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Research paper

Diagnosis and treatment of patients with pulmonary nontuberculous mycobacterial diseases in Arkhangelsk, Russia

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ABSTRACT

Background: Nontuberculous mycobacteria (NTM) are acid-fast bacilli (AFB) that can cause disease in human. Patients with NTM pulmonary disease can be falsely diagnosed with pulmonary tuberculosis (TB) due detection of AFB in sputum and similar clinical and chest X-ray picture. Laboratory detection of NTM is complicated and does not always mean presence of the disease, but can be attributed to colonization or sample contamination. Molecular tests, such as Genotype Mycobacterium CM/AS, allow quick and reliable detection of NTM.
Objective: To assess the NTM identification rate, to estimate the incidence of pulmonary NTM disease and to report the treatment outcomes among patients with NTM disease.
Design: Retrospective cohort design.
Results: NTM were detected among 92 (0.98 per 100,000 population) presumptive pulmonary TB patients in Arkhangelsk region in 2010–2017 among who 39 (0.42 per 100,000 population) patients were diagnosed with NTM disease. The most prevalent species found in our study were *M. avium* (33%) and *M.intracellulare* (11%). 69% of patients with NTM disease completed their treatment, 15% died, 13% were lost to follow up and 3% failed treatment.
Conclusion: A system of diagnostics and treatment for NTM disease was set up in the Arkhangelsk region in Russia. Average NTM identification rate and incidence of pulmonary NTM disease were 0.98 per 100,000 and 0.42 per 100,000 population accordingly and were lower than reported in other studies. Treatment success rate in our study was 69% encouraging further improvements in diagnostics and treatment of patients with NTM.

1. Introduction

Nontuberculous mycobacteria (NTM) comprise a large number of Mycobacterium species that are found in the environment and are detected increasingly in patients around the world, including in the Nordic countries (Hermansen et al., 2017; Tuberkulose i Norge 2015 - Med Behandlingsresultater for 2014, 2016). According to the American Thoracic society the incidence of NTM diseases in industrial countries varies from 1.0 to 1.8 cases per 100,000 population (Griffith et al., 2007). However, it is complicated to accurately determine the incidence and prevalence of mycobacteria in a population because cases of NTM diseases are not subject to mandatory registration and reporting (Johnson and Odel, 2014). Some species are frequently associated with disease, while others only rarely. NTM diseases most commonly involve the lungs and have a clinical-radiological picture similar to tuberculosis (TB) (Griffith et al., 2007; Johnson and Odel, 2014) making diagnosis of NTM disease difficult. NTM are acid-fast bacilli (AFB) detected by microscopy of sputum and look similar to *M.tuberculosis*, but have natural resistance to important anti-TB drugs. Treatment of NTM diseases is different from TB. NTM are currently not considered communicable so no contact tracing or isolation of the patient is needed, therefore it is important to know whether detected AFB are NTM or *M.tuberculosis*. There are no standardized treatment regimens for all species of NTM, creating high risk of poor treatment outcomes (Griffith et al., 2007; Johnson and Odel, 2014). Laboratory detection of NTM does not always indicate disease, but can be laboratory findings due to colonization or sample contamination (Johnson and Odel, 2014). Criteria for diagnosing NTM disease were developed that included recommendation for

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treatment (Griffith et al., 2007). In order to detect and treat cases of NTM disease it is important to know the burden of NTM and how they are managed in the health system.

The burden of TB is falling in the Russian Federation (World Health Organization. Global tuberculosis report, 2018) and as a notifiable disease it has a fairly complete registration of cases; however, information on incidence and treatment outcomes of NTM in the country is scarce (Zhuravlev et al., 2014; Petrova et al., 2018; Smirnova et al., 2017; Ergeshov et al., 2016). Arkhangelsk region, located in the northwest of Russian Federation, is characterized by reduction of TB incidence, prevalence and mortality rate (Eliseev et al., 2016). A regional TB register includes all presumptive TB patients of the region. It also includes cases of NTM and the results of their investigations and treatment since 2010 when Genotype Mycobacterium CM/AS (Hain Lifescience, Germany) assay was introduced for detection of NTM, replacing phenotypical methods such as rate of growth, incubation temperature, morphology of colonies and biochemical tests (Vincent et al., 2003), providing more accurate detection of NTM.

