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Original Paper

Nontuberculous mycobacteria among pulmonary tuberculosis patients: a retrospective Belgian multicenter study

Steven De Keukeleire[1], Vanessa Mathys[2], Sigi Van den Wijngaert[3], Martine Van De Vyvere[4], Stijn Jonckheere[5], Hans De Beenhouwer[5], Annelies De Bel[6], Wouter Arrazola de Onate[7], Maryse Wanlin[7], Denis Piérard[1], Eric Nulens[8], Veroniek Saegeman[9,10]

[1]Department of Microbiology and Infection Control, University Hospital Brussel, Vrije Universiteit Brussel (VUB), Brussels, Belgium, [2]National Reference Centre of Tuberculosis and Mycobacteria, Communicable and Infectious Diseases, Scientific Institute of Public Health (WIV-ISP), Brussels, Belgium, [3]Iris-Lab, Department of Microbiology, Iris-Brussels Public Hospitals Network, Brussels, Belgium, [4]Department of Microbiology, ZNA Hospitals, Antwerp, Belgium, [5]Clinical Laboratory of Microbiology, OLVZ Aalst, Aalst, Belgium, [6]Department of Microbiology, AZ Groeninge, Kortrijk, Belgium, [7]Belgian Lung and Tuberculosis Association BELTA, Brussels, Belgium, [8]Department of Microbiology, AZ Sint-Jan Brugge-Ostend AV, Bruges, Belgium, [9]Department of Laboratory Medicine, UZ Leuven, Leuven, Belgium, [10]Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium

Objectives: Currently, there are no European data about the frequency and clinical significance of nontuberculous mycobacteria (NTM) grown from respiratory samples during the treatment of tuberculosis (TB). We determined the frequency and clinical significance of NTM isolated before or during pulmonary tuberculosis treatment in Belgian laboratories.

Methods: We conducted a nationwide retrospective multicenter cohort study on the co-isolation of TB and NTM in Belgium. Starting from laboratory data between 2006 and 2013, possible TB–NTM co-isolations were searched for.

Results: A total of 2569 unique culture-positive pulmonary tuberculosis cases were included in the study. Only 35 (1.4%) of these TB cases had an NTM co-isolated, and two of these 35 fulfilled the ATS criteria for NTM lung disease.

Conclusion: A very low prevalence of 1.4% NTM co-isolations was found in Belgian patients with culture-proven pulmonary TB.

Keywords: Nontuberculous mycobacteria, Multicenter study, *Mycobacterium tuberculosis* complex, Belgium

Introduction

Nontuberculous mycobacteria (NTM) are typically ubiquitous environmental and biologically diverse microorganisms residing in soil and natural environment as well as treated water. Although, generally of low human pathogenicity, NTM may cause pulmonary disease, lymphadenitis, cutaneous disease, or disseminated disease.[1] Little is known about the frequency and clinical significance of NTM grown from respiratory samples during tuberculosis (TB) treatment. Limited studies report a high frequency (7–14%) and clinical relevance of NTM co-isolation in culture-proven pulmonary tuberculosis patients.[2–6] It has been suggested that the incidence of both NTM laboratory isolation and disease prevalence is increasing.[7] Hitherto, no European data are available.

Correspondence to: Steven De Keukeleire, Department of Microbiology and Infection Control, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel (VUB), Laarbeeklaan 101, 1090 Brussels, Belgium. Email: stevendekeukeleire@hotmail.com

Material and Methods

A seven-year retrospective multicenter cohort study was performed to determine possible TB–NTM co-isolations. Data were included from seven participating Belgian (hospital) laboratories: University Hospital Brussel, Brussels; iris-Lab, Brussels; ZNA Hospitals, Antwerp; OLV Hospital, Aalst; AZ Sint-Jan Bruges-Ostend, Bruges; UZ Leuven, Leuven, and from the national reference laboratory of tuberculosis and mycobacteria (NRL), Brussels. Patients were selected from the participating laboratories' information systems

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to include those with a culture-proven TB from respiratory source in the period 1 January 2007 to 31 December 2011, defined by the date of isolation of the first *M. tuberculosis* complex strain ('index TB' isolate). Among these TB patients, NTM isolates were searched in the period 1 November 2006 to 31 December 2013. Those patients whose first NTM isolate from respiratory source ('index NTM isolate') was collected within two months before the sampling date of the TB index isolate and before the end of TB treatment (two years) were retained as the 'TB/NTM co-isolation' group. The respiratory specimens included sputum, bronchial aspirates, lung biopsies, and bronchoalveolar lavage fluids. Patients fulfilling the ATS microbiological criteria for NTM lung disease were classified as significant.[1] Specimen culture for mycobacteria was performed using the Bactec MGIT 960/320 system (BD, Sparks, MD, USA) and Löwenstein–Jensen medium. Species were identified via detection of the IS6110 insertion sequence or 16S-23S rRNA spacer region for *M. tuberculosis* and 16S rRNA sequencing for NTM. Comparisons between groups were performed using unpaired *t*-tests (MedCalc® software, version 11.4.4.0, Ostend, Belgium). The study was approved by the UZ Brussels' Ethics Committee (B.U.N. 143201421655).

