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Original Article

A decade of challenges: ten-year analysis of non-tuberculous Mycobacteria infections in Vojvodina, Serbia

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Abstract

Introduction: Non-tuberculous mycobacteria (NTM) are a diverse group of environmental microorganisms, most non-pathogenic. Most people come into contact with NTM during their lives. Still, the infection occurs in people with previous lung comorbidities, weakened immune systems, and the elderly. This study aims to analyze the clinical characteristics of patients with NTM.

Methodology: The research was conducted in the form of a retrospective study, which included 23 patients with a diagnosis of NTM who were treated at the Clinic for Tuberculosis and Interstitial Lung Diseases of the Institute of Pulmonary Diseases of Vojvodina in Sremska Kamenica from 2014 to 2023.

Results: Patients were predominantly male (15, 65.2%). The most common type of NTM was *Mycobacterium xenopi* in 8 patients (34.8%), as well as the way of establishing the diagnosis in patients who had negative direct microscopy and culture confirmation (12, 52.2%), followed by positive direct microscopy and culture confirmation (10, 43.5%). Smokers were the most frequent (10, 55.6%). The most common comorbidity was chronic obstructive pulmonary disease (10, 43.5%). A statistically significant difference was found in the frequency of the method of proof across different types of non-tuberculous mycobacterium infections (Fisher's exact test = 21.928; $p = 0.006$). Fatal outcomes were seen in 17.6% of patients.

Conclusions: A detailed history, evaluation of clinical features, radiological findings, and microbiological samples are required in patients with suspected NTM infection. Raising suspicion and speeding up diagnostic procedures in these patients is of great importance for the timely initiation of treatment and reduction of mortality.

Key words: Non-tuberculous mycobacteria; infection; diagnostic; therapy.

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Introduction

Non-tuberculous mycobacteria (NTM) are airborne, waterborne, and soil-dwelling organisms. Most of the 190 species and subspecies of environmental microorganisms known as NTM are non-pathogenic. All individuals come into contact with NTM at some point in their lives. However, the infection is more common in older individuals, those with compromised immune systems, and those with pulmonary comorbidities such as bronchiectasis or chronic obstructive pulmonary disease (COPD). They are not typically spread by contact with an infected person (except in some cases of cystic fibrosis) [1-3]. *Mycobacterium avium complex* (MAC), *Mycobacterium xenopi,* and *Mycobacterium kansasii*, which are slow-growing NTMs, and *Mycobacterium abscessus*, a fast-growing NTM, are the NTMs that cause lung infections. *Mycobacterium avium complex* (MAC), then *Mycobacterium abscessus* and *Mycobacterium kansasii*, is the most frequent causal agent of pulmonary NTM disease [1,2,4].

Skin, soft tissue, lymph nodes, and bones are among the numerous body areas where some NTM can cause disease. Fast-growing mycobacteria, including *Mycobacterium abscessus*, *Mycobacterium chelonae,* and *Mycobacterium fortuitum,* can cause soft tissue and skin diseases in adults, including nosocomial surgery site infections. Severe extrapulmonary or disseminated

NTM infections occur in immunosuppressed adults-patients with human immunodeficiency virus (HIV), posttransplantation, or other cellular immunity deficiencies [1-3].

Individual differences exist in the clinical picture of NTM lung disease, including differences in severity and symptoms. Some individuals may not have any symptoms at all. Most NTM lung disease patients have symptoms comparable to numerous other lung diseases. Identifying the symptoms of NTM lung disease might be challenging since many persons infected with the virus already have chronic lung disease. The most typical symptom is a persistent cough that expectorates mucus. If the disease progresses, blood may occasionally be coughed up. Other typical symptoms that can aid in differentiating NTM from other lung disorders include low-grade fever, night sweats, persistent weariness, inexplicable weight loss and appetite reduction, breathing difficulties, chest discomfort, and recurrent respiratory infections, which may also occur despite antibiotic therapy [2].

