries, particularly in patients with sputum smears negative for alcohol acid fast bacilli (AAFB), owing to the lack of fiberoptic investigations.[16,17,18] Therefore centres with capabilities to perform these advanced diagnostic techniques should monitor disease trends and obtain data on aetiological pathogens to improve treatment algorithms.

Flexible bronchoscopy with bronchoalveolar lavage is simple, safe and reliable. It has been used extensively and established as a diagnostic procedure for assessing immunosuppressed patients with pulmonary infiltrates on chest radiograph.[19-25] In spite of this, there have been few studies in developing countries and none in Zambia about the utility of bronchoalveolar lavage in the setting of HIV. This study therefore aimed to ascertain the causes of pulmonary diseases in severely immunocompromised

HIV-infected patients yet to initiate Highly Active Anti-Retroviral Treatment (HAART).

METHODOLOGY

Study Population

In this cross-sectional study, we conducted intensive tuberculosis screening of every second HAART-naïve HIV-infected patient with CD4+ count less than 200/μL presenting to the University Teaching Hospital medical wards with pulmonary symptoms. The University Teaching Hospital is the largest referral hospital in Zambia, with a total of 1600 in-patient beds.

The study was approved by the University of Zambia Biomedical Research Ethics Committee (Assurance No. FWA 00000338). Wilful written informed consent was obtained from all study participants and none of them received remuneration. No related serious adverse events were recorded during the study.

Our study algorithm comprised initial sputum screening with Ziehl-Neelsen stain. Patients with AAFB sputum-smear positive were assigned a diagnosis of TB, whereas patients who were unable to expectorate sputum and those in whom sputum smear for tuberculosis was negative underwent bronchoscopy with a fiberoptic scope by standard procedure (Olympus^{TM}EvisLucera-BF 260). Bronchoalveolar lavage (BAL) specimens were collectedand assessed for bacteria, fungi, mycobacteria and *Pneumocystis jirovecii* by microscopy and culture. Microbiological diagnoses were correlated with clinical and radiological findings

Data Entry and Analysis

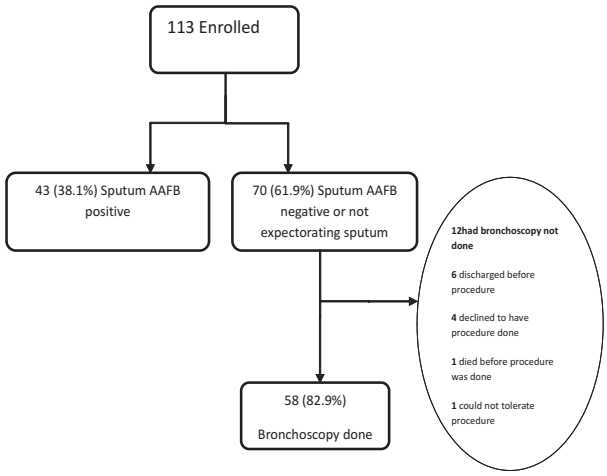
Gathered data was entered onto a specially designed form for onward transmission onto a Microsoft Office Excel (2007) spread sheet and epi-info version 6.0 for analysis.Quantitative variables were expressed as means (±Standard deviation) for normally-distributed values and as medians (inter-quartile ranges) for values not normally distributed. Qualitative variables were expressed as percentages.Chi square test was used to quantify correlations between dichotomous variables. Outcome variables were dichotomised (e.g. all-TB vs. non-TB) to determine association with exposure variables (e.g. chest radiograph feature).Mann-Whitney U test and t-test were used to compare medians and means respectively.A two-tailed p-value equal or less than 0.05 was considered statistically significant.

