Letter to the Editor

A Retrospective Study of Culture-confirmed Mycobacterial Infection among Hospitalized HIV-infected Patients in Beijing, China*

ZHAO Xiu Ying[1,&,#], ZENG Zhao Ying[2,&], HUA Wen Hao[3,&], YU Yan Hua[4], GUO Cai Ping[4], ZHAO Xiu Qin[5], DONG Hai Yan[5], LIU Jie[5], and WAN Kang Lin[5,#]

A retrospective analysis was performed in two major HIV/AIDS referral hospitals in Beijing to evaluate the prevalence of *Mycobacterium tuberculosis* (MTB) and non-tuberculous mycobacterial (NTM) infections in HIV-infected patients. A total of 627 patients' data were reviewed, and 102 (16.3%) patients were diagnosed with culture-confirmed mycobacterial infection, including 84 with MTB, 16 with NTM, and 2 with both MTB and NTM. The most frequent clinical complication by mycobacterial infection was pulmonary infection (48/102, 47.1%). The overall rates of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) were 11.9% and 3.4%, respectively. This study underlines the urgent need to intensify screening for mycobacteria coinfection with HIV and to prevent the spread of drug-resistant TB among HIV-infected patients.

Tuberculosis (TB) ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV). *Mycobacterium tuberculosis* (MTB) is a very common opportunistic pathogen in HIV-infected patients, while non-tuberculous mycobacteria (NTM) are reported with increasing frequency in cases of acquired immunodeficiency syndrome (AIDS). Drug-resistant TB is a threat to global TB control and a major public health concern in many countries[1]. To date, the prevalence of TB, multiple drug-resistant (MDR)-TB, and extensively drug-resistant TB (XDR-TB) and treatment outcomes in the population with HIV and TB coinfection have not been systematically investigated. This knowledge gap has delayed TB/HIV control efforts in China.

Although acid-fast staining is the fastest and simplest bacteriological diagnostic procedure, its sensitivity and specificity are limited. In recent years, the fast liquid culture system had become globally available. Compared with traditional Löwenstein-Jensen's (L-J) medium, this liquid culture system can shorten the recovery time of mycobacteria to approximately 8-13 days. Since 2008, two major HIV/AIDS referral hospitals in Beijing have been equipped with the BACTEC MGIT 960 and BACTEC 9120 mycobacteria detection systems (Becton Dickinson, Sparks, MD, USA), combined with renewed clinical processes to improve the identification of TB. The two BACTEC systems are fully automated and utilized fluorescence combined with an oxygen sensor to detect the growth of mycobacteria in culture. In this setting, we undertook a retrospective study to estimate the prevalence of mycobacterial infection based on laboratory evidence and to further analyze the prevalence of MDR/XDR-TB infection in HIV-infected patients.

In this retrospective study, we reviewed the clinical and laboratory results of hospitalized HIV-infected patients at two HIV/AIDS referral hospitals in Beijing (You'an Hospital and Di'tan Hospital) between January 2009 and December 2012. Each hospitalized HIV-infected patient was screened for mycobacterial infection. Serial sputum sample collection on consecutive days and at least one blood sample were required from each HIV inpatient.

doi: 10.3967/bes2018.060

*This study was supported by the Beijing Municipal Natural Science Foundation [No.5072021]; Capital Medical Development Scientific Research Fund [No. 2009-1057]; and the 11th Five Years Key Programs for Science and Technology Development of China [No. 2013ZX10003006 and No. 2013ZX10003002-001].

1. Beijing Tsinghua Changgung Hospital, School of Clinic Medicine, Tsinghua University, Beijing 102218, China; 2. Department of Clinical Laboratory, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China; 3. Beijing Di'tan Hospital, Capital Medical University, Beijing 102206, China; 4. Beijing You'an Hospital, Capital Medical University, Beijing 100069, China; 5. National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, State Key Laboratory for Infectious Disease Prevention and Control, Beijing 102206, China

Other types of clinical samples (pleural fluid, abdominal fluid, feces, cerebrospinal fluid, etc.) from suspected infection sites were also obtained for mycobacterial cultures. Multi-locus PCR was used to differentiate NTM from MTB. Drug susceptibility testing (DST) of first-line (Isoniazid, Rifampin, Streptomycin, Ethambutol) and second-line TB drugs (Fluoroquinolones, Kanamycin, Capreomycin, Ethionamide) for MTB isolates was tested with proportional method by the National Reference Laboratory of Tuberculosis, Chinese Center for Disease Control and Prevention (CCDC). Data are presented as the median of quantitative variables. The χ^2 test or Fisher's exact test were used to analyze differences; results were considered statistically significant at $P < 0.05$. All statistical analyses were performed with statistical software (SPSS 16.0; SPSS; Chicago, IL).

