**УДК 616.98:579.873.2]-078**

# NONTUBERCULOUS MYCOBACTERIOSES: EPIDEMIOLOGY, CLINIC AND POSSIBILITIES OF LABORATORY DIAGNOSTICS IN MODERN CONDITIONS

**Shevchenko O.S.[1], Novohatska M.F.[2], Sharapova O.V.[3], Pogorelova O.O.[1]**

1Kharkiv national medical university, department of phtisiatry and pulmonology, Nauky av. 4, diva5002007@yahoo.com, http://www.knmu.kharkov.ua
[2] Kharkiv regional TB dispensary №1, Nyutona str. 145
[3] Kharkiv city TB dispensary №7, Moskovskyi av. 197

**Introduction.** Today there are more than 150 species of nontuberculous mycobacteria (NTMB) known, of which 99 were registered in Europe. Due to the similar clinical picture with tuberculosis, mycobacterioses are difficult to diagnose and often occur with TB as a mixed infection. Also, there are cases of NTMB detection in the sputum of persons previously suffered with tuberculosis, which can lead to a false diagnosis of recurrent disease. Currently, the role of atypical mycobacteria in human pathology is increasing. Atypical mycobacteria are characterized by a wide spectrum of sustainability and potential pathogenicity to humans and animals [1-2]. It is generally accepted that the reservoir of infection are animals and the environment (water, soil). NTMB presence in water can lead to the erroneous diagnosis of mycobacteriosis due to laboratory contamination of samples with nontuberculous mycobacteria from the outside. Moreover recently it has been found the possibility of transferring NTMB from person to person on the background of existing lung disease [3]. For many types of infections reservoir still has not been found.

Mycobacteriosis diagnostic criteria have been developed for the US and the European Region, including clinical, radiological (including CT) and laboratory signs (Table 1). [4]

**Table 1. Diagnostic criteria for lung mycobacteriosis**

| **Clinical**<br>**Pulmonary symptoms** |
|---|
| Radiological<br>nodular or cavitary opacities on chest radiograph, or a high-resolution computed tomography scan that shows multifocal bronchiectasis with multiple small nodules |
| Laboratory<br>1. Positive culture results from at least two separate expectorated sputum samples.<br>or<br>2. Positive culture result from at least one bronchial wash or lavage<br>or<br>3. Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or AFB) and positive culture for NTM or biopsy<br>showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washings that are culture positive<br>for NTMB |

According to the criteria listed above, non-tuberculous mycobacteriosis is diagnosed, then the identification of the type of pathogen is carried out using different methods (DNA strip technology (Hein test), DNA sequencing (16S rRNA and / or gene hsp65 for slow growing NTMB, rpoB gene for rapid growing NTMB, ITS1 for M. avium complex), «Rapid and slow growing mycobacterium mic plates», TREK Diagnostic systems, high performance liquid chromatography) for individual selection of chemotherapy regimens. Additionally drug sensitivity tests with minimum inhibitory doses are conducted [5-8].

Despite the availability of laboratory diagnostic techniques and the importance of studying epidemiology of NTMB, mandatory registration of NTMB cases in European Region is implemented only in Finland.

According to the literature NTMB are isolated from 0.4-2.0% of sputum samples. In European region prevail M. avium, M. gordonae, M. xenopi, M. intracellulare, M. fortuitum, M. kansasii (Table 2) [9-10].

**Table 2 - Incidence of different types of NTMB in European Region**

| **Type** | **%** |
|---|---|
| *M. avium* | 23,9 |
| *M. gordonae* | 18,1 |
| *M. xenopi* | 8,7 |
| *M. intracellulare* | 9,3 |
| *M. fortuitum* | 7,6 |
| *M. kansasii* | 7,6 |
| *M. chelonae* | 3,7 |

| | |
|---|---|
| *M. abscessus* | 3,3 |
| *M. malmoense* | 2,1 |
| *M. lentiflavum* | 1,8 |
| *M. lentiflavum* | 1,3 |
| *M. terrae* | 1,3 |
| *M. peregrinum* | 0,9 |
| *M. simiae* | 0,9 |
| *M. bohemicum* | 0,7 |
| *M. chimaera* | 0,7 |
| *M. interjectum* | 0,7 |
| *M. nonchromogenicum* | 0,7 |
| *M. arupense* | 0,6 |
| Другие | 6,1 |