RESULTS

Study process

We enrolled 113 patients.43 (38.1%) had sputum smears positive for AAFB, 53 (46.9%) had sputum-smears negative for AAFB and 17 (15.0%) were unable to expectorate sputum. 58 of 70 (82.9%) sputum AAFB-negative or sputum-scarce-patients underwent bronchoscopy, while 12 of those eligible for bronchoscopy did not have the procedure done for varying reasons; 6 were discharged by their attending physicians before procedure could be done, following clinical response to presumptive anti-tuberculosis treatment and 4 declined to have procedure done despite having earlier consented to it. Onepatient died before procedure could be done and another could not tolerate it following introduction of the bronchoscope into the upper airway [Figure 1]. Median time to bronchoscopy (from date of hospital admission) was 6 days (IQR 4-9)

Figure 1: Patient Enrolment



Baseline characteristics of study participants

54% of enrolled patients were female. The mean agewas 34.9 years (SD±8.9), with amedian CD4+ count and haemoglobin of 55cells/μL (IQR 21-75)and 7.8g/dL (IQR 6.1-9.8), respectively. Most patients presented with cough (86.7%) while the commonest clinical sign was chest crackles (65.5%). Median oxygen saturation was 92% (IQR 87.5-96.0). [Table1]

Of the 113 patients, 100 (97.3%) used empirical antibiotics prior to enrolment, 95 (84.1%) for less than a week prior to enrolment. The most-commonly-used antibiotics were the penicillins (57%). 20 (17.7%) patients were on co-trimoxazole prophylactic treatment and only three (15%) of these had taken it for more than a week at time of enrolment. 23 (20.4%) patients were on anti-tuberculosis treatment. Other antimicrobials used were macrolides (29.2%), cephalosporins (21.2%), high-dose co-trimoxazole (13%), fluconazole (9%) and fluoroquinolones (2%).

Table 1: Clinical and laboratory features

Feature	No. (n=113)	(%)
Patient demographics/Symptoms		
Female	61	54.0
Age in years, mean (SD)	34.9 (±8.9)	
Cough	98	86.7
Chest pain	19	16.8
Dyspnoea	20	17.7
Symptom duration ≥2 weeks	95	84.1
Use of antibiotics	100	97.3
PJP prophylaxis	20	17.7
Previous TB	25	22.1
Signs		
Crackles	74	65.5
Bronchial breath sounds	15	13.3
Normal auscultatory findings	4	3.5
Cyanosis	6	5.3
Respiratory Rate, median (IQR)	32 (24.0-40.0)	
SpO_2 in %, median (IQR)	92 (87.5-96.0)	
SBP in mmHg, mean (SD)	102.5 (±19.5)	
DBP in mmHg, mean (SD)	64.2 (±13.8)	
Laboratory tests		
Hb in g/dL, median (IQR)	7.8 (6.1-9.8)	
CD4+ count in units/μL(IQR)	55.0 (21.0-75.0)	

Aetiology of Pulmonary Disease

43 (38.1%) patients had sputum smears positive for AAFB on initial screen and thus not subjected to bronchoscopy. Seven (12.1%) of the BAL specimens were positive for TB on smear, while 14 (24.2%) had TB diagnosed on culture alone. Cumulatively, 64 (56.6%) patients were diagnosed with TB using this algorithm. Two (1.8%) patients had *Mycobacteria intracellulare* and one (0.9%) had *Mycobacterium avium*cultured on BAL. *Pneumocystis jirovecii* was found in five (4.4%) patients, *Candida* species in six (5.3%), *Klebsiella* in five (4.4%) and gram negative enteric bacteria in two (1.8%). One (0.9%) each of *Staphylococcus aureus*, *Streptococcus pneumonia* and *Proteus mirabilis* were cultured. Additionally, Kaposi's sarcoma was diagnosed in three (2.7%) patients on visual inspection at bronchoscopy. The cause of the pulmonary pathology was not determined in 34 (30.1%) patients.