Clinical, radiological, and microbiological diagnostic criteria help to diagnose non-tuberculous mycobacterium infection. Clinical signs include systemic or pulmonary symptoms, as mentioned in the text previously. Radiological signs such as nodular or cavitary opacities on chest radiographs or a high-resolution computed tomography scan (HRCT) that shows bronchiectasis with multiple small nodules occur in these patients. Microbiological diagnosis is crucial-positive culture results from at least two separate expectorated sputum samples. If the results are non-diagnostic, consider repeat sputum *acid-fast bacillus* (AFB) smears and cultures or positive culture results from at least one bronchial wash or lavage or transbronchial or other lung biopsy with mycobacterial histologic features (granulomatous inflammation or AFB) and positive culture for NTM or biopsy showing mycobacterial histological features (granulomatous inflammation or AFB) and one or more sputum or bronchial washings that are culture positive for NTM is needed for the diagnosis. One of the three microbiological, clinical, and radiological criteria is needed to establish a diagnosis of NTM infection and eliminate alternative diagnoses. The most recent ATS/ERS/ESCMID/IDSA guidance from 2020 offers therapy for various forms of NTM. Knowledge of tuberculosis and NTM infections among patients with the disease is crucial for early disease recognition, patients' full cooperation during the treatment, and prevention of future relapses [4,5].

This study aims to examine the clinical features of NTM patients treated at the Institute for Pulmonary Diseases of Vojvodina for ten years.

Methodology

Study setting and period

This retrospective ten-year analysis included 23 patients with NTM infection who received treatment at the Clinic for Granulomatous and Interstitial Lung Diseases of the Institute for Pulmonary Diseases of Vojvodina (IPDV) in Sremska Kamenica from January 2014 to December 2023.

Data collection

Data on patients with NTM infection in the IPDV electronic database were gathered for the study's initial phase. The Integrated Health Information System was the computerized database used to gather data. The required data were collected by looking through the electronic database, and in subsequent work, they were statistically processed. The following information was taken from the data: sex, type of NTM, method of detection, radiological findings, smoking status, alcohol consumption, comorbidities, therapy, and death outcome.

Statistical analysis

Descriptive statistical methods and statistical hypothesis testing methods were used for primary data analysis. Relative numbers and percentages were used for the descriptive statistical methods. For testing statistical hypotheses, non-parametric statistical analysis - Fisher's exact test was used. Hypotheses were tested at the level of statistical significance (α level) of 0.05. The results are tabulated. Data was processed using standard statistical packages (IBM SPSS Statistics 26).

Results

Among the patients, the number of male patients was 15 (65.2%), and the number of females was 8 (34.8%). The most common type of NTM was *Mycobacterium xenopi* in 8 patients (34.8%). The most common way of establishing the diagnosis was culture confirmation. We divided the patients into groups: those who had negative direct microscopy and culture confirmation, 12 (52.2%), and those with positive direct microscopy and culture confirmation, 10 (43.5%). In 9 (42.0%) patients, there were cavernous changes in radiological findings; bilateral changes were found in 8 (38.1%) and unilateral in 4 (19.0%). Smokers were the most frequent– 10 (55.6%), and patients with no previous alcohol consumption anamnesis– 18 (88.9%).

The most common comorbidity was COPD in 10 (43.5%) (Table 1).

A statistically significant difference was found in the frequency of the method of proof across different types of non-tuberculous mycobacterium infections (Fisher's exact test = 21.928; $p = 0.006$). Positive direct microscopy with culture confirmation was the most frequent proof method for patients with *Mycobacterium xenopi* infection, while negative direct microscopy with culture confirmation was the most frequent for patients with *Mycobacterium avium* infection. There was no statistically significant difference in the frequency of radiological findings among patients with non-tuberculous mycobacterium infections (Fisher's exact test = 11.357; $p = 0.539$) (Table 2).

Three patients (17.6%) died as a result of the infection, one in each case involving *Mycobacterium abscessus*, *Mycobacterium xenopi*, and *Mycobacterium malmoense* infection. No statistically significant difference was found in the distribution of non-tuberculous Mycobacterium in patients who died (Fisher's exact test = 9.625; $p = 0.076$).

Different methods of proof and therapy for patients with non-tuberculous Mycobacterium infections can be found in Table 3.

Table 1. Frequency and percentage of patients with non-tuberculous mycobacterium infections across different variables.

