

CHAPTER 1

1.0 INTRODUCTION

Sub-Saharan Africa carries a disproportionate 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/ μ L.⁴

Due to a lack of extensive diagnostic facilities, the specific causes of mortality remain unknown and are being investigated in studies such as the Centres for Diseases Control (CDC)-sponsored clinical trial; Causes of Early Mortality during Antiretroviral Therapy (CEMART). 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} They are a frequent reason for referral to respiratory specialists for diagnosis and treatment, and have varied causes including mycobacteria, bacteria, fungi, viruses and parasites.⁵

In the developed world, rates of pneumonia are much higher in the HIV-infected population⁸ and particularly so in those with a CD4+ count less than 200/ μ L,^{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 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).

CHAPTER 2

2.0 LITERATURE REVIEW

In spite of the reductions in overall morbidity and mortality due to HIV-related illnesses in the HAART era, pulmonary disease has continued to be a burden particularly in the developing world and among those patients with CD4+ count less than 200/ μ L.¹ Since the original description of AIDS, the respiratory tract has been the most commonly affected site of pathology.^{26,27} At necropsy, the lungs of 90% of HIV-infected patients are affected by AIDS-related pneumonia.²⁷ A 14-year study of the causes and patterns of mortality in HIV-infected, hospitalized patients in a tertiary hospital in India reported respiratory tract infections as the commonest causes of death ahead of meningitis.²⁸ The PULMONARY COMPLICATIONS OF HIV INFECTION study-cohort revealed that respiratory infections were more common at all CD4+cell strata in HIV seropositive patients than in HIV-uninfected controls.²⁹

Outside sub-Saharan Africa, PJP is a common infection among untreated HIV-infected patients.³⁰ It was the second most common opportunistic infection in Brazil³¹ and Kuala Lumpur.³² This pattern is similar in European and American cohorts.^{33,34,35} In Africa, reported rates of PJP have varied from 0-33%,³⁶⁻⁴⁷ in contrast to reported rates of tuberculosis, which have been more consistently higher.^{5,47}

Tuberculosis is seen as the major pulmonary infection in HIV-infected patients⁴⁸ and is often the first manifestation of HIV-infection.⁴⁹⁻⁵¹ It is also the most common cause of death in HIV-infected patients not on anti-retroviral treatment.⁵² In 2006, the World Health Organization's African Region accounted for 85% of HIV-associated tuberculosis.⁴⁸ In hospital-based series in South Africa and Zimbabwe, 40-65% of HIV-infected patients with respiratory disease had tuberculosis.^{53,54} The commonest causes of pneumonia in Zimbabwe, Kenya and Malawi in both in-and-out-patient series are *Mycobacterium tuberculosis* and *Streptococcus pneumoniae*.^{55,56,57} In Tanzania, an HIV-infected cohort at bronchoscopy showed a tuberculosis prevalence of 23.3%,⁵⁸ while prevalence of tuberculosis in Rwanda was 25% in patients with pulmonary disease of unknown aetiology, with PJP accounting for 5% of cause.⁴⁶

In Zambia, Malibata et al (unpublished University of Zambia MMED dissertation) found a tuberculosis prevalence of 26% (on sputum smear) in patients of unknown HIV-status presenting with cough of three weeks' duration or less. They also found chest radiograph features consistent with tuberculosis in 53% of those with microbiologically-proven disease. According to 2008 hospital records (unpublished data) from the Department of Internal Medicine of the University Teaching Hospital, a tertiary-level hospital in Zambia, pulmonary tuberculosis and pneumonia (of unspecified cause) rank second and fifth respectively in case-fatality rates, and second and fourth respectively among causes of death in HIV-infected patients. Pneumonia was also found to be the leading cause of unexpected deaths. However, most of these diagnoses were made on clinical and radiological basis, without microbiological evidence.