Of the five patients with *Klebsiella*, two had co-morbidity with *Mycobacterium intracellulare*and two with *Mycobacterium tuberculosis*. The remaining one had no co-morbidity. Additionally, *Mycobacterium tuberculosis* had co-morbidities with *Candida* (2), *Pneumocystis jirovecii*(1), gram negative enteric bacteria (1), *Proteus mirabilis* (1), *Staphylococcus aureus* (1) and *Streptococcus pneumoniae* (1). [Table 2]

Table 2a: Causative Agents

Agent	Frequency (n=113)	(%)
Mycobacteria tuberculosis	64	56.6
*Candida*species	6	5.3
Pneumocystis jirovecii	5	4.4
Klebsiellapneumoniae	5	4.4
Kaposi's sarcoma	3	2.7
Mycobacteria intracellulare	2	1.8
Staphylococcus aureus	1	0.9
Mycobacteria avium	1	0.9
Gram negative enteric organisms	1	0.9
Streptococcus pneumoniae	1	0.9
Proteus mirabilis	1	0.9
Unidentified	34	30.1

Table 2b: Co-morbidities

Agents	Frequency
*Klebsiellapneumoniae*and *Mycobacteria intracellulare*	2
*Klebsiellapneumoniae*and *Mycobacteria tuberculosis*	2
*Mycobacteria tuberculosis*and *Candida* species	2
*Mycobacteria tuberculosis*and *Pneumocystis jirovecii*	1
*Mycobacteria tuberculosis*and Gram negative enteric organisms	1
Mycobacteria tuberculosis and *Proteus mirabilis*	1
Mycobacteria tuberculosis and *Staphylococcus aureus*	1
*Mycobacteria tuberculosis*and *Streptococcus pneumoniae*	1

Correlation of aetiological agent with radiological and clinical parameters

95 (84.1%) patients had symptoms lasting for two weeks or more prior to presentation to hospital. This finding did not statistically significantly associate with a particular primary outcome.

Four patients had normal chest radiographs. None of these had bacterial pneumonia or Kaposis sarcoma. Three of these had tuberculosis (χ^2 0.564; p=0.45). 16 (80%) of the patients with miliary picture on chest radiograph had tuberculosis (χ^2 5.353; p=0.02). A diagnosis of tuberculosis was also associated with both nodular (χ^2 7.8639; p=0.001)and micro-nodular (χ^2 4.557; p=0.03) infiltrates on chest radiograph, as well as bilateral hilar lymphadenopathy (χ^2 4.105; p=0.03). Consolidation was however not associated with TB (χ^2 4.105; p=0.44). No radiographic picture was statistically significantly associated with Pneumocystis pneumonia. None of the five patients with PJP had prior co-trimoxazole prophylactic treatment. However this association was not statistically significant (χ^2 1.115; p=0.29). A respiratory rate of 40/minute or more was associated with a diagnosis of PJP (χ^2 5.595; p=0.02) [Tables 3 and 4]

Table 3: Clinical and radiological correlates of tuberculosis

Variable	TB		No TB		Odds Ratio 95% CI	P
	n=64	%	n=49	%		
Female	37	57.8	24	49.0	1.43 (0.63-3.23)	0.36
Illness>2 weeks	55	85.9	40	81.6	1.38 (0.50-3.77)	0.54
RR≥40	11	17.2	28	57.1	0.16(0.07-0.37)	<0.01*
SpO_2<85%	4	6.3	12	24.5	0.21 (0.06-0.69)	0.01*
Hb<8g/dL	40	62.5	14	28.6	4.17 (1.87-9.27)	<0.01*
Miliary	16	25.0	4	8.2	3.75 (1.17-12.07)	0.02*
Micro-nodular	14	21.9	3	6.1	4.29 (1.16-15.91)	0.03*
Nodular	8	12.5	17	34.7	0.27 (0.10-0.69)	0.01*
BHL	8	12.5	1	2.0	7.85 (0.96-64.27)	0.03*
Normal CXR	3	4.7	1	2.0	2.56 (0.26-25.34)	0.45

