

## Etiology of Suspected Pneumonia in Adults Admitted to a High-Dependency Unit in Blantyre, Malawi

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**Abstract.** The microbiologic etiology of severe pneumonia in hospitalized patients is rarely known in sub-Saharan Africa. Through a comprehensive diagnostic work-up, we aimed to identify the causative agent in severely ill patients with a clinical picture of pneumonia admitted to a high-dependency unit. A final diagnosis was made and categorized as confirmed or probable by using predefined criteria. Fifty-one patients were recruited (45% females), with a mean age of 35 years (range = 17–88 years), of whom 11 (22%) died. Forty-eight (94%) of the patients were seropositive for human immunodeficiency virus; 14 (29%) of these patients were receiving antiretroviral treatment. Final diagnoses were bacterial pneumonia (29%), *Pneumocystis jirovecii* pneumonia (27%), pulmonary tuberculosis (22%), and pulmonary Kaposi's sarcoma (16%); 39 (77%) of these cases were confirmed cases. Fifteen (29%) patients had multiple isolates. At least 3 of 11 viral-positive polymerase chain reaction (PCR) results of bronchoalveolar lavage fluid were attributed clinical relevance. No atypical bacterial organisms were found.

### INTRODUCTION

Severe pulmonary infections in sub-Saharan African adults are common and frequently lead to hospitalization.<sup>1</sup> Because of the unavailability of expensive and technically demanding diagnostic facilities, the etiology often remains unclear, and antimicrobial treatment is therefore empirical rather than being directed against an identified pathogen. Such a clinically based diagnosis lacks accuracy and carries the risk of inadequate antibiotic treatment, in particular because physical signs and radiographic manifestations in advanced immunodeficiency and the immune reconstitution inflammatory syndrome (IRIS) are commonly insensitive and non-specific.<sup>2–4</sup> In the few reported studies on the clinical and microbiologic spectrum of lower respiratory infections in adults in sub-Saharan Africa, bacterial pneumonia, pulmonary tuberculosis (PTB), and *Pneumocystis jirovecii* pneumonia (PcP) were among the most common causes but studies differed in methods used and the diagnostic work-up was frequently non-exhaustive.<sup>1,5–8</sup> Even fewer data are available, especially from patients with the most severe pneumonia, on the causative roles of viral, fungal, and atypical bacterial pathogens, and on the contribution of Kaposi's sarcoma (KS) to apparently pneumonic syndromes.<sup>1,8</sup>

Pulmonary tuberculosis and pneumococcal pneumonia are frequent diagnoses in patients admitted to the Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi.<sup>9–11</sup> The importance of *Pneumocystis jirovecii* pneumonia has been described for hospitalized children and in adult community and outpatient-based settings in Malawi but is less clear in hospitalized adults.<sup>12–14</sup> In 2004, we set up a medical high dependency unit (MHDU) at QECH to improve care for severely ill adult medical patients, in particular those with respiratory distress and hypoxia. An audit of patients admitted

to this MHDU showed that under routine circumstances with limited diagnostic capacity, microbiologic confirmation could not be achieved in any of the suspected PcP cases and in only in 6 of 40 bacterial pneumonia cases, thus frequently resulting in diagnostic and therapeutic uncertainty (Ajdukiewicz KM, Zijlstra EE, unpublished data).

We therefore conducted a prospective study carried out at a time of ongoing antiretroviral therapy (ART) scale-up in Malawi by using a comprehensive diagnostic work-up to determine final diagnoses in severely ill patients who were admitted to a MHDU with a clinical picture of pneumonia.

### MATERIALS AND METHODS

**Setting, patients, and case definition.** The study was carried out at QECH in Blantyre, the largest health care facility in the country, which serves approximately one million persons. Approximately 70% of adult medical in-patients are infected with human immunodeficiency virus (HIV) and 40% have acquired immunodeficiency syndrome (AIDS).<sup>15</sup> The hospital has a six-bed MHDU that is equipped with oxygen concentrators providing a maximum flow of 5 liters/minute.

All patients admitted to the MHDU during February–September 2006 were screened for enrollment. Inclusion criteria were an age  $\geq 18$  years, at least one sample of expectorated sputum examined that was negative for acid-fast bacilli (AFB), and a clinical diagnosis of pneumonia severe enough to warrant admission to MHDU for supplemental oxygen. The case definition of pneumonia entailed at least one symptom of cough, sputum production, breathlessness, chest pain, and hemoptysis, and an abnormality on a chest radiograph consistent with infection and prescription of empirical antimicrobial therapy for suspected pneumonia by the admitting clinician.

A person was excluded from the study if the diagnosis on admission was not pneumonia, if written informed consent could not be obtained, or if the patient was unfit for bronchoscopy. Patients were followed-up until they were discharged from the hospital.