Despite the fact that the recent cases of non-tuberculous mycobacteriosis in Ukraine occur more often, there is no statistical data on this issue. Diagnosis and treatment standards also are not introduced, which often leads to ineffectiveness of chemotherapy.

**Aim** of this work was to study identified in the Kharkiv region cases of non-tuberculous mycobacterioses, features of their clinical manifestations and laboratory diagnostic options.

**Materials and methods.** We examined 32 patients (25 men and 7 women), residents of Kharkiv and Kharkiv region, Ukraine, who were diagnosed with "non-tuberculous mycobacteriosis of lungs" during 2014-2016. Average patients' age was 38±15 years. 30 patients were diagnosed with "newly diagnosed tuberculosis: (NDTB) and 2 patients – with "recurrent tuberculosis" (RTB). Comorbidities: 3 patients had HIV, 1 – bronchial asthma, 1 – COPD, 1 - duodenal ulcer combined with chronic hepatitis in remission, 1 - congenital stenosis of pulmonary artery, 2 – chronic bronchitis. 20 patients had cough and shortness of breathing, 9 patients had intoxication signs (table 3).

All patients were examined by standard diagnostic algorithm, approved by Order of the Ministry of Health of Ukraine №620 from 09.04.2014, which included chest X-ray, two sputum smears, sputum culture in VASTES system and on Lowenstein-Jensen medium, molecular genetic testing of sputum (GeneXpert MTB / RIF), and routine laboratory studies [11].

**Table 3 – Patients' characteristics**

| Criterion | Characteristics | Number |
|---|---|---|
| Number of patients | | 32 |
| Sex | men | 25 (78,1%) |
| | women | 7 (21,9%) |
| Average age | | 38±15 years |
| Case type | NDTB | 30 (93,7%) |
| | RTB | 2 (6,3%) |
| Comorbidities | HIV | 3 (9,3%) |
| | BA | 1 (3,1%) |
| | COPD | 1 (3,1%) |
| | Duodenal ulcer combined with chronic hepatitis in remission | 1 (3,1%) |
| | Congenital stenosis of pulmonary artery | 1(3,1%) |
| | Chronic bronchitis | 2 (6,2%) |
| Bad habits | Smoking | 12 (37,5%) |
| Complaints | Cough, shortness of breathing | 20 (62,5%) |
| | Intoxication (fever, weight loss, weakness) | 9 (28,1%) |
| Destruction of lung tissue | | 13 (40,6%) |
| Bacterioexcretion (smear) | Scanty | 10 (31,2%) |
| | Moderate | 2 (6,2%) |
| | Massive | 1 (3,1%) |

Nontuberculous mycobacteriosis was diagnosed on the basis of NTMB growth in BACTEC system, after which the diagnosis was verified by the following criteria:

- Smear: the absence of formation of Cord-factor (in the smear NTMB are located scattering)
- Negative immunoassay (ID-test)
- Negative GeneXpert MTB/RIF

Identification of NTMB type was carried out on the basis of Runyon classification (Table 4) [12] and the modified classification, approved by the Order Ministry of Health of Ukraine №45 from 06.02.2002 [13], which takes into account the NTMB growth rate, character and color of colonies.

Statistical analysis was performed using the program Microsoft Office Excel 2007.

