AETIOLOGY AND PRESENTATION OF HIV/AIDS-ASSOCIATED PULMONARY INFECTIONS IN PATIENTS PRESENTING FOR BRONCHOSCOPY AT A REFERRAL HOSPITAL IN NORTHERN TANZANIA
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ABSTRACT

Objectives: To determine the aetiological agents of pulmonary infections in HIV-infected Tanzanians and to correlate the causative agents with clinical, radiographic features, and mortality.

Design: A prospective study.

Setting: Kilimanjaro Christian Medical Centre (KCMC), Tanzania.

Subjects: Bronchoalveolar lavage fluid (BAL) were obtained from 120 HIV infected patients with pulmonary infections. BAL for causative agents was analysed and correlated with clinical and radiographic features, and one-month outcome.

Results: Causative agents were identified in 71 (59.2%) patients and in 16 of these patients, multiple agents were found. Common bacteria were identified in 35 (29.2%) patients , *Mycobacterium tuberculosis* in 28 (23.3%), *Human Herpes Virus 8* (HHV8) in 12 (10%), *Pneumocystis jiroveci* in nine (7.5%) and fungi in five (4.2%) patients. Median CD4 T cell count of the patients with identified causes was 47 cells/μl (IQR 14-91) and in the 49 patients with undetermined aetiology was 100 cells/μl (IQR 36-188; p=0.01). Micronodular chest radiographic lesions were associated with presence of *M. tuberculosis* (p=0.002). The one-month mortality was 20 (16.7%). The highest mortality was associated with HHV8 (41.7%) and *M. tuberculosis* (32.1%). Mortality in patients with undetermined aetiology was 11.3%. No death occurred in patients with PCP.

Conclusion: **In this population of severely immunosuppressed HIV-infected patients with pulmonary infection a variety of causative agents was identified. Micronodular radiographic lesions were indicative of TB. High mortality was associated with *M. tuberculosis* or HHV8. No death occurred in patients with *P. jiroveci* infection.**

INTRODUCTION

Sub-Saharan Africa is the region of the world most affected by HIV/AIDS, more than 70% of HIV infected individuals live in this African sub-continent, (1) and the HIV pandemic is still increasing. Despite this, data pertaining to the disease presentation from this region compared to the developed world are insufficient and require regular updating. The clinical presentation of HIV/AIDS and the occurrence of opportunistic infections depend on different factors such as the presence of endemic diseases, quality of health services, availability of and access to anti-retroviral treatment, and levels of education of the population. Pulmonary infections are the leading causes of morbidity and mortality in HIV-infected individuals (2,3). A microbiological diagnosis is of crucial importance in HIV infected patients with pulmonary infections because of atypical clinical and radiographic manifestations and high mortality (4). Because in these settings resources are limited, treatment is largely empirical, but to be effective, it should be tailored to locally prevailing causative organisms. Therefore health facilities equipped with advanced diagnostic techniques should monitor the trend of diseases and obtain data on aetiological pathogens, so that empirical treatment regimens can be improved.

The aim of this study was to find the aetiological pathogens of pulmonary infections in HIV/AIDS patients who presented with features of pulmonary infection for bronchoscopy.

Furthermore, we correlated the pathogens identified with clinical and radiographic presentations, level of immunity, and outcome after one month.

MATERIALS AND METHODS

At Kilimanjaro Christian Medical Center (KCMC) in northern Tanzania we enrolled 120 HIV infected patients aged 18 years and above, who presented with features of chest infection such as cough (dry or productive) and/or chest pain, dyspnoea, fever and chest radiographic abnormalities. These patients were recruited at the endoscopy unit where bronchoscopic procedures are done. The patients were referred by their attending clinicians for bronchoscopy as part of patient's management after failing to establish the causative agent by other methods and/or following failure to respond to empirical treatment. Pregnant women and patients with oxygen saturation less than 90% under 61/mm of oxygen were excluded from the study.