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**Investigations and laboratory methods.** All samples were processed and analyzed at the Malawi-Liverpool Wellcome Trust research laboratories in Blantyre unless stated otherwise. An expectorated sputum sample was examined with modified Ziehl-Neelsen stain for AFB. Venous blood samples were obtained at admission for aerobic culture (BacTec, Becton-Dickinson, Sparks, MD), complete blood count (automated HmX; Beckman Coulter, Brea, CA), HIV test (2 rapid tests: Uni-Gold; Trinity Biotech, Wicklow, Ireland and Determine; Abbott Laboratories, Abbott Park, IL, conducted at the QECH laboratory), and CD4 count (fluorescein-activated cell sorter count; Becton Dickinson, conducted at the QECH laboratory). Flexible bronchoscopy (Olympus, Center Valley, PA) and bronchoalveolar lavage (BAL) were carried out as soon as feasible after admission and if peripheral oxygen saturation measured by pulse-oxymetry was > 90% with supplemental oxygen.<sup>16</sup> The endoscope was unprotected for bacterial contamination when passing through the nasopharynx. A maximum of 200 mL of sterile water in aliquots of 50 mL was instilled into the affected lobar bronchus or, in diffuse disease, into the right middle lobe bronchus, and re-aspirated to the extend possible, to provide a BAL sample.

The BAL fluid was used for *Pneumocystis* indirect immunofluorescence testing (Detect IF; Axis-Shield Diagnostics Ltd., Dundee, Scotland). Bacterial, fungal, and mycobacterial cultures were set up on blood and chocolate agar plates (incubated at 37°C in a CO<sub>2</sub> incubator for 48 hours), cystine-lactose electrolyte deficient agar plates (incubated aerobically for 24 hours), Sabouraud's agar plates (incubated aerobically for up to 2 weeks) and Lowenstein-Jensen slopes (incubated aerobically at 37°C for 10 weeks).

A single slide was prepared for modified Ziehl-Neelsen staining, and 2 mL of BAL fluid was stored at -20°C. This specimen was used for a polymerase chain reaction (PCR) of 15 respiratory viral pathogens including influenza A and B virus; parainfluenza virus type 1-4; adenovirus; rhinovirus; respiratory syncytial virus type A and B; human metapneumovirus; coronavirus 229E and OC43; human coronavirus NL; bocavirus (Department of Virology, Erasmus Medical Center, Rotterdam, Netherlands); and *Pneumocystis jirovecii*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Legionella pneumophila*, and *Chlamydia psittaci* (Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands). A detailed description of the PCR methods used has been reported.<sup>17,18</sup> Real-time PCR analysis enabled quantification of *Pneumocystis*

*jirovecii*. A threshold cycle (C<sub>t</sub>) value ≤ 35 has been associated with infection and a C<sub>t</sub> value > 35 indicates colonization.<sup>18,19</sup> The PCR and mycobacterial culture results were received after the study was completed and therefore did not influence patient management.

**Outcome.** A final diagnosis was established retrospectively for each patient after reviewing all available clinical and microbiologic data. The final confirmed and probable diagnoses definitions are shown in Table 1. Clinical features dictated which final diagnosis was considered primary, if multiple diagnoses were made in a patient. When detected, non-pathogenic bacteria such as alpha-hemolytic streptococci and *Staphylococcus epidermidis* were reported as contaminants.

**Case management.** Patients received empirical antibiotic treatment for pneumonia and sepsis, which depending on availability, included benzylpenicillin, ampicillin, amoxicillin, third-generation cephalosporins, chloramphenicol, macrolides, or ciprofloxacin. Patients with PcP were treated with high dose co-trimoxazole (trimethoprim/sulfamethoxazole) together with oral corticosteroids, and those considered to have PTB were started on standard four-drug anti-tuberculous treatment. Patients with pulmonary Kaposi's sarcoma (PKS) were assessed by the hospital palliative care team and received vincristine mono-chemotherapy and high-dose corticosteroids if deemed appropriate. Administration of oxygen by nasal prongs aimed to maintain peripheral oxygen saturation above 90%. In agreement with hospital policy, commencement of ART was deferred until after stabilization and discharge from hospital. Patients who were already receiving ART continued this therapy during hospitalization.

**Ethical approval.** Ethical approval for the study was received from the College of Medicine Research and Ethics Committee, Blantyre, Malawi. This committee is formally mandated by the National Research Council of Malawi to review proposals emanating from or linked to the College of Medicine. Written informed consent was obtained from all patients.