**Table 4 – Classification of NTMB by Runyon**

| **Slow-growing (>7 d.)** | | | **Rapid-growing (<7d.) (Group IV)** |
|---|---|---|---|
| **Photo-chromogenic (Group I)** *form yellow pigment under the influence of light* | **Scoto-chromogenic (Group II)** *foem yellow pigment without light* | **Non-chromogenic (Group III)** *don't form yellow pigment* | |
| *M. kansasii*<br>*M.simiae*<br>*M. szulgai*<br>*M. marinum* | *M. gordonae*<br>*M. scrofulaceum* | *MAC*<br>*M. intracellulare*<br>*M. haemophilum* | *M. fortuitum*<br>*M. abscessus*<br>*M. chelonae*<br>*M. mucogenicum* |

**Results and discussion.** In all 32 reported cases infiltrative changes were identified radiographically. In 13 patients (40.6%) cavities were found. In addition in smear of 10 patients (31.2%) was found scant bacterial excretion, 2 (6.2%) - moderate, and 1 (3.1%) - massive. According to the results of culture NTMB were identified.

Based on laboratory data, taking into account the speed and character of colony growth, patients were assigned to the main groups of suspected pathogens (Table 5). In 6 patients it was unable to identify the causative agent.

**Table 5 – Probable distribution of obtained pathogens**

| **NTMB group** | **Number** |
|---|---|
| Chromogenic slow-growing NTMB (*M. kansasii, M.gordonae, M. marinum)* | 17 (65,4%) |
| Non-chromogenic slow-growing NTMB *(M. avium, M. intracellulare, M.xenopi)* | 9 (34,6%) |

Thus we can see that chromogenic slow-growing NTMB prevailed (65,4%), which significantly differs from the situation in European region, where they account for only about 25,7%. A high percentage of pathogens belonging to M. avium complex, is generally consistent with the epidemiological situation in the European region, and around the world.

Then in groups we have compared the severity of clinical manifestations. It was noted that the heaviest lesions were caused by chromogenic slow-growing NTMB, as 41,1% of these patients had extensive lung damage, and 64,7%% - destruction of lung tissue. Radiographic changes correlated with the severity of clinical manifestations - patients had cough, shortness of breath, symptoms of intoxication. Also in this group most often was found detectable by smear bacterial excretion, including moderate and massive (Table 6).

**Table 6 - Characteristic of lesions' severity**

| | **Chromogenic slow-growing NTMB** | **Non-chromogenic slow-growing NTMB** |
|---|---|---|
| Lesion of 1 upper lobe | 6 (35,3%) | 7 (77,8%) |
| Lesion of 2 upper lobes | 4 (23,5%) | 1 (11,1%) |
| Lesion of 1 lung | 3 (17,6%) | 0 |
| Lesion of 2 lungs | 4 (23,5%) | 1 (11,1%) |
| Destruction of lung parenchyma | 11 (64,7%) | 2 (50%) |
| Bacterial excretion (smear) | 8 (47,0%) | 2 (22,2%) |

According to the literature, there are 2 main clinical and radiological variants of nontuberculosis mycobacterioses: nodular bronchiectatic and cavernous forms [14].

Bronchoectatic form often develops in elderly non-smoking women with no previous lung diseases, proceeds more likely easily and is characterized by X-ray picture of the "tree in buds," formation of cylindrical bronchiectases. In most cases, visualization of changes in lung tissue from these patients require CT examination.

Cavernous form is typical for smoking middle-aged men, often develops on the background of previous lung disease - cystic fibrosis (M. abscessus), COPD, bronchoectatic disease, tuberculosis in the past, aspergillosis (M. xenopi), as well as on the background of HIV infection and drug treatment that suppress the immune system (corticosteroids, TNF-alpha inhibitors). Clinical and radiographic this form is almost indistinguishable from pulmonary tuberculosis (infiltrates in upper lobes with formation of cavities), more severe, often with a syndrome of intoxication, cough, shortness of breath, sometimes hemoptysis. Without treatment, the disease progresses rapidly and leads to formation of large cavities in the lungs and respiratory failure. The most common pathogens that cause such clinical and radiological picture are M. kansasii, M. xenopi and Mycobacterium avium complex [15-17]. Exactly this form was more common in our patients.