Results

From 2007 to 2011, 2569 culture-positive non-duplicate pulmonary TB cases were detected. Of these 2569 index-TB patients, 35 (1.4%) had one or more NTM isolated from respiratory origin during TB treatment ('TB/NTM co-isolation' group), corresponding to 58 culture-positive specimens. The TB/NTM co-isolation group was distributed among the different laboratories as follows: 1/72 from University Hospital Brussel, 4/648 from iris-Lab, 6/191 from ZNA Hospitals, and 24/1322 patients from the NRL. Neither AZ Sint-Jan Bruges-Ostend nor UZ Leuven or OLV Hospital had patients with TB/NTM co-isolates. The median age of these 35 patients was 48.4 years (95% CI 41.359.2) and 54.2% of them were men (Table 1).

The most frequently isolated NTM species were *M. gordonae* ($n = 15$ patients; 43%), *M. fortuitum* ($n = 5$; 14%), and *M. avium* ($n = 4$; 11%). For two patients, the isolated NTM species were not further identified to the species level. Twenty-one out of 35 (60%) patients had a first NTM co-isolate within six months following initial TB-positive culture, more specifically at a median of 42 days (range 0–164) after the first positive TB culture. NTM were isolated in the first 31 days in 12 (34%) patients, with *M. gordonae* ($n = 6$ patients, 50%) as most common species in that period. Eight (23%) patients had NTM isolated more than six months after culture-proven TB, and six (17%) had NTM isolated in the two months prior to the isolation of TB. Only one positive NTM culture was obtained from 30 (86%) of the 'TB/NTM co-isolation' group of patients. The isolated species were *M. gordonae* ($n = 13$), *M. avium* ($n = 4$), *M. fortuitum* ($n = 3$), *M. xenopi* ($n = 2$), *M. chelonae/abscessus* ($n = 2$), *M. intracellulare* ($n = 1$), *M. fortuitum – M. peregrinum – M. septicum* coplex ($n = 1$), *M. gordonae/xenopi* ($n = 1$), two unidentified NTM's, and one mixture with *M. nonchromogenicum* and *M. terrae* mixture. The other five (12%) patients had at least two respiratory cultures positive for NTM. One patient had *M. gordonae* and *M. fortuitum* isolated from two different samples. Only four out of the 35 patients (11%) from the 'TB/NTM co-isolation' group fulfilled the ATS criteria for NTM lung disease (Table 2).

A 'TB/NTM co-infection' was identified with a high degree of certainty among two patients: patient 1 with four isolates of *M. fortuitum – M. peregrinum – M. septicum* complex, and patient 2 with repeatedly ($n = 2$) positive cultures for *M. fortuitum*. There was a third patient from whom *M. gordonae* was isolated twice. Since *M. gordonae* has very low pathogenicity and accordingly considered as contaminant, the clinical relevance is doubtful. Patient 4 was diagnosed with a *M. kansasii* infection, the strain being isolated nineteen times. Isolation of *M. tuberculosis* complex in this patient was considered as a patient mix-up since the cultures over the following three months remained negative for *M. tuberculosis* complex (Table 2).

Table 1 Characteristics of culture-proven pulmonary TB cases by NTM co-isolation status

	Culture-positive pulmonary TB cases (no. = 2569)	TB/NTM co-isolation group[a] (no. = 35)	*p* Value*
Sex, no. (%)			
Female	838 (32.6)	16 (45.7)	
Male	1688 (65.7)	19 (54.2)	
Not reported	43 (1.7)	/	
Median age, y (95% CI)			
Total	39.0 (38.0–40.0)	48.4 (41.3–59.2)	0.15
Female	35.0 (34.0–37.0)	41.3 (26.1–67.1)	0.23
Male	42.0 (40.0–43.0)	49.7 (45.5–60.7)	0.31
Not reported (no.)	34		

[a]TB/NTM co-isolation patients with first NTM isolate collected within two months before the sampling date of the TB index isolate and before the end of TB treatment.
*Comparison between TB/NTM co-isolation group and culture-positive pulmonary TB cases with unpaired *t*-test.