**Statistical analysis.** Statistical analysis was carried out by using SPSS 11.0 (SPSS, Chicago, IL) for windows. Categorical data were compared by using the chi-square test. Continuous data were compared by using *t* tests for measures that were normally distributed and the Mann-Whitney test for measures that were skewed in distribution (as assessed by Kolmogorov-Smirnov statistics). Levene's test for equality of variances was performed to ensure homogeneity of variances. A *P* value < 0.05 indicated statistical significance. Adjustment of blood hemoglobin concentration for sex was performed by binary logistic regression analysis.

TABLE 1  
Diagnostic criteria of final diagnoses in patients with suspected pneumonia, Blantyre, Malawi\*

Disease	Confirmed	Probable
Bacterial pneumonia	- Positive culture of BAL and/or blood - Positive BAL PCR result for atypical bacteria	- Suggestive clinical picture and improvement after receiving antibiotics
<i>Pneumocystis</i> pneumonia	- Positive BAL immuno-fluorescence (> 5 oocysts per high-power field)	- Positive BAL PCR result (cycle threshold value < 35) <sup>18,19</sup> and suggestive clinical picture and improvement after treatment for PcP
Pulmonary tuberculosis	- Positive BAL mycobacterial culture and/or AFB ZN smear	- Suggestive clinical picture and improvement after treatment for tuberculosis
Pulmonary Kaposi's sarcoma	- Characteristic tracheo-bronchial lesions on bronchoscopy and suggestive chest radiograph	
Viral pneumonitis	- Positive BAL viral PCR result and suggestive clinical picture	

\* BAL = bronchoalveolar lavage; PCR = polymerase chain reaction; PCP = *Pneumocystis jirovecii* pneumonia; AFB = acid-fast bacilli; ZN = Ziehl-Neelsen.

## RESULTS

**Patient characteristics, clinical features, and mortality.**

During the 28-week study period, 159 patients were admitted to the MHDU, of whom 51 were recruited as shown in Table 2. A total of 45% of the study participants were women (mean age = 35 years, range = 17–88 years).

At admission, 43 (84%) patients were presumed to have a bacterial pneumonia and 7 (14%) had *Pneumocystis jirovecii* pneumonia on the basis of clinical impression alone. Most (66%) patients had symptoms of at least three weeks duration (Table 3). A total of 94% of the participants were infected with HIV, of whom 29% were receiving ART that had been started less than 2 months before admission in 71% of the patients. The mean CD4 count at admission was not significantly different between HIV-infected persons who were receiving ART and those who were not receiving ART (135 versus 116 cells/ $\mu$ L;  $P = 0.77$ ). The mean duration of hospitalization was shorter in those receiving ART than in those not receiving ART (11 versus 16 days;  $P = 0.05$ ) but survival rates were similar. Nine patients died during the MHDU stay after a mean duration of stay of 8 days (range = 4–16 days). An additional two inpatient deaths occurred after discharge from the MHDU, one caused by cryptococcal meningitis and one caused by suspected wasting syndrome and severe anemia. Inpatient mortality was not associated with any of the baseline characteristics, clinical features, or blood results, except hemoglobin concentration, which was higher in survivors.

**Role of bronchoscopy.** Thirty-three patients who were admitted to the MHDU with clinical features of pneumonia were not included in the study because they could not undergo bronchoscopy, either because of early death or because bronchoscopy was contraindicated, usually because of profound hypoxia in the patient (Table 2). Fifty-one patients underwent one bronchoscopy a median of 3 days (range = 1–21 days) after admission to the hospital. Nine (18%) bronchoscopy procedures had to be postponed by 3–5 days because of severe hypoxia (4), writhing/vomiting (2), confusion (1), non-fasted state (1), and unavailability of theatre space (1). Adverse events directly related to the procedure were oxygen desaturation to < 90% in 2 patients (of whom 1 died of confirmed PcP 8 days after bronchoscopy) and a vasovagal reaction in 1 patient.

Results of bronchoscopy and microbiologic investigations of BAL fluid conducted in the context of this study had direct treatment consequences in individual cases when they either confirmed or changed the clinical diagnosis. This finding

occurred in 15 (29%) patients who had bacterial pneumonia ( $n = 4$ ), PCP ( $n = 2$ ), PTB ( $n = 3$ ), and PKS ( $n = 6$ ).

**Microbiologic isolates.** Results of microbiologic investigations are shown in Table 4. Eighty-nine positive results from BAL fluid (81) and blood cultures (8) were obtained. Of these results, 15 BAL cultures (13 alpha-haemolytic streptococci, 2 *Staphylococcus epidermidis*) and 3 blood cultures (2 alpha-haemolytic streptococci, 1 *Staphylococcus epidermidis*) were considered to be contamination or colonization (not shown in Table 4) because alpha-haemolytic streptococci and *Staphylococcus epidermidis* are not common causes of pneumonia in patients with advanced HIV infection.<sup>20</sup> Additionally, 2 *Pneumocystis* PCR (Ct > 35) results were also indicative of colonization. Multiple isolates were seen in 15 (29%) patients, 13 of whom had 2 pathogens and 2 patients had 3 pathogens detected.

