

Mycobacterium colombiense Pneumonia in HIV-Infected Patients: Three Case Reports and a Literature Review

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Background: *Mycobacterium colombiense* pneumonia in HIV-infected patients is relatively unusual but is associated with a high mortality rate, as well as high rates of misdiagnosis and delayed diagnosis. Clinical metagenome next-generation sequencing (mNGS) may have potential for its accurate and timely diagnosis.

Case Presentation: We retrospectively reviewed the medical records of three HIV-infected patients who presented with *M. colombiense* pneumonia in Zhejiang Province between January 2019 and December 2020. No specific clinical presentations or radiological manifestations were found in any of the patients. The detection of *M. colombiense* is 28–55 days earlier using mNGS on bronchoalveolar lavage fluid (BALF) compared to traditional culture methods. A combined treatment of rifabutin, clarithromycin, or azithromycin, and ethambutol did not provide timely relief of symptoms in these three patients. In the early stage of treatment, moxifloxacin and linezolid were used for several weeks. The average course of treatment for all three patients was close to 17 months.

Conclusion: We recommend early BALF mNGS for fast and accurate diagnosis of *M. colombiense* pneumonia in HIV-infected patients with low CD4 counts and long duration of symptoms. Further, moxifloxacin and linezolid may be beneficial in the early stage of treatment.

Keywords: metagenomic next-generation sequencing, nontuberculous mycobacteria, *Mycobacterium colombiense*, pneumonitis, HIV, AIDS

Introduction

Nontuberculous mycobacterium (NTM) infections can be life-threatening and are associated with a mortality rate of nearly 30% in HIV-infected patients, even in this era of highly active antiretroviral therapy.^{1,2} Pulmonary NTMs mainly affect HIV-infected patients with severe immunosuppression, and its delayed diagnosis is associated with poor prognosis.^{3–5} Thus, earlier diagnosis could help shorten the clinical course and improve survival outcomes. Currently, the diagnosis of NTM is mainly verified by polymerase chain reaction (PCR) and culture.⁶ However, the culture and PCR methods are time-consuming and have low sensitivity. Moreover, NTMs need to be identified at the species level, as different species usually require different therapeutic strategies. As a promising alternative, unbiased metagenomic next-generation sequencing (mNGS) offers a faster, culture-independent, and high-throughput methodology to detect potential pathogens from clinical specimens directly.^{7–11} mNGS has been used to identify uncommon or slow-growing pathogens that cause pneumonia.^{7–12} In our previous study, we found that the sensitivity of mNGS in the diagnosis of pneumonia in HIV-infected patients was significantly higher than that of conventional microbiological tests (79.6% vs 61.1%, $P < 0.05$).¹³ Thus, this technique could be useful for the accurate and timely diagnosis of NTM infections in HIV-infected patients.

Mycobacterium colombiense is a rare pathogen that infects patients with HIV infection, and it is associated with high rates of misdiagnosis, underdiagnosis, and delayed diagnosis. According to the existing case reports, the overall mortality rate of *M. colombiense* pulmonary infection or bacteraemia is significantly high.^{14–16} However, so far, reports on the identification of *M. colombiense* by mNGS in HIV-infected patients have been rare. The use of mNGS in the diagnosis of NTM in HIV-infected patients has been gradually increasing in our ward.¹³ Therefore, here we report three HIV-infected patients who had *M. colombiense* pneumonia and were diagnosed by mNGS.

Case Presentation

The demographic and clinical data of the three patients included in this report were obtained from the hospital electronic medical record system. Their written informed consent was obtained for the use of their data. Further, this study was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University School of Medicine.

