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Bronchoscopic study on aetiology of chronic cough in HIV-infected adults with negative sputum smears for Mycobacterium tuberculosis at Kenyatta National Hospital, Nairobi

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ABSTRACT

Objective: **To establish the aetiology of chronic cough in HIV-infected patients with negative sputum smears for Acid Fast Bacilli (AFB).**

Design: **A cross-sectional descriptive study.**

Setting: **Kenyatta National Hospital, a tertiary referral centre in Kenya**

Subjects: **Sixty five HIV-infected adults presenting with chronic cough and negative sputum smears for AFBs.**

Results: **Sixty-two patients were included in the final analysis. Aetiology of chronic cough was established in 42 (68%) patients. *Pneumocystis jiroveci,* bacterial pneumonia and *Mycobacterium tuberculosis* were diagnosed in 22 (35.5%), 17 (27.4%) and 14 (22.5%) patients respectively. Majority (98%) of patients with a diagnosis had multiple causes established in them. Ciprofloxacin had activity against 91% of the isolated organisms while Penicillin was active against 35% only.**

Conclusion: **This study documents *Pneumocystis jiroveci* pneumonia as a common cause of morbidity in a subset of HIV infected patients with chronic cough and negative sputum smears for AFB in Kenya.**

INTRODUCTION

Kenya remains amongst the top ten African countries affected by HIV (1). By June 2005, an estimated 1.5 million Kenyans were infected by HIV, close to 250,000 were in need of Highly Active Antiretroviral Therapy (HAART) and a cumulative total of 1.5 million Kenyans have died since the epidemic began (2,3). Close to 700 Kenyans die daily because of AIDS (4).

Socio-demographic analysis shows that life expectancy for Kenyans was only 54.7 in the period 1995-2000, which is more than ten years less than the projected figure of 65.4 years if Kenya were without AIDS (5).

Pulmonary complications, both infectious and non-infectious, are a leading cause of morbidity and mortality in HIV infected patients and are seen in virtually every patient with HIV infection (6). In developed countries, and before the advent of HAART, the most common opportunistic pulmonary infection was *Pneumocystis jiroveci* which occurred at presentation in 40% of HIV-infected patients and in >80% during the course of their

illness (7). In Africa, on the other hand, tuberculosis is the most common pulmonary infection (8,9) with *Pneumocystis jiroveci* being considered a relatively rare opportunistic infection in HIV infected patients with pneumonia (10-12).

To date, few, if any, rigorous studies on the causes of chronic cough in African, treatment-naïve, HIV-infected patients have been performed. Clinical features and chest radiographs may point towards a diagnosis but cannot reliably establish one. Detection of the actual pathogen(s) involved in the disease process is important in order for pathogen-specific therapies to be instituted. Spontaneously expectorated sputum examination, though important, has inherent limitations such as a low yield for *Pneumocystis jiroveci*, bacterial and other pathogens and contamination of cultures by naso-pharyngeal and oro-pharyngeal commensals (13-15). Flexible fibre-optic bronchoscopy, on the other hand, allows for direct visualisation of the tracheo-bronchial tree and has a high yield for bacterial, mycobacterial and fungal (including *Pneumocystis jiroveci*) elements and is now a well-established diagnostic modality for pulmonary complications in HIV infected patients in the developed world (16-17). The procedure has also been evaluated in some African countries and found to be equally useful (18). Unfortunately bronchoscopy has not been used widely in sub-Saharan Africa for diagnostic purposes due to expense as well as the need for technological expertise, both of which are scarce. We therefore performed intensive and invasive diagnostic testing in a cohort of HIV infected patients with undiagnosed chronic cough to provide guidance to routine clinical diagnostic and therapeutic activities.

MATERIALS AND METHODS

Study site: This study was conducted at the General Medical Wards of the Kenyatta National Hospital (KNH), a 2000-bed tertiary referral hospital in Nairobi, Kenya. It also serves as the teaching hospital for undergraduate, postgraduate and specialist training for the University of Nairobi's College of Health Sciences. All microbiological analysis was carried out at the Kenya Medical Research Institute (KEMRI)/Japan International Cooperation Agency Acute Respiratory Infections Laboratory at the Centre for Respiratory Disease Research. CD4 cell counts were done at the Virus Research Centre, KEMRI. Histopathological analysis of biopsy specimens was carried out at the Department of Pathology, The Nairobi Hospital. The KNH Ethics and Scientific Review Committee approved the study.