**Final diagnoses.** The final clinical diagnoses in 51 patients were bacterial pneumonia,  $n = 15$  (29%); PcP,  $n = 14$  (27%); PTB,  $n = 11$  (22%); PKS,  $n = 8$  (16%), and viral pneumonitis,  $n = 3$  (6%). In 39 (77%) of the cases, the diagnosis could be confirmed (Table 4). Diagnoses of viral pneumonitis and two cases of smear-negative but mycobacterial-culture positive PTB were made retrospectively because these test results were not available in real-time. Diagnoses for the three HIV-negative persons were confirmed pneumococcal pneumonias in two and probable influenza A viral pneumonitis in the third patient. All PCRs in BAL fluid for the atypical agents *Chlamydomydia pneumoniae*, *Chlamydomydia psittaci*, *Mycoplasma pneumoniae*, and *Legionella pneumophila* were negative.

**Clinical features and outcome.** Clinical features and outcome of all 51 final diagnoses are shown in Table 5. Hemoptysis and pleural effusion were not seen in any patients with PcP, and patients with PcP differed from those with other diagnoses in their mean hemoglobin concentration (11.8 g/dL versus 9.1 g/dL;  $P < 0.0001$ ) and mean CD4 count (17 versus 169 cells/ $\mu$ L;  $P < 0.0001$ ). None of the 14 patients with PcP were receiving ART, and only one had been taking trimethoprim/sulfamethoxazole prophylaxis, which he had stopped two weeks before admission. In contrast, most patients with PKS had hemoptysis and pleural effusion and 6 of 8 had been established on ART. In-hospital mortality was similar for all diagnoses. Only one (8%) of 13 patients with PKS had no dermal or oral KS lesions.

## DISCUSSION

We comprehensively described microbiologic etiology and clinical features of adult Malawians who were hospitalized with clinical features of pneumonia severe enough to require admission to a MHDU. We confined the study to patients who were judged by traditional criteria to be able to tolerate bronchoscopy and bronchoalveolar lavage, and in these patients the procedure caused no major adverse events and contributed importantly to obtaining a final diagnosis. We were able to confirm a final diagnosis by detection of an infectious pathogen or PKS in a high proportion of the study population ( $n = 39$ , 77%), which is comparable to percentages of 59–90% reported in some studies of pneumonia etiology in similar African settings and in the developed world.<sup>1,5,6,21</sup> Four main diagnoses of bacterial pneumonia (29%), PcP (27%), PTB (22%), and PKS (16%) in all 51 patients emerged. In

TABLE 2

Enrollment of 51 of a total of 159 patients admitted to a medical high-dependency unit and reasons for exclusion, Blantyre, Malawi

No. patients	Reasons for exclusion
48	Non-respiratory diagnoses*
19	Non-pneumonic respiratory diagnoses†
8	Sputum smear-positive pulmonary tuberculosis
33	Pneumonic diagnoses but unsuitable for bronchoscopy‡
51	Recruited

\*Non-respiratory diagnoses were cardiac disease (18), meningitis (6), tuberculous pericarditis (5), severe anemia (2), pyelonephritis (2), and other (15).

†Non-pneumonic respiratory diagnoses were extra pulmonary tuberculosis (10), chronic airways disease (3), pleural sepsis (3), bronchiectasis (1), and malignancy (2).

‡Unsuitable for bronchoscopy due to early death < 24 hours (15), hypoxia < 90% (6), confusion (2), refusal (2), cough (1), unavailability of staff (6), and under age (1).

TABLE 3  
Patient characteristics, clinical features, and outcomes, Blantyre, Malawi\*