Case 1

A 58-year-old man was hospitalized with a 4-month history of fever, expectoration, and weight loss of 7 kg. Three months before hospitalization, he had started antiretroviral therapy (ART) with a regime comprising dolutegravir (DTG), tenofovir (TDF), and lamivudine (3TC). His CD4 count was 9 cells/mm³. A pulmonary computed tomography (CT) scan showed interstitial lesions with infection, multiple nodules, and enlarged mediastinal lymph nodes (Figure 1A). The results of the serum T-SPOT, cryptococcal antigen, (1,3)- β -D-glucan, and galactomannan test were all negative. The results of ZN staining were positive for both the protected brush catheter (PBC) and bronchoalveolar lavage fluid (BALF). *M. colombiense* was identified by mNGS of BALF based on 10 reads. Moxifloxacin (Mfx), azithromycin (AZI), rifabutin (Rfb), and ethambutol (EMB) were initially administered, but the symptoms of the patient were not relieved. Then, his blood, sputum, and BALF samples were collected again for pathogen culture. *M. colombiense* was identified from sputum culture 28 days after it was detected with mNGS. Drug susceptibility tests indicated that *M. colombiense* was susceptible to clarithromycin (Clr), EMB, Rfb, and Mfx. The clinical symptoms and radiological findings of the patient both improved after 2 months of combined treatment with Clr, EMB, Rfb, and Mfx. He completed 18 months of treatment, and the disease had resolved based on clinical and imaging evidence (Figure 1D).

Case 2

A 45-year-old HIV-infected man was hospitalized with a 3-month history of fever, expectoration, night sweats, and weight loss of 20 kg. His CD4 count was 112 cells/mm³. His pulmonary CT scan showed multiple nodular, flaky high-density shadows, and cavities in the lower lobe of the right lung (Figure 1B). The assay of serum, PBC, and BALF samples revealed negative results for common opportunistic microorganisms, such as *Mycobacteria tuberculosis*, NTM, *Cryptococcus neoformans*, *Pneumocystis jirovecii*, *Candida*, *Aspergillus*, *Toxoplasma gondii*, and *Human Cytomegalovirus*, among others. The results of BALF mNGS revealed the presence of *M. colombiense* based on 307 reads. The patient's condition improved gradually after a 2-week combined regimen of Clr, EMB, Rfb, Mfx, and linezolid (LNZ). This was followed by an ART regime comprising DTG, TDF, and 3TC. Further, an oral Clr, EMB, Rfb, and Mfx regimen was prescribed after the patient was discharged. However, after initiation of ART for 18 days, the patient developed paradoxical NTM-immune reconstitution inflammatory syndrome (IRIS) and his CD4 count increased to 168 cells/mm³. CT-guided lung puncture biopsy was performed, and the sample was found to be positive for *M. colombiense* after 16 days of culture. LNZ was added to the anti-NTM therapy again, and the patient was discharged in good condition after a 12-day combined regimen of Clr, EMB, Rfb, Mfx, and LNZ. The anti-NTM treatment was continued for nearly 18 months, and his clinical and imaging results showed that the disease had resolved (Figure 1E).

Case 3

A 36-year-old young man was admitted to the hospital because he had high fever, dry cough, nausea, night sweats, and progressive fatigue over 3 weeks. He had started on ART with efavirenz (EFV), TDF, and 3TC 10 months ago. His pulmonary CT scan showed multiple flaky, nodular, high-density shadows in the right lung, along with enlarged mediastinal lymph nodes (Figure 1C). The routine screening of fungi, protozoa, and viruses through methods, such as