Study subjects: All patients studied were admitted to the general medical wards of the Kenyatta National Hospital, Nairobi. Entry into the study required fulfillment of all of the following criteria:

(i) HIV positive on Enzyme Linked Immune Assay.
(ii) Presence of cough for a period equal to or greater than 21 days.
(iii) An abnormal chest radiograph.
(iv) A minimum of three sputum smear samples negative for Acid Fast Bacilli (AFB).
(v) Not known to have been diagnosed with any condition associated with chronic cough in the present and/or past admissions e.g. asthma, COPD, carcinoma of the bronchus.
(vi) Written informed consent duly signed after explanation in the language best understood by the patient.

A detailed history and physical examination were carried out on each patient and the results were entered in a standard encounter form. Chest radiograph reports were reported by two independent radiologists blinded to other patient data. These were grouped into five categories:

(i) Diffuse or patchy lung shadowing in all lung zones.
(ii) Fine or coarse reticulonodular shadowing (fine indicating a nodular component < 1mm).
(iii) Peri-hilar distribution of shadowing with sparing of apices and bases.
(iv) Presence of hilar lymph node enlargement.
(v) Multiple abnormalities i.e. presence of two or more of the categories above.

Blood was drawn for confirmation of the HIV status by use of the Vironostika HIV Uniform II *plus O* test kit (Organon Teknika bv, Boxtel, Belgium), an Enzyme Linked Immune Assay. A Cell-Dyn 1300 Coulter Counter (Abbott Laboratories, USA) was used for complete blood counts. Fifty nine patients had a CD4 cell count done using a FACSCAN (Becton Dickinson

& Co, Cockeysville, USA). Erythrocyte Sedimentation Rate (ESR) was measured using the Westergren method. In addition, two 10ml samples of venous blood were collected aseptically from different sites for bacterial culture. An early morning sputum sample was collected from all patients able to spontaneously expectorate (42/62) on the day they were scheduled for bronchoscopy. Patients then underwent flexible fibre-optic bronchoscopy (Karl Storz GmbH & Co, Tuttlingen, Germany) under light sedation (2.5 – 5mg of midazolam IV) and tracheo-bronchial anaesthesia (2ml aliquots of 2% xylocaine). Following inspection of the tracheobronchial tree, bronchoalveolar lavage (BAL) was done on all patients and transbronchial biopsies (TBB) on 41/62 patients. Specimens collected from the study subjects were analysed as follows:

(i) Microbiological examination for detection of bacteria in sputum and BAL: Sputum and alveolar fluid were diluted in brain heart infusion broth and quantitative cultures done on blood agar, chocolate agar and bromothymol blue (BTB) for species identification. The blood agar plate was incubated aerobically and the chocolate agar in a CO_2 enriched medium at 35-37°C for 48 hours. BTB was incubated in room air. Cultures were considered positive if there were greater than 10^4 colonies per milliliter and if these were in pure culture. All bacterial isolates had sensitivity testing using the Kerby-Bauer disc diffusion method. Susceptibility results were generated automatically using the WHONET 4 microbiology lab database software.

Causality was inferred to if the isolated organism is known to be pathogenic, if the two specimens yielded similar isolates or if it was also isolated in blood.

(ii) Blood culture: Trypticase Soy broth supplemented with Para-amino benzoic (PAB) (Organon Tecknica Corp) was used as the liquid medium. Each bottle was inoculated with 10 ml of blood and incubated at 35-37° C and routinely inspected for signs of microbial growth. Subcultures were done on blood agar and chocolate blood agar. All blood cultures were incubated for a total of seven days. If at the end of seven days the blood culture medium was still clear, blind sub-cultures were done. True infection was suspected if the organism grew in two bottles of the same blood specimen, growth was rapid and/or the same organism grew in culture from other specimens. All 'true infections' were considered to be causally related to the patients' illness (septicaemic spread from the lungs).