Characteristic	All (n = 51)	Alive (n = 40)	Dead (n = 11)	OR (95% CI)†
Female	23 (39%)	19 (48%)	4 (36%)	1.58 (0.40–6.27)
HIV status known at study entry	31 (61%)	23 (58%)	8 (73%)	0.50 (0.11–2.20)
HIV positive	48 (94%)	37 (93%)	11 (100%)	–
WHO stage 4	32 (63%)	26 (65%)	6 (55%)	1.54 (0.40–5.98)
Previous medication				
Antiretroviral treatment	14/48 (29%)	11 (28%)	3 (27%)	1.01 (0.22–4.52)
TPM/SMZ > 1 month	8/48 (17%)	7 (14%)	1 (9%)	2.12 (0.23–19.36)
Antibiotics	31 (61%)	26 (65%)	5 (45%)	2.49 (0.64–9.69)
Symptom duration, weeks				
< 1	12 (24%)	10 (25%)	2 (18%)	1.50 (0.27–8.13)
1–3	5 (10%)	4 (10%)	1 (9%)	1.11 (0.11–11.08)
> 3	34 (66%)	26 (65%)	8 (73%)	0.69 (0.15–3.05)
				<i>P</i> (95% CI)‡
Mean age, years	35	34	37	0.32§
Mean vital signs at first examinations				
O <sub>2</sub> saturation (%)	76	75	78	0.25§
Heart rate/minute	123	123	120	0.63 (–15.73 to 9.71)
Respiratory rate/minute	46	47	45	0.68 (–10.40 to 6.92)
Systolic blood pressure, mm of Hg	97	97	96	0.45 (–12.64 to 5.69)
Mean blood results				
Hemoglobin, g/dL	9.8	10.2	8.5	0.043 (–3.42 to –0.05)
Leukocyte count × 10 <sup>3</sup> /μL	6.7	6.9	5.8	0.52§
Platelet count × 10 <sup>3</sup> /μL	222	235	171	0.18 (–161.45 to 32.63)
CD4 cells/μL	121	105 (n = 37)	204 (n = 7)	0.66§

\* OR = odds ratio; CI = confidence interval; HIV = human immunodeficiency virus; WHO = World Health Organization; TPM/SMZ = trimethoprim/sulfamethoxazole.

† Significant if 95% CI does not include 1.

‡ Significant if 95% CI does not include 0.

§ Not normally distributed.

one-third of patients multiple pulmonary morbidities were detected exceeding percentages of 10–13% reported from Kenya, Uganda and Tanzania.<sup>1,5,6</sup>

Despite HIV/AIDS public awareness campaigns and the ongoing ART scale-up in Malawi, a high number of patients (40%) claimed to be unaware of their HIV status at presentation. Twenty-nine percent had started ART, but only 16% were receiving trimethoprim-sulphamethoxazole prophylaxis, an intervention shown to reduce HIV-related mortality.<sup>22</sup> At the time of the study, the national trimethoprim/sulfamethoxazole prophylaxis program had not been widely implemented.

The high PcP prevalence among HIV-positive patients hospitalized for severe pneumonia has not been previously documented in Malawi. Two other studies have determined PcP rates in adults in Malawi in different settings. Hargreaves et al. identified PcP by indirect immunofluorescence or PCR of BAL fluid in 9% of 186 AFB smear-negative outpatients who were about to start anti-tuberculous treatment.<sup>14</sup> In a prospective community-based cohort study of HIV-positive persons, we previously found an overall incidence rate of 1.0 per 100 person-years observation, and based diagnoses on immunofluorescent staining and *Pneumocystis* PCR of induced sputum specimens.<sup>13</sup> In the subgroup of persons with low CD4 counts (< 100/mm<sup>3</sup>) PcP was more common (5.0/100 person-years observation). In the present study, one-fourth of our severely ill patients had confirmed or probable *Pneumocystis* infection, a percentage similar to the 27–38% reported in cross-sectional studies of various designs among AFB smear-negative persons from Uganda, Kenya, Ethiopia, and Zimbabwe.<sup>5,7,23,24</sup> These findings confirm that PcP is an important diagnosis in hospitalized HIV-infected patients with severe respiratory illness in sub-Saharan Africa.

Well-documented clinical features of PcP including hypoxemia, unremarkable chest auscultation, and absence of hemop-

tysis and pleural effusion were also seen in the present study.<sup>25</sup> The most helpful laboratory indicator other than BAL IF for the presence of PcP was a CD4 count < 100 cells/mm<sup>3</sup>. Of the 16 clinically relevant positive PcP IF and/or PCR results for which a CD4 count was available, 14 (88%) were measured as < 25/mm<sup>3</sup> whereas this was observed in only 2 (7%) of 28 patients who had no evidence of PcP infection, which supports the routine use of CD4 counts when evaluating HIV-infected patients with suspected severe pneumonia in settings in sub-Saharan Africa. Most patients had begun high-dose trimethoprim/sulfamethoxazole treatment before the positive result of the real-time IF stain became available, indicating a high index of clinical suspicion among clinicians. Inpatient mortality of patients with PcP was 21% (n = 3) which is comparable to mortality figures reported by others and similar to patients in our study with diagnoses other than PcP.<sup>7,24</sup> On-site and real-time IF methods for confirmation of PcP proved to be a useful diagnostic tool, especially because half of the patients with PcP had a secondary infection or PKS. Three patients who already had another primary diagnosis were also treated with trimethoprim/sulfamethoxazole for PcP on clinical grounds, and proved on examination of BAL fluid to have a negative IF test result but a positive PCR result for *Pneumocystis* (Ct < 35). The interpretation of these findings is uncertain because colonization of airways with *Pneumocystis* is well-described in immunocompromised persons.<sup>26</sup>