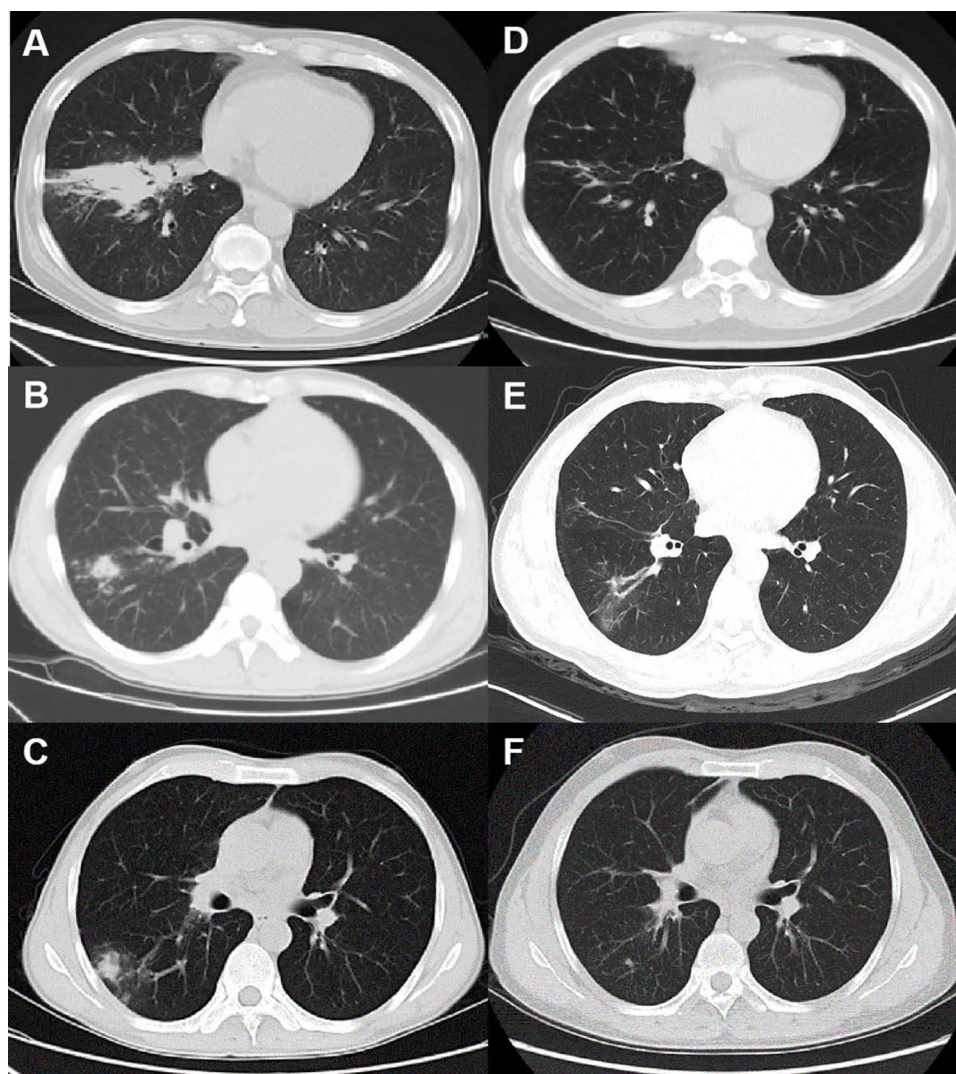


Figure 1 Paired axial unenhanced CT scans of the three patients performed at admission and at the last follow-up (**A–C**) Lung CT scans taken at admission, showing similar observations for the three patients: nodules, patches, cavities, and ground-glass or sheet-like high-density opacities. (**D–F**) Corresponding CT scans taken at the last follow-up showing a significant reduction in the extent of ground-glass opacification and residual bronchiectasis in the three patients.

blood culture, PCR, and serological tests, among others, revealed negative results. *M. colombiense* was identified based on 34 reads by mNGS of BALF. *M. colombiense* was identified from blood culture at 38 days after hospitalization. An anti-mycobacterial regimen comprising Mfx, AZI, Rfb, and EMB was prescribed, with emtricitabine/tenofovir (FTC/TDF) plus DTG for ART. The patient's condition gradually improved, but he developed paradoxical NTM-IRIS approximately 1 month later. LNZ was then added to the anti-NTM therapy for about 2 weeks. The patient completed 14 months of treatment, after which the disease had resolved based on clinical and imaging observations (Figure 1F).

Discussion

Here, we have described three cases of *M. colombiense* pneumonia in HIV-infected patients at an advanced-stage. *M. colombiense* is classified as a slow-growing mycobacterium that is closely similar to the *M. avium* complex (MAC) based on its genetic sequence.^{16,17} Only a few reports of HIV-infected patients with *M. colombiense* infection have been published. The first cases were reported in three HIV-infected Colombian patients in 2006 (Table 1).^{14–16,18} No specific clinical and radiological manifestations have been identified in the reported cases so far, as observed in our cases too. In our series, two of the patients exhibited respiratory symptoms and various pulmonary lesions for three to four months before they were presented to the hospital, and one patient had been experiencing these symptoms for three weeks

Table 1 Ten Previously Published Case Reports of HIV-Infected Patients with *Mycobacterium colombiense* Infection