Since the spectrum of pulmonary manifestation in HIV-infected patients is broad, a definitive diagnosis is preferred over empiric treatment in the evaluation of the HIV-infected patient with diffuse pulmonary disease.^{59,60} Bronchoscopy with bronchoalveolar lavage has been established as a safe and reliable diagnostic tool. It has also been found to have a high diagnostic yield in immunocompromised patients with suspected pneumonia.⁶¹

In a retrospective evaluation of diagnostic effect and safety of fiberoptic bronchoscopy in 153 patients with late-stage HIV and clinical signs of pulmonary infection or abnormal chest radiograph, bronchoscopy led to diagnosis in 82.4% and changed therapy in 54%.⁶² Only 2% had transient hypoxaemia as a complication. Jimenez and colleagues in a fiberoptic bronchoscopy study of 151 HIV-infected patients with pneumonia demonstrated a high diagnostic rate and a low degree of contamination (10%).⁶² Bronchoscopy with bronchoalveolar lavage is also regarded as gold standard for PJP diagnosis in HIV-infected patients owing to its high specificity and sensitivity.^{64,65}

CHAPTER 3

3.1 STATEMENT OF THE PROBLEM

Many HIV-infected patients present to health care facilities with pulmonary disease. In Zambia most of these are treated empirically for bacterial pneumonia, based on algorithms adopted from the World Health Organization. However, pulmonary disease in HIV/AIDS often has non-bacterial causes. The causes are also indistinguishable by clinical or radiological means and these correlations have rarely been studied in third-world settings. In the absence of clinical algorithms based on accurate pathological or microbiological information, diagnoses are missed. Consequently there is a paucity of knowledge about local pulmonary disease patterns and increased mortality among Zambian patients.

3.2 STUDY JUSTIFICATION

Knowledge of the specific causes of pulmonary disease in severely immunosuppressed HIV-infected patients will allow revision of current treatment algorithms to include previously unconsidered but potentially important conditions. This knowledge will enable prioritization of primary prophylaxis for these patients against imminent infections and place into perspective the importance of specific causes of pulmonary morbidity. Depending on the diagnostic yields, this study would also make a case for the increased use of bronchoscopy in HIV-infected patients with pulmonary symptoms, particularly in the absence of sputum expectoration, in third world settings.

3.3 RESEARCH QUESTION

What is the commonest cause of pulmonary disease in HIV-infected patients with severe immunosuppression in Zambia?

3.4 HYPOTHESIS

Tuberculosis is the commonest cause of pulmonary disease in severely immunosuppressed HIV-infected patients presenting with pulmonary symptoms in Zambia.

3.5 OBJECTIVES

3.5.1 General

- To characterize the types of pulmonary disease in severely immunocompromised HIV-infected patients

3.5.2 Specific

- To establish the pathological and microbiological causes of pulmonary disease
- To correlate the pathogens identified with clinical and radiological presentations
- To assess the significance of BAL samples and bronchoscopy in the diagnosis of pulmonary disease

CHAPTER 4

4.0 METHODOLOGY

4.1 Design

This was a cross-sectional study that was conducted from 6th February, 2012 to 5th January, 2013.

4.2 Study Site

The University Teaching Hospital (UTH) is the largest referral hospital in Zambia. It has a total of 1600 in-patient beds and specialized out-patient clinics. Patients in the Department of Internal Medicine are admitted via the Adult Medical Emergency Unit (AMEU) into the Medical Admission Ward (MAW) for onward transfer to the main medical wards for definitive care. Patients are also admitted via the out-patient HIV-clinic at the Adult Infectious Diseases Clinic (AIDC). This clinic handles HIV-infected patients (both treatment-naive and treatment-experienced) referred from the various adult in-patient facilities as well as from outside the hospital.

Being the main points of admission of HIV-infected patients to the UTH, AMEU and AIDC were selected as sites for recruitment of patients for the study.

4.3 Study Population

Target population was HIV-infected patients with CD4+ count less than 200/ μ L presenting with cough, dyspnoea, haemoptysis or abnormal chest auscultatory findings.

4.4 Sampling

Systematic sampling of every second patient fulfilling inclusion criteria at presentation to the study sites was done.

4.5 Sample size

With an estimated proportion of tuberculosis of 25% as a cause of pulmonary disease in the sample population (from previous work),^{4,55,56,57} the sample size was calculated at 113 at a precision of 8% using the prevalence formula.