Variables	n	%
Sex		
Male	15	65.2
Female	8	34.8
Type of NTM		
M. xenopi	8	34.8
M. avium	7	30.4
M. kansasi	2	8.7
M. chelonae	2	8.7
M. intracellulare	2	8.7
M. malmoense	1	4.3
M. abscessus	1	4.3
Method of detection		
Negative DM and culture confirmation	12	52.2
Positive DM and culture confirmation	10	43.5
Molecular testing	1	4.3
Radiological findings		
Cavernous changes	9	42.9
Bilateral changes	8	38.1
Unilateral changes	4	19.0
Smoking status		
Smoker	10	55.6
Non-smoker	5	27.8
Former smoker	3	16.7
Alcohol consumption		
No	18	88.9
Yes	2	11.1
Comorbidities		
COPD	10	43.5
HTA	8	34.8
Others	5	21.7

NTM: non-tuberculous Mycobacterium; DM: direct microscopy; COPD: chronic obstructive pulmonary disease; HTA: hypertension.

Discussion

Based on our examination of the gender structure, male patients were predominant. According to Gopalaswamy *et al.* in a 12-year study (2008-2019) done in the United States, patients with NTM-pulmonary disease (NTM-PD) were more frequently female. Santos *et al.* conducted a study in Portugal and found no statistically significant gender differences, but patients were more frequently male [6,7].

Mycobacterium xenopi and *Mycobacterium avium* were most frequently isolated in patients who had positive direct microscopy with culture confirmation. *Gopalaswamy* and associates identified the most prevalent was *Mycobacterium avium*, which was followed by *Mycobacterium Fortuitum*, *Mycobacterium abscessus*, *Mycobacterium chelonae,* and the rarest was *Mycobacterium kansasii*. According to Santos *et al.,* most infections are caused by the *Mycobacterium avium complex*, *Mycobacterium abscessus-chelonae complex* (MABC), and *Mycobacterium fortuitum* [4,7].

The most recent 2020 guidelines state that clinical, radiographic, and microbiological techniques are necessary to diagnose NTM. More than one positive sputum culture is necessary to diagnose, while two or more positive sputum cultures are advised for *Mycobacterium abscessus* diagnosis. In individuals with one positive sputum culture at initial evaluation, clinically significant MAC lung disease is rare but may reach up to 98% in those with two or more positive cultures [4].

Table 2. Bacterial detection and radiological findings in patients with non-tuberculous Mycobacterium infections, n (%).

Type of NTM	Method of detection			Radiological findings		
	Positive DM and culture confirmation	Negative DM and culture confirmation	Molecular testing	Unilateral	Bilateral	Cavernous changes
M. malmoense	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)
M. intracellulare	1 (50.0%)	0 (0.0%)	1 (50.0%)	0 (0.0%)	1 (50.0%)	1 (50.0%)
M. xenopi	6 (75.0%)	2 (25.0%)	0 (0.0%)	3 (42.9%)	1 (14.3%)	3 (42.9%)
M. abscessus	0 (0,0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)
M. kansasi	1 (50.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)	1 (50.0%)
M. avium	0 (0.0%)	7 (100.0%)	0 (0.0%)	0 (0,0%)	4 (66.7%)	2 (33.3%)
M. chelonae	1 (50.0%)	1 (50.0%)	0 (0.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)

NTM: non-tuberculous Mycobacterium; DM: direct microscopy.

Table 3. Bacterial detection and therapy in patients with non-tuberculous Mycobacterium infections.

Type of NTM	Method of detection	Therapy
M. xenopi	Positive DM and Culture Confirmation (6) Negative DM and Culture Confirmation Cultures (2)	R, E, Azithromycin
M. avium	Cultures (7)	H, R, E, Ofloxacin, Clarithromycin, Azithromycin
M. kansasi	Positive DM and Culture Confirmation (1) Negative DM and Culture Confirmation (1)	H, R, Z, E, Clarithromycin, Ofloxacin
M. chelonae	Positive DM and Culture Confirmation (1) Negative DM and Culture Confirmation (1)	Amikacin, Azithromycin, Ciprofloxacin, Levofloxacin
M. intracellulare	Molecular Analysis (1) Positive DM and Culture Confirmation (1)	R, E, Azithromycin
M. malmoense	Positive DM and Culture Confirmation (1)	H, R, Z, E, Clarithromycin, Ofloxacin
M. abscessus	Negative DM and Culture Confirmation (1)	Ciprofloxacin, Clarithromycin

NTM: non-tuberculous Mycobacterium; DM: direct microscopy; H: Isonisazid, R: Rifampicin, E: Ethambutol, Z: Pyrazinamide.