(iii) Microbiological examination for mycobacterium (BAL only): Alveolar fluid was centrifuged and the sediment stained using the Zeihl Neelsen technique. Mycobacterium culture was done on Lowenstein Jensen medium for a period of 8-12 weeks after decontamination of the specimen with 2% sodium hydroxide and neutralised with phosphate buffer. Positive smear specimens and cultures were subjected to PCR for species confirmation.

(iv) Polymerase chain reaction for confirmation of mycobacterium tuberculosis: The Amplicor *mycobacterium tuberculosis* (MTB) test kit (Roche Diagnostics Corporation, Indianapolis, IN 46256 USA) was used to confirm mycobacterium isolates as being *mycobacterium tuberculosis*. The Amplicor MTB test permits the simultaneous amplification of MTB target DNA and *mycobacterium* internal control DNA. The master mix reagent contains a biotynilated primer pair specific for MTB and *mycobacterium* internal control. Negative and positive external controls were included to verify the results.

(v) Microbiological examination for fungi (sputum and BAL): An amount of sputum and alveolar fluid was inoculated onto SDA + 2% chloramphenical plus brain heart agar (enriched for the growth of dimorphic fungi) and incubated at 35-37° C for 28 days. Daily checks for growth of colonies were done. Any colonies that grew were described and microscopic tests done to identify the species. Association with disease was only considered if the organism is known to cause pulmonary disease, there was evidence of invasion of the pulmonary tree (e.g. visualisation of *Candida albicans* in the broncho-pulmonary tree on bronchoscopy) or there was additional identification of the organism in blood.

(vi) Microbiological examination for Pneumocystis jiroveci (BAL only): The FIPC 100 Detect Immunofluorescence for PCP kit (Shield Diagnostics Ltd, UK) was used. This is an indirect immunofluorescence test kit for the *in vitro* diagnosis of *Pneumocystis jiroveci* in human BAL specimens. If the positive control well

demonstrated obvious oocysts, test specimens containing five or more fluorescent oocysts over the whole slide were termed positive. FIPC 100 kit has a sensitivity of 90.5% and a specificity of 100% for *Pneumocystis jiroveci.*

(vii) Histology for TBB: TBB specimens were stained using Haematoxyline/ Eosin stain and Grocott's stain for identification of yeast cells, fungal hyphae and *Pneumocystis jiroveci.* All identified organisms were considered causally related to the patients' illness.

Statistical analysis: All data were entered into and analysed using the Statistical Package for Social Sciences (SPSS version 11). Continuous data, which is presented as mean or median and range, were analysed using Student's t-test while discrete variables were analysed by use of chi-square test. The level of significance is taken to be at a p-value less than 0.05. The relationships between CD4 counts with selected binary variables were assessed using group t-tests. The distribution of the CD4 counts was skewed to the right so a logarithmic transformation was done before analysis. The logs of the CD counts were compared in the two groups using t-tests. In order to convert means in the SPSS output to geometric means, it was necessary to calculate the antilog of the mean as given; i.e. 10^{mean}. The relationships between CD4 counts and continuous variables were assessed using Spearman's Rank Correlation since the two variables tested do not have a normal distribution.

RESULTS

Between August 2001 and July 2002, 160 patients that satisfied the inclusion criteria were recruited into the study. Out of these 65 (41%) patients underwent fibre-optic bronchoscopy. The rest were excluded for the following reasons: 36 (22.5%) patients had positive sputum smear stains for *mycobacterium tuberculosis;* 16 (10%) patients had either abnormal INR or low haemoglobin levels; 14 (8.75%) patients were considered too sick to withstand the procedure; 12 (7.5%) patients missed the procedure; 10 (6.25%) patients died before the procedure could be carried out while seven (4%) patients declined to give consent. Results of three patients were excluded from the final analysis because their clinical and laboratory data were incomplete.