Bronchoscopy and bronchoalveolar lavage (BAL) was performed by standard procedure using flexible fiberoptic bronchoscope. Briefly, the bronchoscope was wedged in one of the heavily involved segmental bronchi as seen on the chest radiograph. In case of diffuse lung involvement, the scope was wedged in one of the segmental bronchi of the right middle lobe. Then aliquots of 50 ml or less of sterile saline at body temperature, up to a maximum of 150 ml, were instilled and at least 40 ml was sucked back into a sterile container. The sediments of BAL fluid obtained by centrifugation at 1500 g for 10 minutes were used for laboratory tests to identify the aetiological agent of the chest infection.

For identification of *M. tuberculosis*, BAL samples were decontaminated with 4% NaOH, centrifuged and cultured using in-house made Lowenstein-Jensen (LJ) solid medium, with a maximum of eight weeks incubation. Diagnosis of *M. tuberculosis* was based on positive culture results showing acid fast bacilli (AFB) in ZN stain.

Diagnosis of pneumocystis pneumonia (PCP) was based on identification of *Pneumocystis (P) jiroveci* with at least one of the following techniques: Giemsa stain, Gomori methenamine silver (GMS) stain or immunofluorescence test (IFT). Also real time PCR was performed for detection of *P. jiroveci* DNA in the BAL fluid, using 40 as the cycle threshold (C_T) cut off value (5).

Conventional PCR was used for the diagnosis of *Human herpes virus 8* (HHV8) as described by Chang and collaborators (6).

Fungi were diagnosed by direct smear and culture for seven days using Sabouraud dextrose

agar and for 21 days in Sabouraud dextrose liquid medium (Oxoid, Basingstoke, Hampshire, England). Cryptococci were identified microscopically in Giemsa stained slides and confirmed by Mucicarmine stain. Murex cryptococcal antigen test in serum was done as described by Jaye *et al* (7).

The presence of common bacteria was determined by routine culture techniques. Clean isolates of bacteria on blood and chocolate agar plates were recorded. No quantitative culture procedures were performed. We had no facilities to identify presence of infections with *Legionella pneumophila*.

Antibacterial susceptibility testing could not be done.

Peripheral blood samples were taken for total and differential white blood cell count (WBC), haemoglobin, HIV test and CD4 T cell count. Blood was tested for anti-HIV antibodies by both Capillus™ HIV-1/HIV-2 rapid test (Trinity Biotech, Bray, Ireland), and Vironostika ® HIV uniform II ag/ab microwell enzyme immunoassay (bioMerieux, Marcy I 'Etoile, France). CD4 T cell counting was done by flow-cytometry technique (Becton Dickinson Facs Count machine with BD Facscount™ reagent).

Chest radiographs were read by an experienced radiologist, who was informed that the patient had symptoms and signs of chest infection and were HIV infected, but who was not given other clinical information. Reading was done in all cases in a systematic way, describing for each chest film presence or absence of the following characteristics: nodules (miliary: <3 mm; micronodules: 3-6 mm; macronodules: 6 mm – 3 cm); infiltrates (alveolar, interstitial or mixed), cavities and their diameter, and other abnormalities (pleural effusion or thickening, masses (>3 cm), hilar and/or mediastinal adenopathy).

Data on outcome of the patients were obtained at the time of discharge and for outpatients after four weeks of follow up. All patients were given appropriate treatment based on the diagnosis reached by the attending physicians. The study in no way interfered with the management of patients. Results obtained from this study during the course of patient's management were used in the management as needed.

Data analysis was done by SPSS version 12-software for Windows. Normally distributed values were presented as mean with standard deviation

(SD). In other cases data were expressed as median with interquartile range (IQR). Chi-square test was used to quantify correlations between dichotomous variables. Mann-Whitney U test and t-test were used to compare medians and means, respectively. P-value equal or less than 0.05 was regarded as statistically significant.

Clearance for the study was given by the Institutional (KCMC) and National (Tanzania) Ethical Review Boards and informed written consent was obtained from each patient or a close relative.