4.6 Inclusion criteria

- Patients 18 years old and above
- HIV positive with CD4+ count < 200 cells/μL (result obtained within 30 days of recruitment)
- Pulmonary symptoms (cough, dyspnoea or haemoptysis) or signs

4.7 Exclusion criteria

- Chronic kidney disease (CKD) stage IV or V^{97,98}
- Heart failure^{97,98}
- Moribund patients^{97,98}
- Oxygen saturation less than 90% while receiving 6L/minute of oxygen administered nasally^{91,92,97,98}

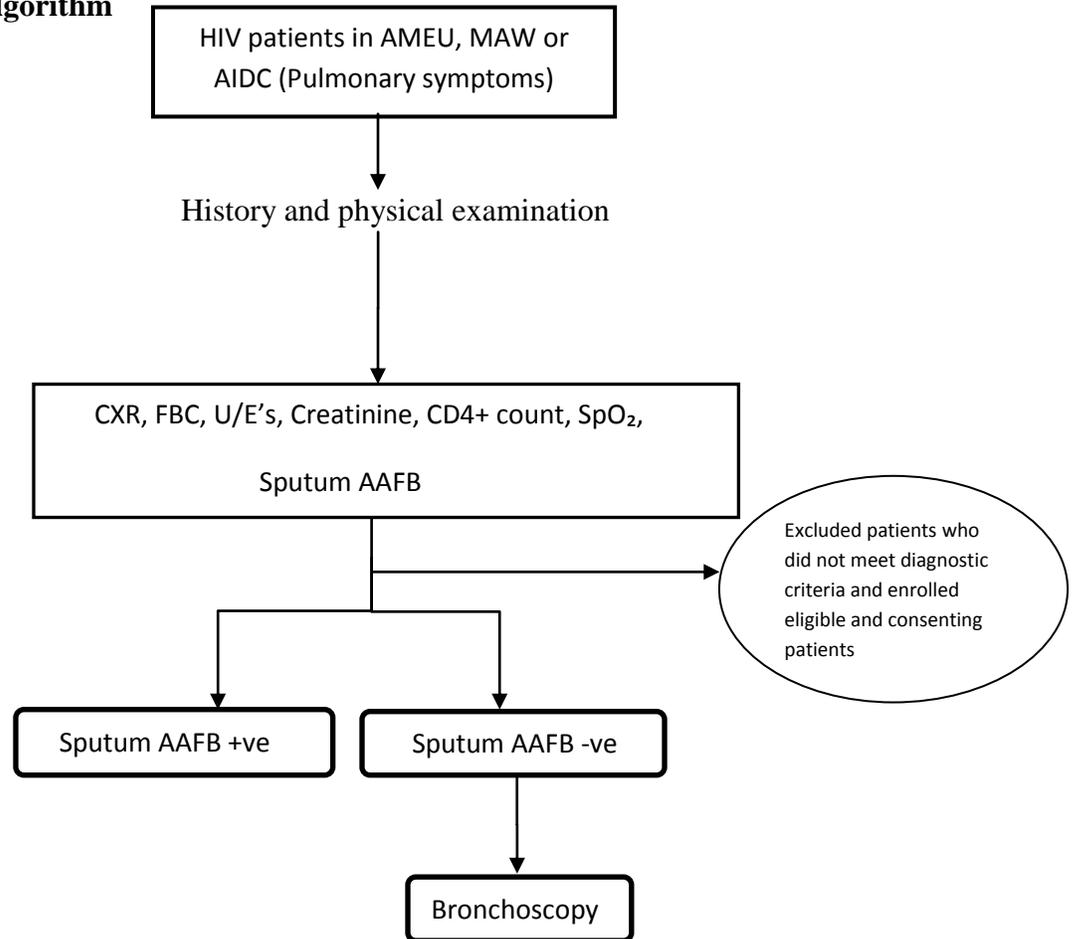
4.8 Patient recruitment

Patient-recruitment was done during working hours on a daily basis in the Adult Medical Emergency Unit, Medical Admission Ward and Adult Infectious Disease Clinic. Screening was done in line with the inclusion and exclusion criteria, before written consent was sought and enrolment determined.