Characteristics of the patients: The base-line characteristics of the study subjects are shown in Table 1. The majority of patients were below the age of 40 years (mean age 36.2 ± 9.9 years; median age 35 years [range 18- 67]) and they were equally split between the sexes (male: female ratio of 1.13). Median duration of cough was eight weeks (range 3 – 78). Median CD4 cell count was 85/ml (range 2 – 1180 cells/ml). Forty two (71.2%) patients had CD4 cell counts < 200/ml. The median CD4 cell count for female and male patients was 91/ml (range 5 - 1180/ml) and 66/ml (range 2 - 753/ml) respectively (p = 0.592; group t-test). Patients with a history of treatment for pneumonia in the past had a median CD4 cell count of 91/ml (range 3 – 621/ml) while those without a similar history had a median CD4 cell count of 178/ml (range 2 – 1180/ml) (p=0.091, group t-test). The median CD4 cell count for patients with a history of being treated for PTB in the past two years was 83/ml (range 3 - 1180/ml). This did not attain statistical significance when compared with the rest of the patients (p=0.435; t-test). Patients were classified into several categories according to the 1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS among Adolescents and Adults. As shown in Table 2, 58 (93.5%) patients either had an AIDS defining condition or had CD4 cell counts less than 200 ml. None of the patients studied had been on either HAART or Co-trimoxazole Preventative Therapy (CPT). All patients had been on at least one antibiotic in the preceding two weeks; 44 (71%) patients had been on two antibiotics, 20 (32%) patients had been on three antibiotics and one (1.6%) patient had been on four different antibiotics. The most frequently used antibiotics were: Crystalline penicillin (60%); Gentamicin (29%); Erythromycin (27%); Co-trimoxazole (24%); Co-amoxiclav (13%); Ceftriaxone (5%); Amikacin (3%) and Amoxicillin (3%).

Aetiology: The aetiology of chronic cough as determined by various diagnostic modalities is presented in Figure 1. A total of 80 diagnoses (25 bacterial, 22 pneumocystis pneumonia, 15 fungal, 14 PTB and four KS) were made from 42 (67.7%) patients on the basis of bronchoscopy findings, microbiology of sputum and alveolar fluid and TBB histology. Of the 42 (67.7%) patients for whom a diagnosis was established 41 (97.6%) had multiple aetiologies for the chronic cough (Table 3). Seven (11.3%) patients

had TB alone, 13 (21%) patients had Pneumocystis pneumonia alone and four (6.5%) patients had both TB and Pneumocystis pneumonia. One (1.6%) patient had KS alone, two (3.2%) had KS and Pneumocystis pneumonia and 1(1.6%) had KS and florid pulmonary candidiasis (visualised on bronchoscopy). One (1.6%) patient had TB and pulmonary invasive candidiasis (histology diagnosis). Two (3.2%%) other patients had pulmonary invasive Candidiasis (histology diagnosis) alone. One (1.6%) patient had both TB and cryptococcosis and one (1.6%) had a combination of TB, Pneumocystis pneumonia and a superadded bacterial infection. Finally one (1.6%) patient was co-infected with Pneumocystis pneumonia and a bacterial infection and one (1.6%) other patient had Pneumocystis pneumonia, Aspergillosis and a bacterial infection. Seven (11.3%) of the patients with positive blood cultures had a positive microbial test from the other respiratory samples. Therefore of the 42 patients that had a diagnosis 41 (98%) patients had at least two pathologies identified in them. The specific contribution of the various specimens and laboratory methods used to determine the diagnosis are given in Table 4.

A total of 33 bacterial organisms isolated from sputum, blood and BAL were tested for antibiotic sensitivities. Presented in Table 5 is a summary of the antibiotic sensitivity test results. Table 6, which is a summary of individual drug performance against the bacterial isolates, indicates that Ciprofloxacin had the best *in vitro* activity with 91% of all the organisms being sensitive to it while Penicillin had the poorest sensitivity pattern (only 35% of the organisms sensitive to it).