RESULTS

General characteristics of the study population: A total of 120 patients were enrolled. The mean age of the study population was 39 years (SD ± 10), 54% were male. Median CD4 T cell count was 65 cells/µl (IQR 20–147). As shown in Table 1, most patients presented with cough, chest pain and difficulty in breathing. Of the 120 patients, 19 (15.8%) presented with signs and symptoms directly related to respiratory disease only, while the remaining 101 (84.2%) had co-morbidities as well, such as oral candidiasis, different dermatological lesions, lymphadenopathy, wasting or diarrhoea, Table 1. Of the 120 patients, 100 (83.3%) used empirical antimicrobial treatment prior to or at the time of enrolment in the study and 44 (36.7%) were on multiple antimicrobial agents. The most commonly used antimicrobial agents were trimethoprin-sulfamethoxazole (TMP-SMX), penicillins and fluconazole, used by 59 (49.2%), 52 (43.3%) and 38 (31.7%) patients, respectively. Some other antimicrobial agents used were: anti-mycobacterial agents 15 (12.5%) patients, and metronidazole, cephalosporins or chloramphenicol in 3-5 patients each.

Aetiology of pulmonary infection: In 71 (59.2%) of the 120 patients causative agents were identified. In the remaining 49 (40.8%) patients no organisms were found. In the 71 patients, 89 causative pathogens were isolated, in 14 two agents and in two three agents. In 35 (29.2%) patients common bacteria were isolated (*Streptococcus pneumoniae* in 14, *S. pyogenesinone*, *Staphylococcus aureus* in seven, coliform bacteria in five, klebsiella species in five and *Pseudomonas aeruginosa* in three), in 28 (23.3%) *M. tuberculosis,* in 12 (10%) HHV8, in nine (7.5%) *P. jiroveci* (identified microscopically) and

in five (4.2%) fungi, (Table 2). The fungi identified were *Cryptococcus neoformans* in three patients and *Aspergillus fumigatus* in two. The cryptococcal cases were identified by a positive cryptococcal antigen test in serum (which was negative in the remaining 117 patients) and in one of the three also cryptococci were seen microscopically in the BAL fluid. In a few patients yeasts (*candida*, other) were seen in low concentrations, but these were regarded as contaminants from the oropharynx.

Real time PCR for *P. jiroveci* was positive in 17 patients, including the nine cases in which *P. jiroveci* was seen microscopically. Of the eight cases positive by real time PCR only, in four no other organisms were isolated and two of the eight were on treatment with TMP-SMX for clinical suspicion of PCP. The mean (SD) CT value for *P. jiroveci* detected by both real time PCR and microscopic examination of stained BAL fluid was 27 (±4) and the mean CT value for *P. jiroveci* detected by real time PCR only was 35 (±4), t-test = -20.4, p = 0.003. Only the nine (7.5%) cases diagnosed microscopically as well were regarded as indicating PCP, thus explaining the pulmonary disease of these patients.

Among the 28 patients in whom *M. tuberculosis* was isolated, in 21 this was the only pathogen, while seven cases had co-infection with common bacteria or (in one case) HHV8. In seven cases *P. jiroveci* was the only pathogen isolated, while two patients had both *P. jiroveci* and coliform bacteria in BAL. Of the 35 patients in whom common bacterial pathogens were isolated, 18 had a single bacterial pathogen, the remaining had multiple types of infections. No cases of *M. tuberculosis* and *P. jiroveci* co-infection were found.