Aims of our study were 1) to assess the NTM identification rate; 2) to estimate the incidence of NTM disease among the persons with identified NTM; 3) to report the treatment outcomes among patients diagnosed with NTM disease.

2. Materials

2.1. Study design

This was a retrospective cohort study.

2.2. Setting

The study area was Arkhangelsk region, northwest Russia, with a population of 1.12 million, and an area of 410,000 km^2. Health services in the 20 districts of the region are provided by > 50 hospitals and outpatient clinics, where sputum microscopy and chest X-ray (CXR) for TB are performed.

2.3. Patient population

Persons with positive results of sputum microscopy for AFB, and/or cough for more than two weeks, and/or CXR abnormalities suggestive of TB were presumed having TB and were referred to the TB program system. The specialized TB control services in the Arkhangelsk region followed the TB control principles in the Russian Federation, provided by The Arkhangelsk Clinical Anti-tuberculosis Dispensary (ACAD) or district ambulatory TB units (Perelman, 2000). ACAD - central facility performing diagnosis and treatment of TB in the region where all persons with presumptive TB were investigated by CXR and sputum microscopy, culture, and line-probe assay (LPA) to detect mycobacteria. ACAD Laboratory participates in national external quality assessment for all methods detecting TB and NTM according to national recommendations. Samples with mycobacteria were tested with LPA (Genotype MTBDR*plus*) for initial identification (MTBDRplus, 2012). Genotype MTBDR*plus* detects DNA of *M.tuberculosis* and targets rpoB, katG and inhA genes associated with drug resistance to rifampicin and isoniazid.

Positive *M.tuberculosis* complex zone of Genotype MTBDR*plus* confirmed presence of *M.tuberculosis*. Negative *M.tuberculosis* complex zone with no evaluable resistance pattern developed suggested that the bacteria did not belong to *M.tuberculosis* complex (MTBDRplus, 2012). Samples positive for AFB where Genotype MTBDR*plus* failed to detect *M.tuberculosis* were cultured using Bactec MGIT 960 system after decontamination using NaOH-NALC and tested for NTM using Genotype Mycobacterium CM/AS to identify mycobacteria.

Genotype Mycobacterium CM/AS is based on a PCR technique targeting a 23S rRNA gene region, followed by reverse hybridization and line probe technology (Richter, 2006; Genotype Mycobacterium and Genotype Mycobacterium, 2011). Genotype Mycobacterium CM identifies following mycobacterial species: *M. avium, M. chelonae, M. abscessus, M. fortuitum, M. gordonae, M. intracellulare, M. scrofulaceum, M. interjectum, M. kansasii, M. malmoense, M. marinum/M. ulcerans, M. peregrinum, and M. xenopi*. Genotype Mycobacterium AS identifies following mycobacterial species: *M. simiae*, *M. mucogenicum*, *M. goodii*, *M. celatum*, *M. smegmatis*, *M. genavense*, *M. lentiflavum*, *M. heckeshornense*, *M. szulgai/M. intermedium*, *M. phlei*, *M. haemophilum*, *M. kansasii*, *M. ulcerans*, *M. gastri*, *M. asiaticum* and *M. shimoidei*.

Presence of Genus control and/or species-specific banding pattern was considered as detection of a specific species of NTM. Absence of genus control and species-specific pattern was considered as a negative result, thus no NTM was detected. In case of positive genus control but absent species-specific pattern test was considered positive and NTM were detected but species of NTM could not be identified. Further identification of those NTM was not available in the setting.

A person in whom NTM was detected in two or more clinical samples, who had a clinical symptoms and CXR signs suggestive of mycobacterial disease was diagnosed with NTM disease according to recommendations (Griffith et al., 2007). Patients with NTM disease were followed up by TB specialists. Culture conversion and negative culture at the end of treatment were the main criteria for treatment success in NTM (van Ingen et al., 2018). 'Treatment success' was registered in patients in whom three or more consecutive cultures taken at least 30 days apart were negative with no positive cultures with the causative species after culture conversion and until the end of treatment. 'Treatment failure' was registered in patients with positive cultures during and at the end of treatment. 'Lost to follow up' - patients who interrupted their treatment for 2 consecutive months or more. 'Died' - a patient who died for any reason during the course of treatment.