Between January 2009 and December 2012, 627 non-repetitive HIV-positive patients were admitted to two HIV/AIDS referral hospitals and underwent *Mycobacterium* screening. The presence of HIV antibodies was detected by enzyme-linked immunosorbent assay and western blot. A total of 102 patients met the criteria for bacteriologically confirmed mycobacterial infection, including 84 cases of MTB (82.3%), 16 cases of NTM (15.7%), and 2 cases (2.0%) of simultaneous infection by MTB and *Mycobacterium avium* (*M. avium*). Among the 18 patients with NTM infection, 14 were infected with *M. avium*, 3 were infected with *Mycobacterium kansasi* (*M. kansasi*), and 1 was infected with *Mycobacterium intracellulare* (*M. intracellulare*).

There were 48 cases (47.1%) reporting only pulmonary involvement and 16 cases (15.7%) reporting localized extrapulmonary involvement. Another 38 (37.3%) patients with positive blood cultures were defined with disseminated mycobacterium infection, either with or without pulmonary and/or extrapulmonary involvement. Among 16 cases with extrapulmonary involvement, the pleura was most often involved (5 cases), followed by the abdomen (3 cases), meninges (3 cases), digestive tract (2 cases), lymph nodes (1 case), liver (1 case), and pericardium (1 case).

Among the 38 patients with blood cultures testing positive for mycobacteria, MTB was the most prominent species, identified in 34 patients, followed by *M. avium* in 3 and *M. intracellulare* in 1. Of the 48 patients with pulmonary involvement, 36 were identified with MTB infection, 8 with *M. avium* infection, 3 with *M. kansasii* infection, and 1 with both MTB and *M. avium* infection. Of the patients with extrapulmonary involvement, 14 were infected with MTB, 1 with *M. avium,* and 1 with both MTB and *M. avium*. No significant difference in clinical manifestation was found between mycobacterial species (Table 1).

As for clinical symptoms, fever was reported in 76 (74.5%) of the 102 mycobacterium cases, followed by weight loss (65, 63.7%), cough and expectoration (32, 31.4%), and night sweats (18, 17.6%). In addition, of the 102 cases, there were 32 (31.4%) with concomitant oral candidiasis, 17 (16.7%) with pneumocystis pneumonia, 9 (8.8%) with cryptococcosis, 6 (5.9%) with herpes zoster, 5 (4.9%) with cytomegalovirus, 4 (3.9%) with hepatitis C virus, and 3 (2.9%) with esophageal candidiasis coinfection. Elevated erythrocyte sedimentation rates (ESR > 20 mm/h) were detected in 88 (86.3%) patients, 67 (65.7%) patients had ESR > 50 mm/h, and 19 (19.0%) had ESR > 100 mm/h.

CD4 cell counts were reviewed, and the results varied from 5 to 893 cells/μL [median: 57, interquartile range (IQR): 26-87]. Most patients (90, 88.2%) had CD4 counts < 200 cells/μL during their initial presentation at the hospital, and 47 (46.1%) had CD4 counts < 50 cells/μL. These results evidence a correlation between low CD4 cell counts and mycobacterial infection. For the 38 patients with disseminated infection, CD4 counts ranged from 6 to 631 cells/μL (median: 45, IQR: 25-87); for the 48 patients with pulmonary involvement, the range was 7 to 893 cells/μL (median: 56, IQR: 26-83); and for the

Table 1. Mycobacteria Species in Different Clinical Mycobacteria Infection

Clinical Feature	Total No. (%)	Mycobacteria Species				
		MTB	MTB + *M. avium*	*M. avium*	*M. kansasii*	*M. intracellulare*
Disseminated	38 (37.3)	34	0	3	0	1
Pulmonary	48 (47.1)	36	1	8	3	0
Localized Extrapulmonary	16 (15.7)	14	1	1	0	0
Total	102 (100.0)	84	2	12	3	1

Note. MTB, *Mycobacterium tuberculosis*.