Table 1

Base-line characteristics of HIV infected patients with undiagnosed chronic cough undergoing bronchoscopy (n = 62)

Patient	No.	(%)
Male	29	(46.8)
Female	33	(53.2)
Age (years)	36.2 ±9.9	
Median duration of cough	8 weeks	(3-78)
Presenting symptoms and signs		
Haemoptysis	15	(22.4)
Chest pain	36	(58.1)
Dyspnoea	39	(62.9)
Fever	50	(80.6)
Night sweats	45	(72.6)
Weight loss	53	(85.5)
Pneumonia in the last two years	23	(37.1)
Treatment for pulmonary tuberculosis in the last two years	24	(38.7)
Antibiotic treatment prior to bronchoscopy	62	(100)
Crystalline penicillin	37	(59.7)
Gentamicin	18	(29.0)
Erythromycin	17	(27.4)
Co-trimoxazole	15	(24.2)
Co-amoxiclav	8	(12.9)
Cephtriaxone	3	(4.8)
Amikacin	2	(3.2)
Amoxicillin	2	(3.2)

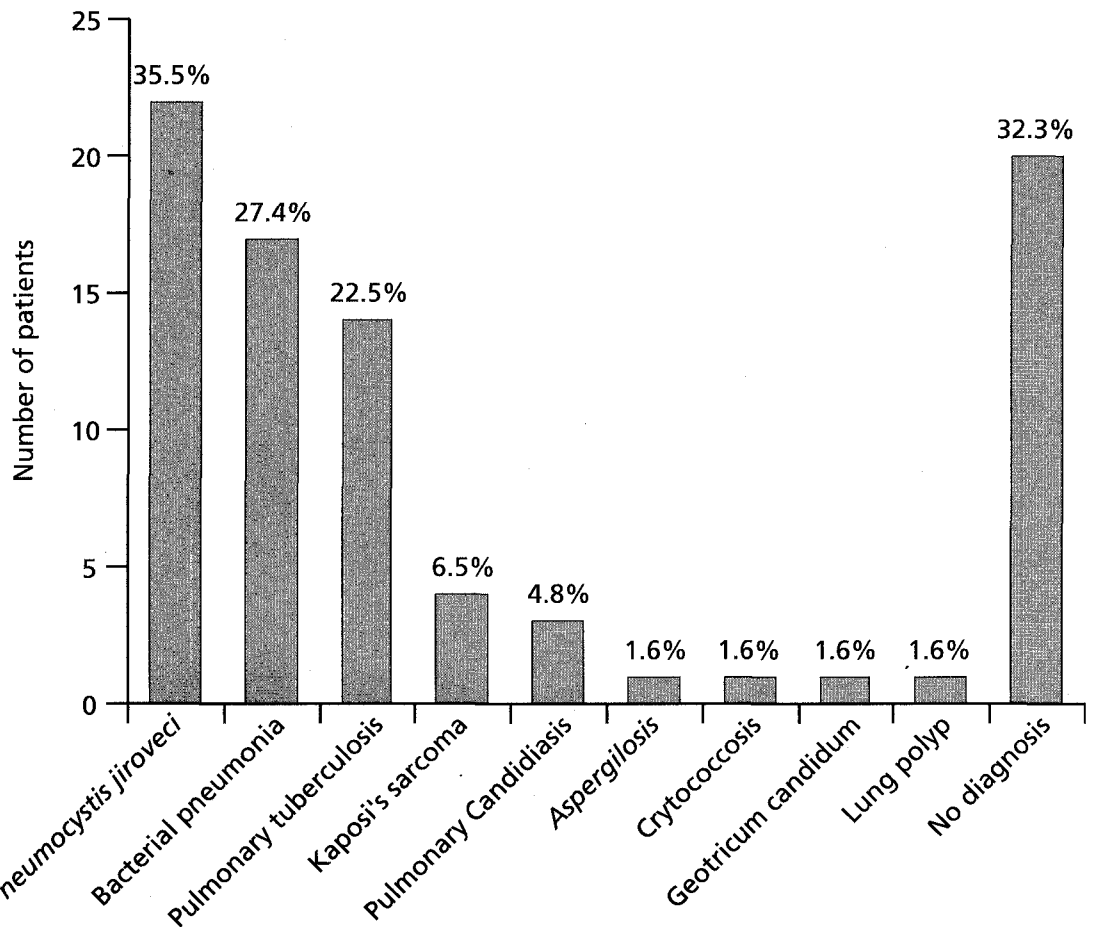
Table 2

Categorisation of HIV positive patients with undiagnosed chronic cough according to the 1993 Revised Classification System for HIV Infection (n = 62)