NTM disease was not registered in a person in whom NTM was detected in only one clinical sample or with a clinical and CXR signs not suggestive of NTM disease.

2.4. Data collection and variables

All persons that were tested for NTM were identified in regional TB register and laboratory registers, with information on NTM disease, treatment and outcome. Additional variables collected were age, sex, HIV status, CXR findings, result of microscopy and culture, result of LPA for TB and NTM, NTM species, number of samples positive for NTM. Chi-square was used to compare difference in CXR findings and *t*-test was used to compare age between cases of NTM disease and persons with no NTM disease in whom NTM were detected. Notification rate of pulmonary NTM diseases was calculated as number of cases of NTM disease notified in a given year per 100,000 population.

2.4.1. Ethics

The study was approved by the ethics committee of Northern State Medical University in Arkhangelsk, Russian Federation (approval protocol № 01/02–18).

3. Results

From 2010 to 2017 overall 10,391 persons were tested for TB with sputum microscopy and/or culture and 3085 new cases and 524 retreatment cases of TB were registered in Arkhangelsk region. During that period 153 presumptive TB patients were tested for NTM using Genotype Mycobacterium CM/AS (Fig. 1), as LPA did not detect *M.tuberculosis*, even though microscopy and/or culture detected the presence of AFB. Average age among all tested for NTM was 49 (SD 19), 99 were male (64.7%) and 54 (35.3%) were female.

AFB - acid-fast bacilli; LPA - line probe assay; NTM - nontuberculous mycobacteria; TB - tuberculosis.

Among 153 persons tested 92 persons had positive result for NTM

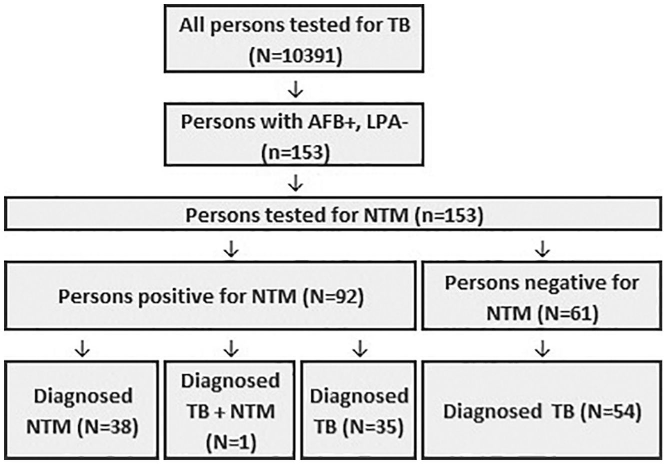

Fig. 1. Diagnostic algorithm of nontuberculous mycobacteria (NTM) diseases used for patients investigated for tuberculosis (TB) in Arkhangelsk region in 2010–2017.

Table 1
Incidence of NTM from 2010 till 2017.

Year	Population	Persons with identified NTM	Persons with NTM disease	NTM identification rate per 100,000 population	Incidence of pulmonary NTM disease per 100,000 population
2010	1,254,449	12	5	0.96	0.40
2011	1,224,880	7	3	0.57	0.24
2012	1,171,096	10	4	0.85	0.34
2013	1,159,506	14	9	1.21	0.78
2014	1,148,760	15	4	1.31	0.35
2015	1,139,950	13	6	1.05	0.53
2016	1,130,240	10	4	0.88	0.35
2017	1,121,813	12	4	1.07	0.36
Total	9,350,694	92	39	0.98	0.42

(60%), out of whom 39 were diagnosed with pulmonary NTM disease, including one patient to have TB and NTM disease comorbidity, resulting in an annual incidence varying from 0.24 to 0.78 (average 0.42) per 100,000 per year (Table 1). The remaining 53 persons were considered not to have NTM disease and 35 of them were later diagnosed with TB (Fig. 1). Patients registered with NTM disease had a mean age of 59 years and 56% were men, whereas patients without NTM disease had a significantly lower ($p < 0.05$) mean age of 46 years and 62% were men.