A detailed history was taken by research staff at recruitment. Information gathered included patient demographics, presenting pulmonary symptoms and past medical and drug history.

Physical examination was done to elicit pulmonary signs and obtain vital signs such as blood pressure, oxygen saturation, respiratory and pulse rates, and body temperature.

Sputum was collected for Ziehl-Neelsen stain. Patients with sputum-smear results positive for AAFB had a diagnosis of TB assigned, while those with sputum smears negative for AAFB and those unable to expectorate sputum underwent bronchoscopy. [Figure 1]

Figure 1: Study Algorithm

4.9 Bronchoscopy and Broncho-alveolar lavage

We conducted bronchoscopy with broncho-alveolar lavage (BAL) at the UTH bronchoscopy suite in medical clinic 5 by standard procedure^{21,97} using a flexible fiberoptic bronchoscope, OlympusTM Evis Lucera-BF 260. Bronchoscopy was immediately preceded by inhalation of nebulised 1% lignocaine by the patient for local (throat) anaesthesia. Then as the bronchoscope was introduced into the airway, instillation of 2% lignocaine on the vocal cords, trachea and distally was done, up to a maximum of 20 mg. The bronchoscope was then briefly wedged in one of the heavily affected segmental bronchi as seen on chest radiograph. Where there was diffuse lung involvement, the bronchoscope was wedged in one of the middle lobe segmental bronchi. Then aliquots of 50mls sterile saline at body temperature were instilled and at least 40 mls sucked back into a sterile container, with care taken to avoid contamination by saliva.

Specimens were immediately sent to the laboratory for identification of causative organisms. No patient had suspicious bronchial lesions requiring biopsy, and no severe adverse events due to procedure occurred.

4.10 Laboratory

Laboratory processing of BAL specimens were done using standardised methods.⁹⁹

For identification of *M.tuberculosis*, BAL samples were digested and decontaminated with 4% NaOH, centrifuged and cultured using Lowenstein-Jensen medium.

Bacteria and fungi were identified by gram stain and cultured on blood, chocolate and MacConkey agar. No quantitative culture procedures were performed. We had no facilities to identify *Legionella pneumophila*, *Mycoplasma pneumoniae* or other atypical bacterial organisms.

For *Pneumocystis jirovecii*, toluidene blue and giemsa stains were used for identification of cysts and trophozoites, respectively.

Peripheral venous blood samples were drawn for full blood count, renal function tests, confirmatory HIV test and CD4 T cell count. Blood was tested for anti-HIV antibodies by two sequential rapid tests; determineTM and unigoldTM. CD4 T-cell counting was done by flow-cytometry technique (Becton Dickinson Facs Count machine with BD Facscount re-agentTM)

4.11 Diagnostic criteria:

The following criteria were used to determine the cause of pulmonary disease:^{60,99}

4.11.1 Pathological and microbiological:

- Bacterial pneumonia: positive culture of known pyogenic bacteria
- Pulmonary mycobacterial infection: identification of AAFB on direct smear examination. Mycobacterial speciation was done by PCR using cultures grown on solid media.
- Fungal pneumonia: positive direct examination of fungal elements on gram stain (e.g., fungal hyphae) or positive culture of pathogenic fungi. BAL fluid was collected in a shielded manner making collection of organisms from the upper tract less likely.
- Pneumocystis pneumonia: presence of *P. jirovecii* cysts and/or trophozoites in BAL fluid on toluidene blue and giemsa stains.
- Bronchopulmonary Kaposi's sarcoma: typical macroscopic lesions on bronchoscopy

4.11.2: Chest radiographs:

Chest radiographs were read by two experienced radiologists who had clinical details of patients withheld apart from the fact that patients had respiratory symptoms and/or signs and were HIV-infected. Reporting criterion used was as below:

- **Normal Chest x-ray:** Absence of infiltrates in lung fields
- **Focal infiltrates:** if involving 1 lung lobe or less