16 patients with extrapulmonary involvement, the range was 5 to 292 cells/μL (median: 63, IQR: 30-145). In MTB-infected patients, CD4 counts ranged from 5 to 631 cells/μL (median: 52, IQR: 25-85), and in NTM-infected patients, it ranged from 7 to 893 cells/μL (median: 63, IQR: 40-216). No significant difference in clinical manifestation was found between levels of CD4 nor between Mycobacterium species (Table 2).

DST results of 59 MTB strains from the Beijing group were summarized, including 44 newly diagnosed and 15 previously treated cases. There were 41 isolates (69.5%) resistant to at least one first-line drug. Twenty-seven isolates showed resistance to streptomycin (45.8%), 19 to isoniazid (32.2%), 13 to rifampin (22.0%), and 8 to ethambutol (13.6%). Isolates from 32 patients (54.2%) were resistant to at least one second-line drug, with resistance to capreomycin being the most frequently reported (24, 40.7%), followed by resistance to fluoroquinolones (10, 16.9%), kanamycin (4, 6.8%), and ethionamide (5, 8.5%). MTB isolates from seven patients were resistant to both isoniazid and rifampin, resulting in an MDR-TB rate of 11.9%. From the seven MDR-TB cases, all with prior TB treatment, two were identified to be XDR-TB (3.4%), and all seven MDR-TB were isolated from patients with prior treatment histories.

The 102 patients with positive mycobacterium cultures consisted of 88 (86.3%) males and 14 (13.7%) females, with a male-to-female ratio of 6.3:1. The age range was from 1 to 90 years, with a median age of 37 years. Homosexual intercourse was the most common route of transmission (40, 41.2%), followed by commercial plasma donation (26, 25.5%), contaminated blood and blood product transfusions (13, 12.7%), heterosexual intercourse (8, 7.8%), intravenous drug use (4, 3.9%), vertical transmission (1, 1.0%), and unknown routes (10, 9.8%). There were 23 (22.5%) patients with previous TB treatment history. Thirty-one (30.4%) had begun antiretroviral therapy (ART) before diagnosis. Only 6 patients (3.9%) received isoniazid preventive therapy before mycobacterial infection was confirmed (Table 3).

We found the rate of laboratory-confirmed mycobacterial infection among hospitalized HIV-infected patients to be 16.3%, in close agreement with another study based on bacterial evidence from TB screening in HIV patients, which reported an HIV/TB coinfection rate of 15.0%[2]. At least 48 cases had pulmonary involvement, demonstrating the importance of preventing the nosocomial and community spread of mycobacteria by HIV patients. Although high rates of disseminated mycobacterial infection have been found, accurate diagnosis of localized extrapulmonary infection is difficult[3], particularly in health facilities with limited resources. The absence of rapid, simple, and accurate diagnostic tools for extrapulmonary mycobacterial infection could result in unnecessary treatment and increased mortality in HIV-infected patients[4].

Of the 102 patients studied, fever and weight loss were the two most frequent symptoms, whereas only 31.4% of patients reported a cough and 17.6% reported night sweats. These results demonstrate the risks of reliance upon non-specific symptoms or signs to diagnose TB in HIV patients. Low CD4 cell counts have been used as an index to identify HIV-infected patients at risk of undiagnosed TB. In this study, there were 88.2% patients with CD4 counts below 200 cells/μL. Among patients with disseminated infections, 58.3% had CD4 counts < 50 cells/μL. This result adds to the accumulating evidence that the high incidence of mycobacterial infection observed in HIV-positive patients results from severe immunosuppression, especially in the case of disseminated infection.

In contrast to NTM infection, MTB was proven to be the most prevalent mycobacterial infection in HIV patients in this study. A study conducted in Guangxi province of southern China reported recovery of MTB in 53% and NTM identification in 47% of HIV patients[5]. Considering that the patients

Table 2. CD4 Cell Counts with Different Clinical Manifestations and Mycobacteria Species

CD4 Counts (cells/μL)	HIV/TB patients No. (%)	Clinical Manifestation (*n*)			Mycobacteria Species (*n*)		
		Disseminated	Pulmonary	Extrapulmonary	MTB	NTM	MTB + NTM
< 50	47 (46.1)	20	21	6	41	6	0
50-200	43 (42.1)	15	19	9	35	6	2
> 200	12 (11.8)	3	8	1	8	4	0
Total	102 (100.0)	38	48	16	84	16	2