No.	Authors	Year of Report	Description	Conventional Assays	Treatment	Outcome
1 ¹⁶	Murcia, M. I.	2006	Pulmonary infection	Unmentioned	Unmentioned	Death
2 ¹⁶	Murcia, M. I.	2006	3 patients with bacteraemia	Unmentioned	Unmentioned	Death
3 ¹⁴	Lee, M.R	2017	Bacteraemia	Blood culture	Tigecycline	Death
4 ¹⁴	Lee, M.R	2017	Bacteraemia	Blood culture	EMB, Clr, Rfp, Lfx, Amk	Death
5 ¹⁴	Lee, M.R	2017	Bacteraemia	Blood culture	EMB, Clr, Mfx	Death
6 ¹⁴	Lee, M.R	2017	Bacteraemia	Blood culture	EMB, AZI, Rfb, Lfx	Survived
7 ¹⁸	Pena, E	2019	Disseminated infection	Abdominal Sample culture	Clr, Rfb and EMB	Survived
8 ¹⁵	Yu, X	2021	Pulmonary infection	Blood culture	Meropenem, SMZ/TMP, caspofungin, immunoglobulin and LN2	Deteriorated and automatic discharge

Abbreviations: Amk, Amikacin; AZI, Azithromycin; Clr, Clarithromycin; EMB, Ethambutol; Lfx, levofloxacin; LN2, Linezolid; Mfx, Moxifloxacin; Rfb, Rifabutin; Rfp, rifampicin; SMZ/TMP, Sulfamethoxazole/trimethoprim.

(Table 2). *M. colombiense* infection usually occurs in the advanced stage of HIV infection and is characterized by CD4 counts less than 50 cells/mm³.^{14,15,18} In our cases, the CD4 count of two patients (case 1 and case 3) was significantly lower than 50 cells/mm³, and the CD4 count in case 2 was slightly higher than 100 cells/mm³. All three patients were in the advanced stage of HIV infection, and their CD4 counts are consistent with those reported in the literature for advanced-stage HIV.^{14,15,18}

Early diagnosis of *M. colombiense* pneumonitis is a challenge because its symptoms are not specific and mimic those of a number of benign and malignant diseases.^{15,19} Moreover, due to the inhibitory effect of antibiotics used before specimen collection or the limited number of selected targets of molecular assays, the causative pathogen cannot be detected in time with the traditional detection methods. Furthermore, the limitations of conventional diagnostic tests may result in an underestimation of the prevalence of pulmonary *M. colombiense*. In our case series, mNGS allowed for

Table 2 Demographic Data, Clinical Characteristics, and Outcome in Three Cases of HIV-Infected Patients with *Mycobacterium colombiense* Pneumonitis

	Case 1	Case 2	Case 3
Sex	Male	Male	Male
Age (y)	58	45	36
Symptoms	Fever, expectoration, weight loss	Fever, expectoration, weight loss, night sweats	Fever, dry cough, fatigue, weight loss
Symptom duration (months)	4	3	0.75
ART before hospitalization (months)	3	None	10
Initial ART regimen	DTG/TDF/3TC	None	EFV/TDF/3TC
Optimized ART regimen	DTG/TDF/3TC	DTG/TDF/3TC	DTG/FTC/3TC
CD4 count at admission (cells/mm ³)	15	112	22
Pulmonary CT scan	Interstitial lesions, multiple nodules, enlarged mediastinal lymph nodes	Multiple nodules, patches, cavities and sheet-like high density opacities, multiple enlarged lymph nodes	Multiple patchy and nodular high-density shadows in the right lung, enlarged mediastinal lymph nodes
mNGS	BALF, 10 reads	BALF, 307 reads	BALF, 34 reads
Anti-NTM therapy	AZI(Clr)+Rfb+EMB+Mfx	Clr+Rfb+EMB+Mfx, LN2 (for 2 months)	AZI+Rfb+EMB+Mfx, LN2 (for 2 weeks)
Positive traditional detection methods versus mNGS	Sputum culture, plus 28 days	Lung tissue culture, plus 55 days	Blood culture, plus 35 days
Total course	18 months	18 months	14 months