- **Diffuse infiltrates:** involving more than 1 lobe (either unilateral or bilateral)
- **Lung nodule:** less than 3cm in diameter (miliary <3mm; micro-nodules 3-6mm; macro-nodules 6mm-3cm)
- **Lung mass:** more than 3cm in diameter

4.12 Outcomes

4.12.1 Primary outcome

Microbiological/pathological aetiologies of pulmonary morbidity

4.12.2 Secondary outcomes

Clinical and radiological features of pulmonary disease

Bronchoscopy diagnostic yield

4.13 Data Analysis

4.13.1 Independent variables

These included age, sex, respiratory rate, oxygen saturation, duration of symptoms, CD4+ count

The independent variables were further categorized as follows;

Sex: Male or Female

Respiratory rate: <40/min or \geq 40/min

Oxygen saturation: <85% and \geq 85% (on room air)

Type of symptoms: Cough, breathlessness, chest pain, 'other'

Duration of symptoms: <2 weeks or \geq 2 weeks⁹³

Duration on antibiotics: < 1 week or \geq 1 week

4.13.2 Dependent variables

Microbiological or pathological findings on bronchoscopy

Clinical/radiological features

4.13.3 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.

4.13.4 Descriptive statistics

Quantitative variables were expressed as means (\pm Standard deviation) for normally-distributed values and as medians (inter-quartile ranges) for values not normally distributed. Qualitative variables were expressed as percentages.

4.13.5 Analytical statistics

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

4.14 Ethical Considerations

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. The purpose of the study was explained to them and they were informed of their right to opt-out without compromise to their medical care. None of the participants received remuneration. No related serious adverse events were recorded during the study.

Information obtained has been kept under lock and key in the Bronchoscopy suite of Clinic 5 of the UTH. Results of investigations have been availed to patients' attending physicians for the purpose of clinical management. Otherwise, access to this information has been restricted to the Principal Investigator and the Study Supervisors. Patient identity numbers and not names were used for confidentiality.