Table 1

Clinical and laboratory features of the study population (n = 120)

Feature	No.	(%)
Symptom		
Cough	118	98.3
Chest pain	99	82.5
Dyspnoea	65	54.2
Wasting	37	30.8
Diarrhoea	15	12.5
Use of antibiotics prior to admission	100	83.3
Signs		
Oral candidiasis	50	41.7
Fever	41	34.2
Skin lesions*	38	31.7
Lymphadenopathy	19	15.8
Kaposi's sarcoma lesion	17	14.2
Herpes zoster lesion	16	13.3
Herpes simplex	3	2.5
Respiratory rate, median (SD)	27	9
Laboratory tests		
Oxygen saturation in %, median (SD)	92	8
CD4 T cell count (cells/μl), median (IQR)	65.0	(20–147)
Haemoglobin (g/l), median (IQR)	90.0	(77–115)
WBC count (x 10^9/l), median (IQR)	5.5	(3.7–8.4)
Neutrophil count (x 10^9/l), median (IQR)	2.5	(1.6–3.0)
Lymphocyte count (x 10^9/l), median (IQR)	1.6	(1.1–3.0)
ESR (mm/hr), median (IQR)	70	(117–136)

* Other than Kaposi's sarcoma and herpes zoster lesions

Correlation of aetiological agents with radiological and clinical parameters: Ten patients (8.3%) had normal chest radiographs. In six of these 10 patients microorganisms were isolated in BAL fluid; one *M. tuberculosis*, four common bacteria, and one *Aspergillus fumigatus* infection. The bacteria isolated included *Staphylococcus aureus, Streptococcus pneumoniae, klebsiella* and coliform bacteria, either single or in combination. In the remaining four patients with normal chest radiograph no organisms were isolated.

In 23 of the 28 TB patients chest radiograph showed nodular lesions (Table 3). Association between microbiological diagnosis of TB with the radiographic presence of nodules was statistically significant, $X^2 = 7.3$, $p = 0.008$. The diagnosis of TB was further statistically significantly associated with presence of the nodular subgroup of micronodules: 21 patients with TB had micronodules on the chest radiographs, $X^2 = 9.9$, $p = 0.002$. No significant association was found between TB and other types of radiographic features or between radiographic features and other identified pathogens.

Table 2

Causative agents in 120 HIV-infected patients with pulmonary infection*

Agent	Frequency	(%)
M. tuberculosis	28	23.3
S. pneumoniae /S. pyogenes**	15	12.5
Human herpes virus 8	12	10.0
Pneumocystis jiroveci	9	7.5
Staphylococcus aureus	7	5.8
Coliform bacteria	5	4.2
Klebsiella species	5	4.2
Cryptococcus neoformans	3	2.5
Pseudomonas aeruginosa	3	2.5
Aspergillus fumigatus	2	1.7
No organism isolated	49	40.8

* More than one causative agent in 16 patients, 14 patients with two causative agents, and two patients with three. ** *Streptococcus pneumoniae* 14; *Streptoccus pyogenes* (1).

Table 3

Radiographic features of different causative pathogens

Pathogen	Miliar Y		Nodule S		Infiltrate S		Cavitie S		Hilar nodes		Pleural Effusion		Normal	
MTB (n = 28)	1	3.6	23	82.1	13	46.4	7	25	6	21.4	6	21.4	1	3.6
Bacteria (n = 35)	2	5.7	15	44.1	14	40.0	3	8.8	2	5.9	4	11.7	4	11.7
HHV8 (n = 12)	1	8.3	5	41.7	7	58.3	1	8.3	2	16.8	5	41.7	0	0
P. jiroveci (n=9)	0	0	5	55.6	5	55.6	0	0	0	0	1	11.1	0	0
Fungi (n = 5)	1	20	2	40	3	60	0	0	1	20	1	20	1	20
No M.O* (n = 49)	2	5.7	30	61.2	30	61.2	13	26.5	5	10.2	10	20.4	4	2.0

* No M.O: no microorganism isolated

MTB: *Mycobacterium tuberculosis*

HHV8: *Human herpes virus 8*

Of 28 TB patients, 23 had low haemoglobin, $X^2 = 9.0$, p = 0.005. PCP was associated with dyspnoea; all nine patients with PCP had dyspnoea, $X^2 = 8.1$, p = 0.004. Seventeen patients (14.2%) had clinical Kaposi's sarcoma (KS) lesions (Table 1); ten on the skin, five on the palate, and two in the airway. Three patients had both detectable KS lesion and HHV8 DNA in the BAL fluid, two patients with lesions in the airway and the other one on the skin. No statistically significant association was found between presence of HHV8 in the BAL fluid and presence of detectable KS lesion.