Sputum samples from the patients in our study were used for diagnosis. According to a study by Martin *et al*., most NTM species were isolated from lung samples- primarily sputum samples, bronchial lavage, and endotracheal aspirate [8].

Nodular or cavitary changes on a chest X-ray or bronchiectasis with many tiny nodules on high-resolution computed tomography (HRCT) are radiological abnormalities that suggest NTM infection [4]. According to a study by *Nonak* and colleagues, consolidation was the rarest finding. In contrast, nodular/granular shadows were the most prevalent radiological appearance, and the prevalence of bronchiectasis was less common [9]. Although bilateral abnormalities were more common than unilateral ones, cavernous changes were still in our study's most common radiological manifestation.

Precisely 55.6% of participants in our study were smokers, and 16.7% were former smokers. When *Nonaka* and associates looked at risk variables in patients with MAC infection, they found that most patients were non-smokers, followed by former smokers [9].

Of the patients in the sample, 88.9% did not drink alcohol, compared to just 11.1% who did. A study on the impact of alcohol consumption on the results of NTM treatment was carried out by Jacob *et al.* The result showed that drinking alcohol was linked to a worse outcome [10].

In our samples, COPD and arterial hypertension (HTA) were the most common comorbidities among patients with confirmed NTM infection; other comorbidities were less common. According to a 2020 literature review and meta-analysis on the relationship between comorbidities and NTM infection, respiratory comorbidities include bronchiectasis, prior pulmonary tuberculosis, interstitial lung disease, COPD, and asthma, which pose the highest risk for NTM pulmonary diseases. According to Uno *et al.* NTM disease was linked to both men and women in cases of comorbidities like aspergillosis, asthma, chronic heart failure, diffuse panbronchiolitis, gastroesophageal reflux, interstitial pneumonia, lung cancer, cancer other than breast, lung, ovarian, or prostate cancer, and rheumatoid arthritis. Men's NTM disease was linked to chronic obstructive pulmonary disease, while women's NTM disease was linked to osteoporosis, Sjogren's syndrome, and chronic kidney disease [10,11].

In our study, fatal outcomes occurred in 17.6% of patients. The cause of death in two individuals was found to be NTM infection combined with respiratory insufficiency brought on by long-term COPD, whereas NTM infection and pulmonary thromboembolism was the cause of death in one patient. In the study by Fleshner *et al.,* fibrocavitary disease and pulmonary hypertension were associated with a significantly elevated risk of mortality [12].

Im *et al.* examined the connection between patients' deaths, diagnostic dates, and therapy start dates. Among all patients, the median waiting period without antibiotics was 4.8 months. Six months after starting therapy, 67% of patients had achieved culture conversion, and 135 patients had passed away. No correlation was seen between the waiting period and death or a 6-month culture conversion. On the other hand, a substantial inverse relationship between lethal results and 6-month culture conversion was observed [13].

The limitations of our study were that the research was conducted in a single institution within the nation—a nation with a tiny population—and our study was limited by the small number of patients. Since some patients have had lung conditions for a long time and cannot discriminate between newly generated and preexisting respiratory symptoms, the data about the length of symptoms was untrustworthy, and we didn't include them in our study. We didn't have access to data regarding risky behaviors, such as alcohol usage and smoking histories, for some of our patients.

Conclusions

Most NTM infections happen to those who have

already had lung tissue damage. The nonspecific symptoms can mimic those of other lung conditions. Patients with suspected NTM infection must have a thorough history, clinical feature evaluation, radiological findings, and microbiological samples. Increasing suspicion and expediting diagnostic processes in these patients is crucial for starting therapy on time and lowering mortality.

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Conflict of interest

No conflict of interest is declared.

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