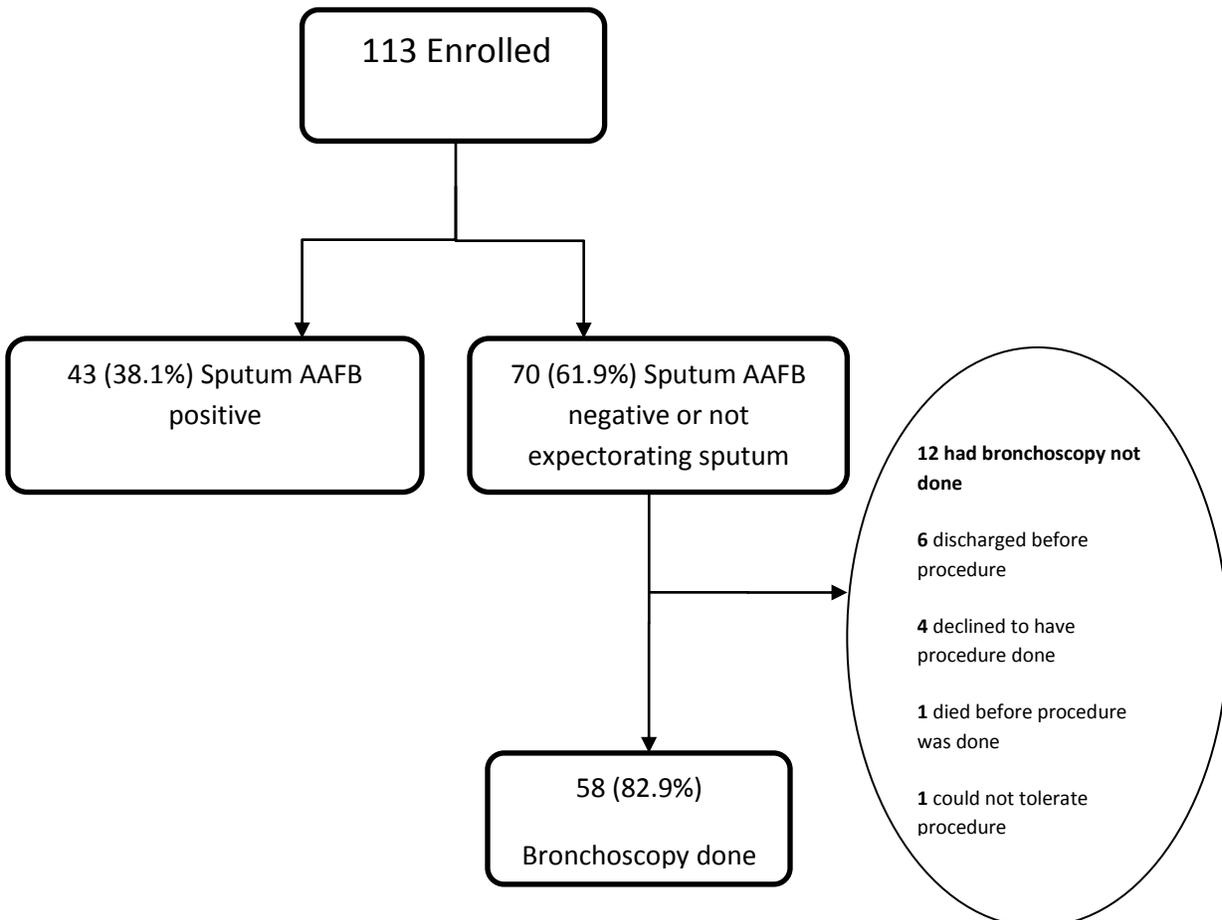
CHAPTER 5

5.0 RESULTS

5.1: Study process

Between February, 2012 and January, 2013, 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. This was due to apprehension about the procedure among the patients and their family members, despite reassurances about its importance and safety. 1 patient died before procedure could be done and another could not tolerate it following introduction of the bronchoscope into the upper airway [Figure 2]. Median time to bronchoscopy (from admission to hospital) was 6 days (IQR 4-9)

Figure 2: Patient Enrolment



5.2 Baseline characteristics of study participants

A total of 113 patients were enrolled. 54% were female. The mean age of study participants was 34.9 years ($SD\pm 8.9$), with a median CD4+ count and haemoglobin of 55cells/ μ L (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). [Table 1]

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 (\pm 8.9)	
Cough	98	86.7
Chest pain	19	16.8
Dyspnoea	20	17.7
Symptom duration \geq 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 (\pm 19.5)	
DBP in mmHg, mean (SD)	64.2 (\pm 13.8)	
Laboratory tests		
Hb in g/dL, median (IQR)	7.8 (6.1-9.8)	
CD4+ count in units/ μ L(IQR)	55.0 (21.0-75.0)	

5.3 Aetiology of Pulmonary Disease

Of 113 enrolled patients, 43 (38.1%) had sputum smears positive for AAFB on initial screen. 53 (46.9%) had sputum smears negative for AAFB and 17 (15.0%) were unable to expectorate sputum; of these, 58 (82.9%) agreed to further screening with 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 pneumoniae* 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)	(%)
<i>Mycobacteria tuberculosis</i>	64	56.6
<i>Candida</i> species	6	5.3
<i>Pneumocystis jirovecii</i>	5	4.4
<i>Klebsiella pneumoniae</i>	5	4.4
Kaposi's sarcoma	3	2.7
<i>Mycobacteria intracellulare</i>	2	1.8
<i>Staphylococcus aureus</i>	1	0.9
<i>Mycobacteria avium</i>	1	0.9
Gram negative enteric organisms	1	0.9
<i>Streptococcus pneumoniae</i>	1	0.9
<i>Proteus mirabilis</i>	1	0.9
Unidentified	34	30.1

*Percentages add up to greater than 100% due to co-morbidities

Table 2b: Co-morbidities

Agents	Frequency
<i>Klebsiella pneumoniae</i> and <i>Mycobacteria intracellulare</i>	2
<i>Klebsiella pneumoniae</i> and <i>Mycobacteria tuberculosis</i>	2
<i>Mycobacteria tuberculosis</i> and <i>Candida</i> species	2
<i>Mycobacteria tuberculosis</i> and <i>Pneumocystis jirovecii</i>	1
<i>Mycobacteria tuberculosis</i> and Gram negative enteric organisms	1
<i>Mycobacteria tuberculosis</i> and <i>Proteus mirabilis</i>	1
<i>Mycobacteria tuberculosis</i> and <i>Staphylococcus aureus</i>	1
<i>Mycobacteria tuberculosis</i> and <i>Streptococcus pneumoniae</i>	1