Sputum smear AFB-negative PTB remains a challenging entity because no validated diagnostic or management algorithms exist.<sup>27</sup> Almost one-fifth of our sputum smear-negative patients had microbiologic proof of tuberculosis upon further investigation, which is comparable to previous reports citing detection rates of up to 33% on BAL or induced sputum specimens.<sup>6,23,28</sup> We may have under-diagnosed PTB by sputum smear examination because we only examined one expectorated spu-

TABLE 4  
Final diagnoses and diagnostic technique of patients and co-infection or PKS, Blantyre, Malawi\*

Patient	Final diagnosis	Confirmed/probable	Co-infection or PKS
<b>Bacterial pneumonia</b>			
1†	<i>Staphylococcus aureus</i> by BAL culture	Confirmed	PcP by PCR
2	<i>Klebsiella pneumoniae</i> by BAL culture	Confirmed	PcP by PCR
3	<i>Streptococcus pneumoniae</i> by BAL culture	Confirmed	
4	<i>Staphylococcus aureus</i> by BAL culture	Confirmed	Parainfluenzavirus type 1
5	<i>Staphylococcus aureus</i> by BAL culture	Confirmed	PKS
6	<i>Staphylococcus aureus</i> by BAL culture	Confirmed	PKS
7	<i>Klebsiella pneumoniae</i> by BAL culture and <i>Streptococcus pneumoniae</i> by BC	Confirmed	Coronavirus OC 43
8	<i>Staphylococcus aureus</i> by BAL culture	Confirmed	
9	<i>Streptococcus pneumoniae</i> by BC	Confirmed	Respiratory syncytial virus A
10–11	<i>Streptococcus pneumoniae</i> by BC	Confirmed	
12–15† (one death)	No isolate	Probable	
<b>Pneumocystis pneumonia</b>			
16†	IF/PCR	Confirmed	<i>Pseudomonas</i> by BAL culture
17	IF/PCR	Confirmed	Rhinovirus
18†	IF/PCR	Confirmed	<i>Cryptococcus neoformans</i> by BC + PKS
19–25	IF/PCR	Confirmed	
26†	IF/no PCR	Confirmed	Rhinovirus + PKS
27	PCR	Probable	Coronavirus NL
28	PCR	Probable	
29	PCR	Probable	
<b>Pulmonary tuberculosis</b>			
30†	ZN/mycobacterial culture	Confirmed	MRSA by BAL culture + rhinovirus
31–33† (two deaths)	ZN/mycobacterial culture	Confirmed	<i>Enterobacter cloacae</i> by BAL culture
34	ZN/mycobacterial culture	Confirmed	
35	ZN	Confirmed	PKS
36†	Mycobacterial culture	Confirmed	<i>Enterobacter cloacae</i> by BAL culture
37	Mycobacterial culture	Confirmed	PcP by PCR
38	Mycobacterial culture	Confirmed	
39–40	No isolate	Probable	
<b>Viral pneumonitis</b>			
41	Influenza A virus	Probable	
42	Bocavirus	Probable	
43†	Adenovirus	Probable	
<b>Pulmonary Kaposi's sarcoma</b>			
44	Bronchoscopy	Confirmed	Rhinovirus
45–51† (one death)	Bronchoscopy	Confirmed	

\* PKS = pulmonary Kaposi's sarcoma; BAL = bronchioalveolar lavage; PcP = *Pneumocystis jirovecii* pneumonia; PCR = polymerase chain reaction; BC = blood culture; IF = immunofluorescence; ZN = Ziehl-Neelsen staining; MRSA = methicillin-resistant *S. aureus*; all viral isolates by PCR.

† Died.

tum sample before performing bronchoscopy. Other factors contributing to the low sensitivity of sputum smear examination of approximately 20–30% include advanced immunosuppression, inability to expectorate, and variable quality of sampling and specimen processing procedures.<sup>29</sup> Mycobacterial culture increases diagnostic sensitivity, but it is rarely available in sub-Saharan Africa. Of five BAL AFB smear-negative patients in our study, three were culture positive, two of whom had not received anti-tuberculous treatment by the time the culture results became known. Non-specific and atypical clinical features in patients who recently began treatment with ART and subsequently having IRIS can complicate the diagnosis of sputum smear-negative PTB. Five of 11 patients with the final diagnosis of PTB were within two months of having started treatment with ART previously, and IRIS could have contributed to their current illness. Profound anemia was a distinguishing clinical marker separating PTB from other diagnoses, a finding that has been reported elsewhere.<sup>6</sup>

Most (84%) patients at admission were treated empirically for suspected bacterial pneumonia. However, only one-third of these cases had a positive BAL or blood culture result. The predominant pyogenic isolates from BAL fluid were *Staphylococcus aureus* (n = 6), *Pseudomonas aeruginosa*