		Clinical Category			
	CD4 cell count/ml	A	B	C	No. of patients (%)
Category 1	>500	1	1	8	10 (16.1)
Category 2	200-499	2	0	5	7 (11.3)
Category 3	<200	2	4	36	42 (67.7)
Missing CD4 counts		0	0	3	3 (4.8)

Figure 1

Aetiology of chronic cough in HIV-infected patients with negative sputum smears for acid fast bacilli (n = 62)



*Note. The percentages in figure 1 exceed 100 because some patients had more than one organism isolated from their specimens.

Table 3

Co-morbidity

Pneumocystis pneumonia	No.	(%)	PTB	No.	(%)
Pneumocystis pneumonia	13	(21)	PTB alone	7	(11)
Pulmonary tuberculosis	4	(6.5)	Pneumocystis carinii pneumonia	4	(6.5)
Pulmonary Kaposi's sarcoma	2		Pulmonary Kaposi's sarcoma	0	
Bacterial pneumonia	1		Bacterial pneumonia	1	
PTB + bacterial pneumonia	1		Pulmonary cryptococcosis	1	
Aspergillosis + bacterial pneumonia	1		Pulmonary invasive candidiasis	1	
Total	22	(35.5)	Total	14	(22.5)

Table 4

Sputum, bronchoalveolar lavage and blood bacterial and fungal cultures, mycobacterium and Pneumocystis jiroveci test results from HIV infected patients with undiagnosed chronic cough

Test Result	Sputum (n = 42)	BAL (n = 62)	Blood (n = 62)	TBB (n = 41)
Bacterial culture				
Klebsiella pneumoniae	4	4	0	-
Staphylococcus aureus	2	5	4	-
Haemophilus influenza	2	2	0	-
Proteus mirabilis	1	0	0	-
Acinetobacter spp	0	1	0	-
Corynebacterium spp	0	1	0	-
Escherichia coli	0	1	0	-
Pseudomonas aeruginosa	0	1	1	-
Streptococcus pneumoniae	0	1	0	-
Salmonella spp	0	0	1	-
Morganella morgani	0	0	1	-
Fungal culture				
Candida albicans	5	7	1	3
Cryptococcus neoformans	0	1	0	0
Aspergillus fumigatus	0	1	0	1
Geotricum candidum	0	1	0	0
Other tests				
BAL Zeihl Neelsen stain	-	4	-	-
BAL Lowenstein Jeensen culture	-	4	-	-
BAL MTB PCR	-	14	-	-
BAL PCP Immunofluorescence	-	18	-	-
TBB Grochott's stain (PCP)	-	-	-	12

Table 5

Antibiotic sensitivity test results of isolated microorganisms to commonly used antibiotics

Organism	Ciprofloxacin	Cefalothin	Cefotaxim	Co-trimoxazole	Gentamicin	Chloramphenicol	Penicillin	Erythromycin
Staphylococcus aureus 1	S	S	S	R	S	S	R	S
Staphylococcus aureus 2	S	S	S	S	S	S	S	S
Staphylococcus aureus 3	R	R	R	R	R	R	R	R
Staphylococcus aureus 4	S	S	S	S	S	S	S	S
Staphylococcus aureus 5	S	S	IR	S	S	S	S	R
Staphylococcus aureus 6	S	S	S	S	S	S	S	S
Staphylococcus aureus 7	S	S	S	S	S	S	R	S
Staphylococcus aureus 8	S	S	S	R	S	R	R	R
Staphylococcus aureus 9	S	S	S	R	S	S	R	S
Staphylococcus aureus 10	R	S	R	R	R	R	R	R
Staphylococcus aureus 11	S	S	S	R	S	S	R	S
Klebsiella pneumoniae 1	S	R	R	R	R	R	R	R
Klebsiella pneumoniae 2	S	S	S	S	R	S	R	R
Klebsiella pneumoniae 3	S	S	S	S	S	R	R	R
Klebsiella pneumoniae 4	S	R	S	R	S	R	R	R
Klebsiella pneumoniae 5	S	R	R	R	R	R	R	R
Klebsiella pneumoniae 6	S	R	R	R	R	R	R	R
Klebsiella pneumoniae 7	S	S	S	S	S	S	S	S
Klebsiella pneumoniae 8	S	S	S	S	S	S	S	S
Haemophilus influenza 1	S	S	S	R	IR	S	R	S
Haemophilus influenza 2	S	S	S	R	IR	S	R	S
Haemophilus influenza 3	S	R	R	R	R	S	R	R
Haemophilus influenza 4	S	R	S	R	R	S	R	R
Pseudomonas aeruginosa 1	S	R	R	R	R	R	R	R
Pseudomonas aeruginosa 2	S	R	R	R	R	R	R	R
Acinetobacter spp	S	S	R	R	S	S	R	R
Corynebacterium spp	S	S	S	S	S	S	S	S
Escherichia coli	NT	NT	NT	NT	NT	NT	NT	NT
Proteus mirabilis	S	R	S	R	R	R		R
Streptococcus pneumoniae	S	S	S	S	S	S	S	S
Salmonella spp	S	S	S	R	IS	R	R	R
Morganella morgani	IS	R	R	R	R	S	R	R
Sphingomonas paucimobilus	S	S	S	S	S	S	S	S