Note. MTB, *Mycobacterium tuberculosis*; NTM, non-tuberculous mycobacterial.

in our study were from northern China, geographical variation might account for the difference in NTM incidence. Studies indicated that the rate of NTM infection in the non-AIDS population varied from 1.6% to 6.38%[6-7]. The high frequency of NTM among HIV-infected patients raises concerns about the accuracy of species identification prior to determination of the appropriate treatment. This study found relatively high rates of MDR-TB (11.9%) and XDR-TB (3.4%). We attribute this to the inappropriate and excessive use of first-line drugs and diagnostic delays. However, the extraction of drug-resistant data from only two hospitals in this report may limit the applicability of the DST results.

In summary, considering the severity of HIV/TB coinfection in China, proper strategies should be in place to assist in the diagnosis and prevention of epidemic mycobacterial infections; moreover, it is necessary to raise awareness of MDR/XDR-TB in HIV-infected patients.

Table 3. Demographic and Clinical Characteristics *Mycobacteria* Infected Patients

Characteristics	No. (%)
Total	102 (100.0)
Sex	
Male	88 (86.3)
Female	14 (13.7)
Age range (years)	
0-14	1 (1.0)
15-25	12 (11.8)
26-35	32 (31.4)
36-45	35 (34.3)
> 45	22 (21.5)
Transmission route	
Homosexual intercourse	40 (39.2)
Commercial plasma donation	26 (25.5)
Contaminated blood transfusion	13 (12.7)
Heterosexual intercourse	8 (7.8)
Injection drug use	4 (3.9)
Vertical transmission	1 (1.0)
Unknown routes	10 (9.8)
Prior TB treatment history	23 (22.5)
ART	31 (30.4)

Note. Antiretooiral Therapy (AR).

We wish to express our gratitude to DAI Fang Fang, ZHU Dong, LU Liang Ping, WANG Hui Zhu, GAO Gui Ju, CHEN Ming, and WANG Chen for their general technical and statistical assistance.

[&]These authors contributed equally to this work.

[#]Correspondence should be addressed to ZHAO Xiu Ying, Tel: 86-10-56119198, E-mail: zhaoxiuying2001@163.com; WAN Kang Lin, Tel: 86-10-58900779, E-mail: wankanglin@icdc.cn

Biographical notes on the authors: ZHAO Xiu Ying, female, PhD, born in 1969, chief physician, majoring in research on infectious diseases; ZENG Zhao Ying, female, MD, born in 1986, attending physician, majoring in clinical diagnosis; HUA Wen Hao, female, M.D, born in 1975, chief physician, majoring in research on infectious diseases.

Received: July 22, 2017;

Accepted: January 24, 2018

REFERENCES

1. Zumla A, George AV, Sharma V, et al. The WHO 2014 global tuberculosis report--further to go. Lancet Glob Health, 2015; 3, e10-2.
2. Cain KP, McCarthy KD, Heilig CM, et al. An algorithm for tuberculosis screening and diagnosis in people with HIV. N Engl J Med, 2010; 362, 707-16.
3. Leeds IL, Magee MJ, Kurbatova EV, et al. Site of extrapulmonary tuberculosis is associated with HIV infection. Clin Infect Dis, 2012; 55, 75-81.
4. Kufa T, Mngomezulu V, Charalambous S, et al. Undiagnosed tuberculosis among HIV clinic attendees: association with antiretroviral therapy and implications for intensified case finding, isoniazid preventive therapy, and infection control. J Acquir Immune Defic Syndr, 2012; 60, e22-8.
5. Lan R, Yang C, Lan L, et al. Mycobacterium tuberculosis and non-tuberculous mycobacteria isolates from HIV-infected patients in Guangxi, China. Int J Tuberc Lung Dis, 2011; 15, 1669-75.
6. Jing H, Wang H, Wang Y, et al. Prevalence of nontuberculous mycobacteria infection, China, 2004-2009. Emerg Infect Dis, 2012; 18, 527-8.
7. Wang HX, Yue J, Han M, et al. Nontuberculous mycobacteria: susceptibility pattern and prevalence rate in Shanghai from 2005 to 2008. Chin Med J (Engl), 2010; 123, 184-7.