All 153 persons tested for NTM were also tested for HIV. 4 (3%) were HIV positive and among them 3 were positive for NTM (*M. avium*) - 2 were registered with NTM disease.

Among the 39 patients with NTM disease, microscopy for AFB was positive in 28 (71.8%) cases and culture in 38 (97.4%) cases. In one case of NTM disease MGIT culturing was not performed. A patient died before treatment and TB was recorded as a main cause of death. During postmortem examination of lung tissue AFB were detected using microscopy (3+ result) in two specimens, but LPA failed to detect NTM. Genotype Mycobacterium CM was performed directly on both tissue specimens detecting *M. interjectum*.

31 (80%) patients with NTM disease had *M. avium* or *M. intracellulare* that were more frequent in patients with NTM diseases than in persons without NTM disease. *M. fortuitum* and *M. lentiflavum* were observed frequently in persons without NTM disease (Table 2). *M.tuberculosis* was also detected in one patient with NTM disease caused by *M.chelonae* resulting in NTM and TB comorbidity. *M. chelonae* was detected in 4 separate samples from that patient.

A result of chest CXR examinations of patients in whom NTM was

Table 2
NTM species detected in persons with NTM in Arkhangelsk region in 2010–2017.

NTM species	Number of persons without NTM disease (%)	Number of persons with NTM disease (%)
M. avium	7 (13)	23 (59)
M. intracellulare	2 (4)	8 (21)
M. fortuitum	9 (17)	1 (3)
M. lentiflavum	6 (11)	1 (3)
M. smegmatis	4 (8)	0
M. gordonae	3 (6)	1 (3)
M. malmoense	1 (2)	2 (5)
M. celatum	3 (6)	0
M. xenopi	2 (4)	0
M. interjectum	0	1 (3)
M. abscessus	1 (2)	0
M. scrofulaceum	1 (2)	0
M.genavense	0	1 (3)
M.chelonae	0	1 (3)
Not identified	14 (27)	0
Total	53 (100)	39 (100)

detected is presented in Table 3. CXR results were available for 36 out of 39 patients with NTM disease and for 50 out of 53 persons without NTM disease in whom NTM were detected. Cavities were observed more frequently ($p < 0{,}05$) in patients with NTM disease compared to persons without NTM disease (Table 3).

33 patients with NTM disease started treatment, as 6 people died before treatment initiation – 5 died from NTM disease and 1 died from other reasons. Combinations of at least 3 medications were used in treatment of all patients. Clarithromycin was used in 94% (31 patients) out of 33 who started treatment. Other medications used for treatment were rifampicin (25 patients; 76%) or rifabutine (3 patients; 9%), ethambutol (24 patients; 73%), levofloxacin (6 patients, 18%), amikacin (5 patients; 15%) moxifloxacin (4 patients; 12%), isoniazid (3 patients; 9%). A patient with NTM and TB comorbidity had multi-drug resistant TB (MDR), resistance to isoniazid and rifampicin, confirmed by Genotype MTBDR*plus*. That patient was treated according to international recommendations for MDR-TB treatment and his regimen included pyrazinamide, ethambutol, cycloserine, capromycin, PAS, protheonamide and moxifloxacin with addition of clarithromycin. Results of treatment are presented in Table 4.

In 69% of patients with NTM disease, including one patient with NTM and TB comormidity, treatment was successful. At the same time 6 patients (15%) died and all of them died before treatment was initiated. 4 patients (10%) were lost to follow up and additionally in one patient no data on treatment outcome was available.