(n = 1), *Enterobacter cloacae* (n = 2), and *Klebsiella pneumoniae* (n = 2). A higher prevalence of community-acquired atypical pyogenic bacteria in patients with advanced HIV infection has been reported by other investigators.<sup>5,6,20</sup> Some of the isolates in our study might have been hospital acquired because for 30% of the patients, BAL sampling was carried out after five days of hospitalization. *Streptococcus pneumoniae* was identified in four blood cultures, which had been obtained at admission, whereas only one BAL sample was positive. We may have under-diagnosed pneumococcal disease on BAL fluid specimens in our population because two-thirds of patients had received antibiotic treatment before hospital admission and further empirical antibiotic therapy was commonly commenced in hospital before bronchoscopy was undertaken. Given the unconventional bacterial spectrum observed in this and other studies, antimicrobial treatment with a narrow-spectrum penicillin for severe community acquire pneumonia in HIV-positive patients might not be sufficient.

Viral causes of community-acquired pneumonia have received heightened attention in the western world and viral detection rates of up to 29% in HIV-negative patients have been reported.<sup>30</sup> Hardly any epidemiologic data have originated from sub-Saharan Africa where sensitive PCR-based

TABLE 5  
Patient characteristics, clinical features, and outcomes of final diagnoses, Blantyre, Malawi\*

Characteristic	Bacterial pneumonia (n = 15)	<i>Pneumocystis</i> pneumonia (n = 14)	Pulmonary tuberculosis (n = 11)	Pulmonary Kaposi's sarcoma (n = 8)
Female sex	7 (47%)	8 (57%)	5 (45%)	2 (25%)
HIV status known at study entry	7 (47%)	7 (50%)	9 (82%)	7 (88%)
Confirmed HIV positive	13 (87%)	4 (100%)	9 (100%)	8 (100%)
ART at study entry	3 (20%)	0; <i>P</i> = 0.005†	5 (45%)	6 (75%; <i>P</i> = 0.003)†
Hemoptysis	4 (27%)	0; <i>P</i> = 0.022†	3 (27%)	4 (50%)
Crackles on chest auscultation	13 (87%)	7 (50%); <i>P</i> = 0.005)†	9 (82%)	8 (100%)
Pleural effusion	6 (40%)	0; <i>P</i> = 0.011†	2 (18%)	7 (88%); <i>P</i> = 0.019†
Survived	13 (87%)	11 (79%)	7 (64%)	7 (88%)
Mean				
Oxygen saturation %‡	71	70	81	85; <i>P</i> = 0.017†
Heart rate/minute	122	132; <i>P</i> = 0.04†	119	116
Respiratory rate/minute	51; <i>P</i> = 0.046†	46	47	42
Systolic blood pressure, mm of Hg	93	94	98	104
Hemoglobin, g/dL	9.6	11.8; <i>P</i> < 0.0001†	8.0; <i>P</i> = 0.03†	8.6
Leukocyte count × 10 <sup>3</sup> μL‡	8.8	6.1	5.3	6.2
Platelet count × 10 <sup>3</sup> μL	237	291; <i>P</i> = 0.034†	171	115
Lymphocyte count × 10 <sup>3</sup> μL‡	1.7	0.8	0.7; <i>P</i> = 0.02†	1.5
CD4 cells/μL‡	168; <i>P</i> = 0.027†	17; <i>P</i> < 0.0001†	157	201
CD4 cells/μL‡				
< 100	5	14	6	5
100–199	2			1
> 200	4		3	2

\* Each diagnosis was compared with a composite of the other four diagnoses (3 cases of viral pneumonia are not shown). HIV = human immunodeficiency virus; ART = antiretroviral therapy.

† Only *P* < 0.05 is shown.

‡ Not normally distributed.

diagnostics are seldom used. Our study is the first to report the results of comprehensive PCR assays for detection of respiratory viruses. Scott et al. retrospectively identified infection with influenza A and B and adenovirus by serologic testing in 5.7% of adult patients in Kenya with acute pneumonia, half of whom had bacterial co-infections.<sup>1</sup> The presence of viral nucleic acid in BAL or upper airway swabs is generally believed to represent infection, although asymptomatic carrier states have been described. The viruses detected in our cohort have been associated with respiratory illness to a variable extent, although it remains controversial whether viruses such as rhinovirus, coronaviruses, or the newly described bocavirus cause pneumonia through invasion and replication in the lower respiratory tract, facilitate bacterial infection or merely are innocent bystanders.<sup>30–33</sup> We documented two deaths in four patients who had evidence of rhinovirus infection, although both had significant co-infections with PcP and PTB/methicillin-resistant *Staphylococcus aureus*. Both patients infected with bocavirus (no other organism isolated) and coronavirus NL (co-infection with PcP) infection survived to discharge.