5.4 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 Kaposi 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 \geq 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

*Statistically significant

BHL=Bilateral Hilar Lymphadenopathy

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 \geq 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	9	8.3	Undefined	0.32
Normal CXR		0	4	3.7	Undefined	0.83

*Statistically significant

BHL=Bilateral Hilar Lymphadenopathy

5.5 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.

5.6 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%.

CHAPTER 6

6.0 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%.^{21,57,68,69,70,71}

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.^{44,55,57} 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.⁹⁰

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.⁷¹⁻⁷⁶

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.^{39,80} 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.⁷⁷⁻⁸¹ 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 ongoing 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 pulmonary symptoms resembling mycobacterial disease are caused by *Mycobacterium tuberculosis*. This largely arises from the lack of appropriate diagnostics as well as the endemic nature of *Mycobacterium tuberculosis* in these areas.^{85,86} However, Maiga and colleagues found that 18% of clinically chronic TB cases could be attributable to non-tuberculous Mycobacteria (NTM).⁸⁸ 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 trachea-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.⁹⁴ 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.^{95,96}

The low incidence of bacterial pneumonia and particularly pneumococcal pneumonia in our study was at odds with findings in earlier African studies.^{82,83} 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.^{55,57,79} The widespread use of antibiotics as well as the incapacity of our laboratory to identify atypical as well as viral organisms could partly explain this.

As expected, miliary as well as micro-nodular chest radiograph pictures were significantly associated with a diagnosis of tuberculosis, and so was BHL.^{46,89} 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.⁸⁹ 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.^{20,69,70,84} 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.

CHAPTER 7

7.1 CONCLUSION

Mycobacterium tuberculosis is the commonest cause of pulmonary disease in our study population and its prevalence higher than in any other similar series. Clinical and radiological correlates of TB can be used in the diagnosis of and subsequent treatment of AAFB smear negative pneumonia, in the absence of bronchoscopy, which has 100% specificity in the diagnosis of AAFB smear-negative pneumonia.

7.2 RECOMMENDATIONS

From our findings the following recommendations are to be made;

1. To enhance the active screening for TB in immunosuppressed patients presenting to health facilities with pulmonary symptoms.
2. To increase and roll out the use of bronchoscopy in diagnosis of AAFB negative sputum smears beyond the University Teaching Hospital
3. To conduct research using less-invasive diagnostic means such as induced sputum for severely hypoxic patients with AAFB smear negative pneumonia in our setting.
4. To increase the use of Mycobacterial cultures for poorly responding pneumonias and invest further in research into faster *Mycobacterial* diagnostic techniques such as Xpert®
5. To isolate potential TB patients admitted to the medical wards to prevent transmission to other in-patients most of whom are immunosuppressed, as well as invest in ultra-violet light equipment

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APPENDIX I**DATA COLLECTION SHEET : Aetiology and presentation of pulmonary disease in HIV-infected patients at risk of early mortality at the University Teaching Hospital in Lusaka, Zambia**

Instruction : Kindly check or tick box where applicable

Hospital File No. _____

PTID/Study No. _____

Township : _____

Phone No : _____

Date of Admission: ____/____/____

HISTORY

1. AGE: _____ years

2. SEX: Male Female

3. Presenting symptom: _____

4. Duration of symptoms:

<2 weeks ≥2 weeks

5. Previous TB history: Sputum CXR None

6. Currently on antibiotics :

Yes No (if no, skip questions 7 and 8)