Correlation between causative agents of the chest infection and level of immunity: The overall median CD4 T cell count for all patients in whom causative pathogens were found was 47 cells/ml (IQR; 14–91) while in patients in whom no organisms were found was 100 cell/ml (IQR; 36–188), (Mann-Whitney U-test, p = 0.01). Patients with *M. tuberculosis* had median CD4 T cell count of 54 cells/μl (IQR, 29–128), patients with HHV8 47 (IQR, 9–185), patients with common bacterial pathogens 44 (IQR, 10-136), with *P. jiroveci* 26 (IQR, 7–91) and patients with fungi 14 cells/μl (IQR, 1– 69). No statistically significant difference in CD4 T cell count was found between these groups.

Outcome: Twenty out of the 120 patients (16.7%) died during the one month follow up period. Nine of the 28 patients (32.1%) with TB died, in six of the nine patients *M. tuberculosis* was the only pathogen isolated, while of the other three, two also had co-infection with HHV8 and one with *Staphylococcus aureus.* Five of the 12 patients (41.7%) with HHV8 died; in two of the five patients HHV8 was the only pathogen isolated, while of the remaining three, two had coinfection with TB and one with *Streptococcus pneumoniae.* One of the three patients with *Cryptococcus neoformans* died; in this patient also *Staphylococcus aureus* and klebsiella bacteria were found. One patient died in whom *Staphylococcus aureus* was identified as the only pathogen. Six (12.2%) of the 49 patients with undetermined aetiology of chest infection died. None of the nine patients with PCP died. *M tuberculosis* and HHV8 were statistically significantly associated with mortality, $X^2 = 5.7$, p = 0.02 and $X^2 = 6.0$, p = 0.03, respectively.

DISCUSSION

In the studied population of severely immuno-suppressed HIV-infected patients with pulmonary infection, the spectrum of pathogens identified was wide, ranging from common bacteria, *M. tuberculosis, P. jiroveci,* HHV8 and fungi. Common bacterial infection was the leading cause of pulmonary infection, however by far the most deadly single causative bacterial pathogen for chest infection was *M. tuberculosis.* One-third of the TB patients in this study died within one month of follow up. The TB burden in countries highly affected by HIV has increased tremendously and has generally resulted into high mortality (2,8-11). PTB/HIV co-infected patients exhibit a higher risk of death than other co-infections in HIV infected patients even when they are matched for CD4 T cell count, which is largely due to adverse interaction of the two infections (12,13).

We found that presence of micronodules on chest radiograph, as well as anaemia, were statistically significantly associated with TB. Generally, features differentiating TB from other common pulmonary infections in HIV infected individuals are few (4,14,15). Given the sputum scarcity in HIV infection and low yield of acid fast bacilli (AFB) smear (16), presence of micronodular lesions in chest radiographs in the absence of other aetiological agents of respiratory infection in these patients should be highly suggestive of TB.

HHV8 was among the most common pathogen found in BAL in this study and presence of HHV8 was significantly associated with mortality. HHV8 DNA in BAL is mainly associated with Kaposi's sarcoma (KS) of the lungs with high sensitivity and specificity than cutaneous KS (17). Lung parenchyma is the primary site of pulmonary KS (18) and lesions are therefore easily missed by bronchoscopy. Pulmonary KS, unlike cutaneous KS, contributes decisively to morbidity and mortality especially in resource-poor setting where specific treatment is lacking (19). To our knowledge, we are the first to report the presence of HHV8 in BAL fluid from HIV seropositive patients from Africa. HHV8 is highly prevalent in sub-Saharan Africa and some parts of southern Europe but less common elsewhere (20). It can therefore be expected that Kaposi's sarcoma also contributes significantly to pulmonary pathology in sub-Saharan Africa. Our findings are in line with

this. Besides that, HHV8 has also been associated with primary effusion lymphoma and multicentric Castleman's disease (21,22), which have very poor prognosis and are associated with HIV infection (23,24).