KEY: S = Sensitive, IS = Intermediate Sensitivity, IR = Intermediate Resistance, R = Resistance, NT = Not Tested

Table 6

Summary of antibiotic-sensitivity pattern of commonly used antibiotics

Antibiotic	Organisms tested	Number Sensitive	(%)
Ciprofloxacin	32	29	91
Cefalothin	31	21	68
Cefotaxime	31	21	68
Co-trimoxazole	31	18	58
Gentamicin	31	17	55
Chloramphenicol	21	10	48
Penicillin	17	6	35
Erythromycin	13	9	69

Two *Staphylococcus aureus* isolates were found to be resistant to methicillin (Methicillin Resistant Staphylococcus aureus - MRSA) but sensitive to vancomycin (Vancomycin Sensitive Staphylococcus aureus - VSSA). The MRSA were also resistant to ciprofloxacin. All organisms except *S. pneumoniae* and *Corynebacterium spp* were found to be Multi-Drug Resistant (MDR).

Chest radiograph findings: Of the 22 (35.5%) patients with proven Pneumocystis pneumonia, 14 (64%) had a fine or coarse reticulonodular pattern, six (27%) had multiple pathology on chest radiograph (three of these had concomitant TB), while two patients (9%) had diffuse lung shadowing in all lung zones. Patients with Pneumocystis pneumonia were more likely to present with a reticulonodular pattern on chest radiograph than patients with a different diagnosis (p=0.03). The most common presentation on chest radiograph for patients with TB was that of multiple pathologies (9 of 14 patients). This finding did not attain any statistical significance. None of the patients with TB presented with a fine or coarse reticulonodular pattern.

All patients with either perihilar consolidation (Chest Radiograph Report Category iii) or mediastinal lymph node enlargement (Category iv) had positive BAL cultures for MTB.

DISCUSSION

From a combination of laboratory tests performed on BAL and TBB the incidence of *Pneumocystis jiroveci* in HIV-infected adults with chronic cough and radiological abnormalities in whom tuberculosis was not initially diagnosed by sputum AFB staining in study was 35.5%. This represents one of the largest series of *Pneumocystis jiroveci* in sub-Saharan Africa indicating that it is an important and perhaps emerging cause of pneumonia in HIV/AIDS patients in Africa. Earlier studies done in Africa that employed bronchoscopy with BAL but not TBB for diagnosis of *Pneumocystis jiroveci* among patients with chronic pneumonia reported varying rates of *Pneumocystis jiroveci* ranging between 0% and 22% (19-21). In a more recent study from South Africa utilising both BAL and TBB, the reported rates of Pneumocystis pneumonia were 27.3% in Blacks and 58.8% in Caucasians (22). More recently and in a bronchoscopy study on clinically suspected Pneumocystis pneumonia in HIV patients in a public hospital in Nairobi, Kenya, the incidence of *Pneumocystis jiroveci* was found to be 37%. This study included all patients with cough, infiltrates on chest radiograph, and no clinical evidence of active TB (23).