4. Discussion

This is the first study to establish the occurrence of the NTM among persons with presumptive pulmonary TB in a region of the Russian Federation, a country with a high burden of MDR-TB. During an 8-year period NTM were detected in a total of 92 persons, resulting in overall pulmonary NTM notification rate of 0.98 per 100,000 per year. NTM disease was diagnosed in 39 and the incidence rate of pulmonary NTM disease was 0.42 per 100,000 per year. Studies performed in other countries report higher rates of NTM occurrence and NTM disease (Hermansen et al., 2017; Griffith et al., 2007). Other studies on NTM in Russia are laboratory based and are difficult to relate to whole populations (Zhuravlev et al., 2014; Petrova et al., 2018; Smirnova et al., 2017; Ergeshov et al., 2016).

The most prevalent species found in our study were *M. avium* (33%) and *M. intracellulare* (11%), and this correlates well with other studies, including studies performed in Russia (Zhuravlev et al., 2014; Petrova et al., 2018; Smirnova et al., 2017; Ergeshov et al., 2016). *M. kansasii* was not detected and *M. abscessus* was detected in only one case,

Table 3
CXR findings in persons with NTM in Arkhangelsk region in 2010–2017.

CXR findings	Number of persons without NTM disease (%) (n = 50)	Number of persons with NTM disease (%) (n = 36)
Focal changes	34 (68)	25 (69)
Fibrotic changes in the lungs	21 (42)	15 (42)
Infiltration	16 (32)	19 (53)
Cavitation	11 (22)	21 (58)
Pleural involvement	7 (14)	6 (17)
Dissemination of the pathology	7 (14)	7 (19)
Cirrhotic changes in the lungs	0	3 (8)
Pathology of intrathoracic lymph nodes	1 (2)	3 (8)
No abnormal findings	4 (8)	0

Table 4
Treatment outcomes of NTM disease (%) in Arkhangelsk region during 2010–2017.

Treatment outcome	Number of persons with NTM disease (%)
Success	27 (69)
Failure	1 (3)
Lost to follow up	4 (10)
Death from any reason:	6 (15)
Death from NTM	5
Death from other reasons	1
Treatment outcome was not evaluated	1 (3)
Total	39 (100)

although they are usually reported in similar studies, performed in other regions of Russia (Zhuravlev et al., 2014; Petrova et al., 2018; Smirnova et al., 2017; Ergeshov et al., 2016). One patient in our study had TB and NTM comorbidity with *M. tuberculosis* and *M. chelonae* detected multiple times in separate sputum samples.

It is well known that not all positive results for NTM are associated with diseases, and sample contamination must be considered (Griffith et al., 2007). Criteria for the diagnosis of NTM disease have been developed (Griffith et al., 2007), but diagnosing NTM disease may be complicated. Evaluation of most prevalent NTM species, including investigation of genotypes and genetic population of NTM, might be useful for identifying NTM disease as well as sources and ways of transmission of NTM (Yano et al., 2017). Results of chest CXR were available for 66 (72%) patients. We found that cavities were frequently observed in patients with NTM disease.

Favorable treatment outcome was registered in 27 out of 39 patients (69%). Clarithromycin based regimen was commonly used for treatment of NTM disease. 5 patients died due to NTM disease before the treatment was initiated encouraging earlier diagnosis of NTM that may contribute to reduction of death due to NTM disease.

The main limitation of our study was that only presumptive pulmonary TB patients were evaluated for NTM. There were risk groups not observed in our study for NTM such as patients with cystic fibrosis, as well as extrapulmonary pathology in HIV-positive and HIV -negative patients. Our calculated incidence of NTM disease should be regarded as a minimum as it only assesses pulmonary diseases, and some risk groups may be missed.

5. Conclusion

A system of diagnostic and treatment for pulmonary NTM disease was set up in the Arkhangelsk region. Average incidence was 0.42 per 100,000 population and was lower than reported in other studies. It is possible that additional cases of NTM disease could be found in risk groups for NTM disease, such as patients with cystic fibrosis and patients with extrapulmonary pathology in HIV-positive and -negative patients that were not routinely tested for NTM in the region. It is clear that cases of NTM disease are difficult to distinguish from contamination and colonization. NTM disease is difficult to treat and may have unfavorable outcomes. Treatment success rate in our study was 69% encouraging further improvements in diagnostics and treatment of patients with NTM.

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