Table 2 Demographic and clinical characteristics of patients with TB/NTM co-isolation from respiratory specimens[a]

Patient	Patient 1	Patient 2	Patient 3	Patient 4
Age (years)	50	48	27	36
Gender	Man	Man	Woman	Woman
Country of origin	Romania	Ghana	N/A	Portugal
Tobacco use	No active or history of smoking	History of smoking	N/A	Active smoker
Underlying lung disease	N/A	N/A	N/A	Previous pulmonary NTM disease (M. kansasii), interstitial pneumonia (*P. jirovecii*)
Other co-morbidities	N/A	N/A	Absent	Recurrent Cytomegalovirus infections, resolved Hepatitis C virus infection, recurrent oropharyngeal Candida infections
Presenting symptom(s)	N/A	Cough, haemoptysis, nausea, vomiting, weight loss	N/A	Cough, nausea, cachexia
HIV status	Negative	Negative	Negative	Positive
TB susceptibility	Multisusceptible	Multisusceptible	Multisusceptible	N/A
Radiology findings	Consolidation area – cavity	Consolidation/ cavity area	N/A	Fibrotic lung changes
NTM species	*M. fortuitum – M. peregrinum – M. septicum*	*M. fortuitum*	*M. gordonae*	*M. kansasii*
No. of positive cultures for NTM	4	2	2	19
Therapy compliance/adherence	Completed therapy	Completed therapy	Lost to follow up	Non-compliance, lost to follow up

Notes: N/A = not available; NTM = non-tuberculous mycobacteria; MTB = *M. tuberculosis* complex.
[a]Patients fulfilling the ATS, 2007 microbiological criteria (*1*).

Description of the TB/NTM Co-infection Cases

Patient 1, a Romanian 50-year old man was imprisoned at diagnosis. He had no tobacco history, no underlying co-morbidities, and was HIV seronegative. Throracic CT-scan was suggestive of tuberculosis with peribronchial consolidation zones in right and left upper lung lobes. There are also calcified lesions diffusely spread over both lungs. Chest X-ray confirmed these bilateral diffuse infiltrates. Therapy was initiated and adapted based on antimycobacterial susceptibility testing, (isoniazid (INH), rifampicin (RIF), ethambutol (EMB), pyrazinamide (PZA), and moxifloxacin, followed by INH, RIF, and PZA, and finally INH and RIF) during a six-month period.

Patient 2 was a Ghanaian 48-year old man, admitted to hospital with complaints of cough, haemoptysis, nausea, sporadic vomiting, and a non-intentional weight loss of 10 kilograms. The patient did not indicate a history of fever or night sweats. He recently stopped smoking, had no underlying pulmonary disease, and was HIV seronegative. Thoracic CT-scan was compatible with open lung tuberculosis; there was a large cavitary lesion in the upper lobe and some smaller lesions apically in the right lung, in the middle of the right lung, and in the left upper lung lobe. Chest X-ray showed a large lesion with cavities in the upper lobe of the right lung. He received standard anti-TB treatment, consisting of RIF, INH, EMB, and PZA during nine months.

Patient 3, a 27-year old female patient, had no underlying co-morbidities, and was HIV seronegative. No information regarding medical imaging was available. Standard quadruple anti-TB therapy was started, but she was lost to follow up.

Patient 4 was a 36-year old HIV-seropositive Portuguese woman, admitted with general malaise and virus deterioration. She complained of a productive cough since two weeks associated with dysphagia and nausea. She presented with cachexia, due to multiple opportunistic infections in the past: interstitial pneumonia with *P. jirovecii*, CMV-driven cholangitis, Hepatitis C infection with viral clearance, and she experienced a pulmonary infection with *M. kansasii* in 2008. Moreover, the patient was non-compliant to her antiretroviral triple therapy and she was an active smoker. She suffered from a chronic pancytopenia due to the HIV infection, CMV viraemia, and drug-induced toxicity to sulfomethoxazol–trimethoprim. Physical, cardiovascular, and neurological examinations were clinically normal, besides an oropharyngeal mycosis. Chest radiography revealed few fibrotic changes and clear costodiafragmatic sinuses. She refused anti-tuberculous treatment, and was lost to follow up (Table 2).