7. Type of antibiotics :

Penicillin Cephalosporin
 Macrolide ATT
 PJP Prophylaxis Other: _____

8. Duration on antibiotic:

< 1 week ≥ 1 week

9. Comorbidities:

Diabetes Hypertension Other, specify: _____

PHYSICAL EXAMINATION

- 10. Weight: _____ Kg
- 11. Height: _____ m
- 12. Blood Pressure: _____ mmHg
- 13. Axillary temperature _____ °C
- 14. Respiratory Rate: _____ breaths/min
- 15. Cyanosis: Yes No
- 16. Auscultatory findings: Crackles Bronchial breathin Reduced air entry
 Other, specify _____
- 17. SpO₂: Room air Supplemental O₂

INVESTIGATIONS

- 18. Haemoglobin: _____ g/dL
- 19. Serum Urea: _____ mmol/L
- 20. Serum creatinine: _____ µmol/L
- 21. CD4+ count _____ cells/µL

CHEST RADIOGRAPH findings:

- 22. Normal: YES
 NO If no; Lesion description: _____
Distribution: foca diffu

BRONCHOSCOPY findings:

- 23. Inspection: _____
- 24. Microbiology: Microscopy _____ Culture _____
Sensitivity _____

APPENDIX II:**CONSENT FORM: Aetiology and presentation of pulmonary disease in HIV-infected patients at risk of early mortality at the University Teaching Hospital in Lusaka, Zambia****Introduction**

I, Kondwelani John Mateyo, an MMED student in the School of Medicine at the University Of Zambia, kindly request your participation in the above mentioned study. This study is in partial fulfilment for the award of a Master of Medicine in Internal Medicine. Before you make up your mind whether to take part in the study or not, I would like to explain to you the purpose of the study and what is expected of you. If you agree to take part in this study, you will be asked to sign this consent form in the presence of a witness.

Nature and purpose of the study

This study is being conducted to determine the causes and patterns of diseases of the lung in patients with advanced HIV who are admitted to the University Teaching Hospital.

Procedure of the study

If you agree to participate in this study, we will obtain information using a data entry sheet. Your contact details will be required. You shall be asked to submit sputum to the laboratory to test for tuberculosis. In the event that you are unable to produce sputum and/or tuberculosis is not found in your sputum, bronchoscopy will be done as part of the process of trying to find out the cause of your illness. Bronchoscopy is a procedure that involves placing a tube through your mouth into the lungs (after your throat is numbed with some medicine) to see the appearance of the lungs and also to collect lung samples for testing in the laboratory. Samples of blood will also be taken from you for laboratory testing. The other test to be done is a chest x-ray. The results of the tests will be communicated to you (if you so wish) and your attending physicians, so that they can treat your illness better.

Possible risks and discomforts

You will not be exposed to any risks by enrolling into the study. However, you will experience some discomfort, in addition to a possible temporary feeling of breathlessness and fever from the bronchoscopy. Further discomfort and some pain may be experienced from routine collection of blood samples and any other procedures during the course of treatment as part of routine hospital care.

Possible benefits

The information obtained in this study will help in the management of yours and other patients' lung disease.

Confidentiality

All the information collected is strictly confidential. Data that will be collected, analysed, and reported on will not include your name and therefore cannot be traced to you.

Consent

Your participation is strictly voluntary. You will not suffer any consequences if you decide not to participate in this study. You may also withdraw from the study at any time for any reason without consequences to you.

Thank you for considering participation in this study. If you have any questions, concerns and clarifications, please contact Dr Kondwelani John Mateyo or The University of Zambia Research Ethics committee on the following addresses;

Dr Kondwelani John Mateyo

Department of Internal Medicine

University Teaching Hospital

P.O Box 51292,

Lusaka.

Phone Number: 0966-611097

The University of Zambia Research Ethics Committee,

School of Medicine

Ridgeway campus

Nationalist Road

Lusaka.

Consent Form

I, _____ hereby confirm that the nature of this clinical study has been sufficiently explained to me. I am aware that my personal details will be kept confidential and I understand that I may voluntarily, at any point, withdraw my participation without suffering any consequences. I have been given sufficient time to ask questions and seek clarifications, and of my own free will declare my participation in this research.

I have received a signed copy of this agreement

_____	_____	_____
Name of Participant (Print)	Participant (Signature or thumbprint)	Date

_____	_____	_____
Witness (Print Name)	Witness (Signature)	Date