There is also a paucity of data from Africa concerning atypical bacterial organisms as a cause of pneumonia. Lockman et al. used PCR testing and convalescence serologic analysis to identify 36 (17%) cases of acute *Mycoplasma pneumoniae* among hospitalized, predominantly HIV-infected adults in urban Botswana, of whom more than two-thirds had a co-pathogen detected.<sup>8</sup> A much lower yield of 2.5%, diagnosed by only by using convalescence serologic analysis, was seen in a cohort of patients with radiologically confirmed acute community-acquired pneumonia in Kenya, 52% of whom were HIV-infected.<sup>1</sup> Neither study found evidence of infection with *Chlamydomphila pneumoniae*. Somewhat unexpectedly, we were unable to identify any *Mycoplasma pneumoniae*, *Chlamydomphila pneumoniae*, *Legionella pneumophila*, or

*Chlamydomphila psittaci* by PCR testing. Although DNA PCR as the sole diagnostic test, as used in our study, may have a lower sensitivity between 78% and 90% compared with serologic analysis, it seems unlikely that an important number of cases in our study were missed.<sup>8</sup> It may be that the data collection occurred outside a *Mycoplasma* epidemic or that the generally less severe clinical features associated with *Mycoplasma* disease obviated hospital and particularly MHDU admission. However, it is reasonable to assume that atypical bacteria are unlikely to represent a common cause of severe respiratory infections in Malawi.

Intrathoracic KS occurs in up to 10% of patients with AIDS and this increases to 25% if mucocutaneous involvement is present.<sup>34</sup> The incidence of KS has decreased in Europe and North America since the advent of ART, a trend that could not have been apparent in Malawi at the time of this study, when ART scale-up was not yet advanced. One-fourth of our patients displayed characteristic features of tracheobronchial KS, all of whom had markedly abnormal chest radiographs. Endobronchial KS (or pulmonary KS once lung parenchyma has been infiltrated) is believed to develop after mucocutaneous KS is established, a fact that can aid the diagnosis in patients with respiratory symptoms in which bronchoscopy for inspection of large airways is not available. All 12 patients in our study with dermal and/or oral mucosal KS (both in 8 patients) also had tracheobronchial disease, and only one patient had endobronchial KS without skin or oral involvement. Therefore, the triad of severe respiratory symptoms, abnormal chest radiograph, and presence of mucocutaneous KS was strongly associated with pulmonary KS in this patient cohort. However, intrathoracic KS associated respiratory symptoms, radiographic infiltrates and/or pleural effusion (present in 53% of all 13 patients with evidence of KS in our study) are non-specific findings and extensive investigations to exclude opportunistic pulmonary infections are

recommended, as highlighted by the fact that 6 of the 13 patients with PKS in our study had co-diagnoses of PcP, *Staphylococcus aureus* pneumonia, PTB, or rhinovirus infection.<sup>35</sup> In five PKS patients respiratory illness developed soon after ART initiation and without co-infection, suggesting the possibility of IRIS.<sup>36</sup>

Our study had several limitations. First, we investigated a select group of severely ill and hypoxic, sputum AFB smear-negative patients who came to an urban tertiary referral center. Therefore, extrapolation of our findings to other settings is not straightforward, although many patient characteristics were comparable to studies carried out elsewhere in sub-Saharan Africa.<sup>5,6</sup> Second, in the absence of validated tools to assess severity of community acquired pneumonia in sub-Saharan Africa such as CURB 65, which is used in the western world, direct comparison of patient cohorts from different studies is fraught with difficulty.<sup>37</sup> Third, pre-admission and in-hospital empirical antibiotic treatment before carrying out study investigations will have influenced the detection rate and spectrum of organisms we found. Fourth, almost one-third of patients had commenced ART recently, yet we were unable to assess formally whether the illness occurred in the context of IRIS. Fifth, because the number of patients described was small, any statistical significance of results pertaining to even smaller subgroups should be viewed with caution. And finally, we did not have an independent review of case notes, radiologic findings, and final diagnoses. Interpretation of results is therefore based on the expert opinion of the authors.

In a time of ongoing ART scale-up, we comprehensively determined the etiology of severe pneumonia in mostly HIV-positive adults in Malawi at a high dependency unit of an urban tertiary hospital. Investigations including bronchoscopy and BAL could be safely performed and provided a confirmed final diagnosis in three-fourths of the patients. Most diagnoses were PcP, PTB, bacterial pneumonia, and PKS, and these occurred at similar frequencies. *Mycoplasma*, *Chlamydia*, and *Legionella* species did not play a role in this patient group. The clinical relevance of observed viral isolates is not entirely clear and requires further study.

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