The finding that Pneumocystis pneumonia is a common opportunistic infection in HIV/AIDS patients in Kenya has several implications. Firstly, it must influence clinicians to consider *Pneumocystis jiroveci* in differential diagnoses for the HIV-infected patient with chronic cough and negative sputum AFB smear stains, to evaluate the patient for it, and to treat it aggressively. The main limitation to diagnosis of *Pneumocystis jiroveci* is that bronchoscopy with BAL and TBB is not in widespread use. In addition, the cost of the procedure is out of reach for most patients. Secondly, the disease is sufficiently common to warrant adoption of widespread preventive therapy, as recommended (24). This calls for use of CD4 cell counts to monitor patients' immune status in order to start deserving patients on prophylactic treatment. Co-trimoxazole, the drug of choice for preventing Pneumocystis pneumonia, is also

efficacious in preventing Toxoplasmosis, some bacterial pneumonia, some diarrhoeas and malaria. Co-trimoxazole has been associated with reduced morbidity and mortality in HIV-infected patients and has beneficial effects on the rate of CD4-cell count decline as well as rise in viral load (24-28). Thirdly, a simple clinical diagnostic algorithm that is appropriate for the Kenyan patient population needs to be developed and validated. This would allow clinicians to treat HIV-infected patients empirically for Pneumocystis pneumonia when a definitive test is delayed or unavailable. Emphasis should also be placed on developing a laboratory diagnostic procedure that is sensitive, specific, non-invasive, and affordable. All these issues call for more extensive studies.

In sub-Saharan Africa several bronchoscopy-based studies have been done in HIV infected patients with chronic pulmonary infections with negative sputum smear stains for MTB. The incidence of *Mycobacterium tuberculosis* in these studies ranges between 5% in Central Africa and 39% in Zimbabwe (9,21). The finding of a significant number of patients with smear negative, culture positive active TB (22.5%) in this and other studies is important. Pulmonary TB should still be considered as a cause of chronic cough even when conventional sputum microscopy is negative. The recommendation to treat latent TB infection in HIV-infected patients must be carried out carefully in order to avoid exposing patients with active but undiagnosed TB to monotherapy leading to the development of resistant strains (24).

Since a high proportion of patients in this study had received beta-lactam antibiotic therapy, the BAL results probably represent an underestimate of the incidence of invasive pneumococcal infection as a cause of chronic pulmonary infection in HIV-infected patients in Kenya. Prior use of antibiotics may also explain the high number of patients (32%) that had no established diagnosis. Sensitivity testing for bacterial organisms isolated from various patient specimens revealed high rates of resistance to commonly used antibiotics except for ciprofloxacin, cefalothin and cefotaxim. Although ciprofloxacin had the best antibiotic sensitivity, it should be used cautiously as monotherapy because of the risk of invasive pneumococcal disease occurring (29). Two *Staphylococcus aureus* isolates resistant to ciprofloxacin were also resistant to methicillin (MRSA) though none was resistant to vancomycin. Resistance to penicillin was high (65%). This is likely due to the high prevalence of pre-treatment with penicillin prior to patients' being enrolled in this study. The level of resistance to penicillin and other drugs could not be determined, as Minimal Inhibitory Concentrations (MICs) were not done. However separate studies in various Kenyan regions have determined that 30% of *Streptococcus pneumoniae* have intermediate resistance to penicillin (30,31).

CONCLUSION

This study confirms earlier findings of the presence of Pneumocystis pneumonia in Kenyan HIV infected patients. It also demonstrates that bronchoscopy has an important role in the diagnosis of both *Pneumocystis jiroveci*, TB and other pathogens in HIV-infected patients in sub-Saharan Africa presenting with chronic cough and radiological abnormalities that have persisted despite empiric antibiotic treatment. The diagnosis of *Pneumocystis jiroveci* and other pulmonary pathology other than tuberculosis should be considered strongly in such patients. Better and less expensive techniques for the diagnosis of Pneumocystic pneumonia as well as empiric clinical algorithmic approaches to patients with chronic cough should also be developed for this setting.

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