Prevalence of *P. jiroveci* in this study was higher than previously reported from Tanzania (25,26). However, this prevalence may still be an underestimation since patients with hypoxaemia despite administration of oxygen could not undergo bronchoscopy and thus were excluded from the study. Also, nearly 50% of patients had used TMP-SMX prior to enrolment into the study. Lower prevalence of PCP in Africa has been largely attributed to diagnostic problems, the current trend however shows an overall increase in PCP in developing countries (27,28) which could be due to increased awareness and improvement in the diagnosis of *P. jiroveci*. Additional use of real time PCR in this study showed a much higher prevalence of PCP than using standard staining techniques alone. This is due to the higher sensitivity of real time PCR compared to staining techniques (29). However, the median CT value of the cases detected by real time PCR alone was significantly higher than that of cases detected by staining techniques as well as real time PCR. There was also no overlapping of the standard deviations of the CT values. This finding suggests that the validity of this rapid molecular technique to distinguish between clinical PCP disease and colonisation lies on the application of an appropriate CT cut off value (29).

High prevalence of PCP in this study was not associated with mortality since none of the patients with PCP died during the one-month follow up. This may be due to our clinical practice of applying early presumptive treatment with TMP-SMX when PCP is suspected. This implies that in this way the high mortality associated with PCP is preventable especially in patients presenting with dyspnoea, a symptom which was found to be significantly associated with detection of *P. jiroveci* in BAL fluid. Instituting presumptive treatment has been found to lower mortality in patients with PCP (30).

Common bacteria were also highly prevalent in this study, despite the fact that the majority of patients used empirical antibiotic treatment prior to enrolment into the study. A similar trend has been observed in other studies in Africa with streptococcal pneumonia being the most common

(3,15,26,31-33). Usually common bacteria occur in patients with relatively preserved CD4 T cell count, but a recent study in north Africa also found that common bacteria were more likely to occur at lower CD4 T cell count, (3) and thus common bacteria should also be suspected even in advanced HIV/AIDS. We did not have the necessary facilities to identify *Legionella pneumophila* infection in our patients. Besides, we had hypothesised that in Tanzania the risk of acquiring legionella infection is low, also in patients not infected with HIV, which was supported by serological data from Kenya (34), and at the same time another study from the region question the clinical relevance of positive serological test for legionella (35). Nevertheless, it would have been interesting to study legionella in our patient population. Few fungal infections (three *Cryptococus neoformans*, two *Aspergillus fumigatus*) were identified in this study. The incubation period of twenty-one days used for fungus culture, however, may have hindered identification of *Histoplasma capsulatum*. In the two patients in whom only cryptococcal antigen test was positive, cryptococcal pulmonary infection was entertained as the cause of morbidity since no other manifestations of cryptococcal infection (such as meningitis) were found.

Other serious co-morbidities not pertaining to pulmonary infection were also quite prevalent in this study. These conditions which usually confound morbidity are quite common in HIV infected patients from this region (15,26).

In more than 40% of patients no causative organisms were identified. Part of this may be attributed to the fact that over 80% used empirical antimicrobial treatment prior to the laboratory investigations, but we may have missed pathogens undetectable by our diagnostic methods. This group of patients had a somewhat higher median CD4 T cell count and a significantly lower mortality rate than the patients in whom causative agents were isolated. In other studies the percentage of patients with undetermined aetiology in HIV infection varies, but may be almost half of the study population (15,33).

In conclusion, this group of severely immunosuppressed patients with pulmonary infection, we found a wide spectrum of aetiological agents. The main identified pathogens were *M. tuberculosis, Streptococcus pneumoniae / S. pyogenes,*

HHV8, *P. jiroveci* and *Staphylococcus aureus*. Mortality was high, particularly in TB patients and in patients with HHV8. Micronodular chest radiographic lesions and anaemia were associated with TB. PCP was significantly associated with dyspnoea.

ACKNOWLEDGEMENTS

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