Abbreviations: 3TC, Lamivudine; ART, antiretroviral therapy; BALF, bronchoalveolar lavage fluid; Clr, Clarithromycin; CT, computed tomography; DTG, Dolutegravir; EFV, Efavirenz; EMB, Ethambutol; FTC, Emtricitabine; LN2, Linezolid; Mfx, Moxifloxacin; mNGS, Metagenomic Next-generation sequencing; NTM, Nontuberculous mycobacteria; TDF, Tenofovir; Rfb, Rifabutin.

a diagnosis of *M. colombiense* 28 to 55 days earlier than the traditional culture method. Thus, mNGS appears to have an advantage over traditional methods as it allows for early detection of pathogens and can, therefore, improve the prognosis of patients.

There is no consensus on the optimal anti-mycobacterial regimen for *M. colombiense* infection.^{16,20} Susceptibility tests have indicated that the drug susceptibility patterns of *M. colombiense* are similar to those of MAC, so therapeutic regimens for *M. colombiense* infections are mainly based on treatment regimens for MAC.^{14,16,20} In our patients, a combination of AZI or Clr, EMB, and Rfb was empirically prescribed as the initial therapy.^{21,22} The vitro susceptibility of Mfx and LNZ to MAC has been proven previously.^{23–25} Accordingly, the addition of Mfx and LNZ to the multidrug regimens were adopted in our study. However, as there are discrepancies between in vitro drug susceptibility test results and in vivo clinical outcomes; further studies are needed to evaluate the efficacy and the optimal course of Mfx and LNZ.⁶

Even though our case observations indicate the potential of mNGS, the results are limited because they were obtained from three different laboratories from different regions. Due to the lack of standard methods and availability of reference materials, the performance of different mNGS laboratories vary in terms of the identification of microbes and differentiation of true pathogens.^{26–28} At present, there is relatively little research in this area. Thus, further research is needed to improve the database setup and standardize the operation process.

Conclusions

In summary, we have reported three cases of HIV-infected adults with pulmonary *M. colombiense* infection who were diagnosed by mNGS and reviewed the associated literature. There were no specific clinical or radiological manifestations in these patients, but mNGS allowed for early diagnosis and identification of the pathogen. Thus, for patients in whom *M. colombiense* infection is suspected, BALF mNGS could allow for fast and accurate diagnosis of NTM. Further, although the patients responded to the administered anti-mycobacterial regime, the lung lesions regressed slowly and the infection recurred in two cases. Based on the observations in our cases, drug susceptibility testing is recommended for NTM infection patients if treatment regimens with Rfb, Clr, or AZI and EMB are ineffective. Alternatively, empirical addition of Mfx and LNZ may yield good results and could be considered.

Abbreviations

mNGS, metagenomic next-generation sequencing; NTM, Nontuberculous mycobacteria; *M. colombiense*, *Mycobacterium colombiense*; MAC, *M. avium* complex; BALF, bronchoalveolar lavage fluid; PBC, protected brush catheter; PCR, polymerase chain reaction; CT, computed tomography; ART, antiretroviral therapy; DTG, Dolutegravir; TDF, Tenofovir; 3TC, Lamivudine; EFV, efavirenz; FTC/TDF, emtricitabine/tenofovir; Mfx, moxifloxacin; AZI, azithromycin; Amk, Amikacin; Rfb, rifabutin; EMB, ethambutol; Clr, clarithromycin; LNZ, linezolid.

Data Sharing Statement

Patient data that were used in this study are provided in the tables.

Ethics Approval

The treatment of three patients was conducted in accordance with the Declaration of Helsinki. This report received the approval of the Ethics Committee of the First Affiliated Hospital of Zhejiang University School of Medicine (IIT20230802A).

Consent for Publication

Written informed consent has been obtained from three patients for the case details and images to be published. The case report does not contain any pictures that could identify the patient.

Acknowledgments

We especially acknowledge all frontline healthcare workers engaged in direct diagnosis, treatment, and care of patients.

Funding

This work was supported by the National Major Scientific and Technological Special Project for “Significant New Drugs Development” during the Thirteenth Five-Year Plan Period (grant number 2017ZX09303004-001). The funding organization had no involvement in the study or in the decision to submit the article for publication.

Disclosure

None of the authors have any conflicts of interest to declare.

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