Table 4: Clinical and radiological correlates of PJP

Characteristic	PJP		No PJP		Odds Ratio 95% CI	P
	n=5	%	n=108	%		
Female	3	60.0	58	53.7	1.29 (0.21-8.05)	0.78
Illness>2 weeks	4	80.0	91	84.3	0.74 (0.08-7.10)	0.80
Co-trimoxazole prophylaxis		0	5	4.6	0 (0.0-5.18)	0.37
RR≥40	4	80.0	35	32.4	8.34 (0.90-77.44)	0.03*
SpO_2<85%	2	40.0	14	13.0	4.48 (0.69-29.2)	0.09
Miliary	3	60.0	17	15.70	10.96 (1.67-71.89)	0.05
Micro-nodular	1	20.0	16	14.8	1.55 (0.16-14.83)	0.21
Nodular	1	20.0	24	22.2	0.88 (0.09-8.20)	0.69
BHL	0	0	9	8.3	Undefined	0.32
Normal CXR	0	0	4	3.7	Undefined	0.83

Causative agent correlates with CD4+ count

The median CD4+ count in patients whom the cause of pulmonary disease was determined was 54 (IQR 20-75) while in those whom the cause was undetermined was 55.5 (IQR 25-68). Median CD4+ count was 50(IQR 9-60), 55(IQR 40-68), 55(IQR 29-77) and 54(IQR 20-75) for bacterial, fungal, Pneumocystis and Tuberculous pneumoniae, respectively.

Significance of BAL

Among the patients who underwent bronchoscopy (i.e. with either negative sputum smears or unable to expectorate sputum), seven (12%) had tuberculosis diagnosed on direct examination of BAL specimens and 14 (24%) on BAL cultures alone**.** Using BAL culture as gold-standard BAL smear had diagnostic sensitivity of 33% and specificity of 100%.

DISCUSSION

Systemic use of our study algorithm (figure 1) led to an accurate diagnosis of aetiology in immunocompromised patients with pulmonary symptoms. We found a diagnostic yield of 69.9% which is higher than in studies done elsewhere, in which the yield was 51-60%.[21,26,27,28,29,30]

In this study, tuberculosis was the commonest cause of pulmonary disease, as was the case in Malawi, Tanzania

and Zimbabwe. However, the prevalence rate of tuberculosis was, at 55.8%, higher than figures in studies in the sub-Saharan region.[31,32,26] This highlights the fact that in the developing world it remains the major cause of morbidity in HIV-infected patients. This was also mirrored by Bates *et al* who found a tuberculosis prevalence of 22.4% in adults admitted to the University Teaching Hospital in Lusaka not suspected to have tuberculosis but able to produce sputum and presenting with different communicable and non-communicable diseases.[33]

Further, our study went on to document the local epidemiology of sputum AAFB smear-negative pneumonia. On bronchoscopy/BAL, 36.2% of this sub-group turned out to have tuberculosis and 8.6% *Pneumocystis* pneumonia. Relative to BAL culture, BAL smears had a specificity of 100% and sensitivity of 33%. This signifies the importance of bronchoscopy as a diagnostic tool for sputum-smear AAFB-negative pulmonary symptoms as shown in previous series.[30,34-38]

Overall, the prevalence *of Pneumocystis jirovecii* pneumonia (PJP) was 4.4%. This contrasts recent data which has been showing a rise in rates of PJP in the developing world, but is more consistent with studies done in the early days of AIDS, including two Zambian prospective studies which showed low prevalence.[39,40] This low prevalence of PJP could still be an underestimate since patients with hypoxia refractory to treatment, who potentially had PJP, were excluded from the study. The fact that none of the patients with PJP had been on co-trimoxazole prophylaxis was an important finding, but not statistically significant. This could be explained by the fact that these patients had only been diagnosed with HIV at the time of enrolment into the study and had been on prophylaxis for a period of less than one week. Only three (15%) of the 20 patients on chemoprophylaxis for PJP had taken it for more than a week.

Research has shown that the percentage of individuals with PJP co-infected with other organisms is higher in the developing world; rates of co-infection with other pathogens ranging from 20-70% have been reported. *Mycobacterium tuberculosis,* which is the commonest co-pathogen, is present in association with 13%–66% of cases of PJP.[41-44]Our study however revealed only one (20%) case of PJP with co-morbidity; with tuberculosis.