Discussion

To our knowledge, this is the first laboratory-based study in Belgium, Europe to examine the proportion of culture-proven pulmonary TB patients in whom NTM were isolated during TB therapy. In this Belgian, multicenter study, NTM and TB were co-isolated in only 35 patients, reaching a prevalence of 1.4% culture-proven pulmonary TB patients having a positive NTM culture two months before the index TB isolate or during TB treatment. In 2012, 987 TB cases were newly diagnosed in Belgium. The Belgian national TB incidence is 8.8/100.000 and is considered low (Belgian Register Tuberculosis 2012, VRGT-FARES-BELTA). Unfortunately, because

there is no mandatory registration of NTM in Belgium, comparison of data of TB/NTM co-infections with national figures is impossible. Compared with data from previous authors, our prevalence of 1.4% culture-proven pulmonary TB/NTM patients is low. Yet, the definitions of co-isolation used in the different studies are variable.[2–4] A possible explanation for the low prevalence of TB/NTM co-infections in this study is the lack of systematical identifications of all consecutive positive cultures from a known TB patient in the first month after the index isolate. Also, some laboratories only performed a *M. tuberculosis* complex-specific PCR directly on samples. This could have caused missing NTM co-isolates during the TB infection. Although, no higher prevalence of co-infections was observed in the laboratories that use solid media enabling the visualization of mixed growth by colony growth and pigmentation.The strength of this study is lying in its multicentric cohort upset and the relative large sample size included. Although, this study also had some limitations. First, routine clinical care led to variable numbers of mycobacterial specimens collected at variable times during and after TB treatment, which may have biased the detection of NTM toward patients who gave more samples. The variable sampling frequency may also have led to an underestimation of the true frequency of NTM co-isolates. Patients from whom more samples were available, may have had more symptoms and thus more examinations.[4] Second, because of the retrospective study design, follow-up cultures were obtained at the discretion of the treating physician. Third, especially knowing the tedious nature of Mycobacteria in culture, the different laboratories used different mycobacteriological sampling, culture, and identification techniques. Fourth, the lack of consistency among clinicians and hospitals in clinically diagnosing patients may have skewed the data. Fifth, since there is no mandatory registration of NTM in Belgium, this could have introduced a selection bias. And sixth, there was no participating laboratory from Limburg and Wallonia. Since the national reference center of tuberculosis and mycobacteria was one of the participating laboratories though, we do have included strains from these regions.

In conclusion, compared to international data, a very low prevalence of 1.4% NTM co-isolations in patients with culture-proven pulmonary TB was found in Belgium between 2006 and 2013. Moreover, the cases found in this multicenter retrospective survey largely represent colonization. In only two patients, TB/NTM co-infection could be considered significant. Current laboratory practices not focusing on the detection of TB/NTM co-infections may be responsible for this low prevalence and warrants reconsideration. This study demonstrates that either the handling of mycobacterial cultures might be limited to expert laboratories or national guidelines for mycobacterial diagnostics could be established in order to guarantee high quality.

Disclaimer statements

Contributors SDK was responsible for the study setup, data analysis, interpretation, and writing of the manuscript. VM, SvDW, MVDV, SJ, HDB, ADB, WAdO, MN, DP, EN, and VS participated in the data acquisition. SDK performed the data analysis, analyzed the data, and wrote the paper. All authors critically evaluated the article and gave their final approval before submission.

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Conflicts of interest The authors report no declarations of interest.

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References

1 Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med. 2007;175(4):367–416.
2 Huang CT, Tsai YJ, Shu CC, Lei YC, Wang JY, Yu CJ, et al. Clinical significance of isolation of nontuberculous mycobacteria in pulmonary tuberculosis patients. Respir Med. 2009;103(10):1484–91.
3 Jun HJ, Jeon K, Um SW, Kwon OJ, Lee NY, Koh WJ. Nontuberculous mycobacteria isolated during the treatment of pulmonary tuberculosis. Respir Med. 2009;103(12):1936–40.
4 Damaraju D, Jamieson F, Chedore P, Marras TK. Isolation of nontuberculous mycobacteria among patients with pulmonary tuberculosis in Ontario, Canada. Int J Tuberc Lung Dis. 2013;17(5):676–81.
5 Kendall BA, Varley CD, Hedberg K, Cassidy PM, Winthrop KL. Isolation of non-tuberculous mycobacteria from the sputum of patients with active tuberculosis. Int J Tuberc Lung Dis. 2010;14(5):654–6.
6 Epstein MD, Aranda CP, Bonk S, Hanna B, Rom WN. The significance of mycobacterium avium complex cultivation in the sputum of patients with pulmonary tuberculosis. Chest. 1997;111(1):142–7.
7 Johnson MM, Odell JA. Nontuberculous mycobacterial pulmonary infections. J Thorac Dis. 2014;6(3):210–2.