The finding of several other co-morbidities in our cohort raises the need for intensive screening for co-morbidity, particularly in patients not responding to empiric treatment. This was exemplified in one of the patients with *Mycobacterium intracellulare* who had been unsuccessfully presumptively treated as tuberculosis and *Pneumocystis* pneumonia leading to eventual death; BAL culture results were only ready after the patient's death. This justifies on-going research into faster diagnostic means for *Mycobacteria*such as the Xpert®. Our cohort had three (2.7%) patients with non-tuberculous mycobacteria. In developing countries, it is often presumed that most pulmonarysymptoms resembling mycobacterial disease are caused by *Mycobacteriumtuberculosis.* This largely arises from the lack of appropriate diagnostics as well as the endemic nature of *Mycobacterium tuberculosis* in these areas.[45,46] However, Maiga and colleagues found that 18% of clinically chronic TB cases could be attributable to non-tuberculous Mycobacteria (NTM).[47] This suggests the need to consider NTM in patients who fail first line and re-treatment regimens for TB.

Candida species found in six (10.3%) of BAL specimens could have been due to colonization of the tracheo-bronchial tree rather than infection. The currently accepted criteria for diagnosis of *Candida* pneumonia require a positive blood culture, a positive culture from a normally sterile site (other than urine, sinuses, or respiratory tract), or a histologically positive biopsy specimen.[48] Therefore, isolation of *Candida* from BAL samples alone does not indicate invasive infection. Nonetheless, various studies show an incidence of 0.23 to 8%, the highest being in immunocompromised patients.[49,]

The low incidence of bacterial pneumonia and particularly pneumococcal pneumonia in our study was at odds with findings in earlier African studies.[51,52] This could be due to the fact that 94% of patients were on presumptive anti-bacterial treatment; 57% on penicillins, 29% on macrolides and 21% on co-trimoxazole. The median time to bronchoscopy (from day of admission to hospital) of 6 days (IQR 4-9) was sufficient to allow sterilization of pulmonary bacterial pathogens.

30.1% of our patients had undetermined aetiology. This is

less than was found in similar studies done elsewhere.[26,32,43] The widespread use of antibiotics as well as the lack of capacity of our laboratory to identify atypical as well as viral organisms could partly explain this finding.

As expected, miliary as well as micro-nodular chest radiograph pictures were significantly associated with a diagnosis of tuberculosis, and so was BHL.[53, 54]No other radiographic picture was as predictive of a particular disease entity. Possibly due to the poor immune status of these patients, cavitations were not seen, as postulated by Lawn and colleagues.[54]36.2% of sputum smear-negative and non-expectorating patients turned out to have tuberculosis on analysis of BAL specimens. It is therefore worth initiating such severely immunosuppressed patients on anti-tuberculosis treatment on the basis of these predictive chest radiograph findings, in the absence of a microbiological diagnosis and bronchoscopy facilities.

Our study had several limitations. First was the inability to evaluate for the presence of 'atypical' organisms and/or respiratory viruses. Previous studies have shown that these have caused pneumonia in 17-19% of immunosuppressed patients.[20,28,29,55] The disease burden of these organisms in our population remains unknown and is therefore worth investigating. Then, the aversion for bronchoscopy despite giving informed consent deprived the study of useful data and also raises the need to further enlighten the general population on research and its importance. As earlier discussed, the exclusion of moribund patients potentially limited the frequency of PJP and other pulmonary opportunistic infections.

No severe adverse events were recorded from bronchoscopy in our study, underlining the safety of this procedure in the diagnosis of lung disease in this cohort; more so that it significantly added to diagnostic yield particularly in patients unable to expectorate sputum and those whose sputum smears were negative for AAFB. However the exclusion of moribund patients and those with poor oxygen saturations could partly explain the absence of severe adverse events.

In conclusion, *Mycobacterium tuberculosis* is the commonest cause of pulmonary disease in HIV-infected patients with low CD4 count, with the prevalence of tuberculosis exceeding 50% in this population. Although clinical and radiological characteristics are helpful in the presumptive diagnosis of tuberculosis, bronchoscopy can frequently identify a definitive aetiology in sputum smear negative patients.

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