**Conclusions.** In Kharkiv region there is an increase of non-tuberculous mycobacterioses, but their diagnostics is difficult due to the lack of diagnostic capabilities: it is impossible to perform CT examination in all patients, no possibility of reliable identification of the type of pathogen (no DNA strip technology, tablet technology, DNA sequencing, high-performance liquid chromatography). Among the identified cases of mycobacterioses prevailed chromogenic slow-growing NTMB. Clinical and radiographic pattern was characterized by severe thoracic (cough, shortness of breath) and intoxication (fever, weight loss, weakness) complaints and extensive lesions of pulmonary system. Also in this group was often found detectable by smear bacterial excretion, including massive one. It was noted that often mycobacterioses developed on the background of existing pulmonary disease.

Failure to conduct complete laboratory diagnostics, identification of pathogen and determination of its individual sensitivity to drugs significantly complicates prescribing of adequate chemotherapy regimens. In this regard, we consider a priority the development of standards for diagnosis and treatment of non-tuberculous mycobacterioses and further strengthening the capacity of laboratory diagnostics.

**References**

1. Piersimoni C, Scarparo C. Pulmonary infections associated with nontuberculous mycobacteria in immunocompetent patients// Lancet Infect Dis 2008. Vol. № 5. P. 323–334.
2. Daley CL, Griffith DE. Pulmonary non-tuberculous mycobacterial infections// Int J Tuberc Lung Dis. 2010. Vol. 14. № 6. P. 665–671.
3. Bryant JM, Grogono DM, Rodriguez-Rincon D. [et al.]. Emergence and spread of a human-transmissible multidrug-resistant nontuberculous mycobacterium// Science. 2016. Vol.354. P. 751-757
4. Nontuberculosis mycobacteria (NTM)// Guidelines for health professionals in the Northern Territory. 2014
5. Pfyffer GE. Mycobacterium : general characteristics, laboratory detection, and staining procedures// Manual of clinical microbiology. 11th ed. Washington, DC: ASM Press. 2015. P. 543-72.
6. Somoskovi A, Hotaling JE, Fitzgerald M, O'Donnell D, Parsons LM, Salfinger M. Lessons from a proficiency testing event for acid-fast microscopy// Chest. 2001. № 120. P. 250-257.
7. Guglielmetti L, Mougari F, Lopes A, Raskine L, Cambau E. Human infections due to nontuberculous mycobacteria: the infectious diseases and clinical microbiology specialists' point of view// Future Microbiol. 2015. № 10. P. 1467-1483.
8. Somoskovi A, Salfinger M. Nontuberculous mycobacteria in respiratory infections: advances in diagnosis and identification// Clin Lab Med. 2014. № 34. P. 271-95.
9. Hoefsloot W, Andrejak C, Angeby K. [et al.]. The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples// Eur Respir J. № 42. P. 1604-1613
10. Werf MJ, Kodmon C, Katalinic-Jankovic V [et al.]. Inventory study of non-tuberculous mycobacteria in the European Union// BMC Inf Dis. 2014. № 14. P. 62
11. Order of Ukrainian Ministry of Health № 620 of 04.09.2014 «Unified clinical protocols of primary, secondary (specialized) and tertiary (highly specialized) medical care for adults: tuberculosis» - 179 c.
12. Runyon EH. Anonymous mycobacteria in pulmonary disease// The Medical clinics of North America. 1959. Vol. 43. № 1. P. 273–290
13. Order of Ukrainian Ministry of Health № 45 of 06.02.2002 «On approval of Instruction on bacteriological diagnosis of tuberculosis infection»
14. BTS Guidelines for Non-Tuberculous Mycobacteria
15. Koh WJ, Kwon OJ, Lee KS. Nontuberculous Mycobacterial Pulmonary Diseases in Immunocompetent Patients// Korean J Radiol. Vol. 3. № 3. 2002. P. 145-157
16. Ryu YJ, Koh WJ, Daley CL. Diagnosis and Treatment of Nontuberculous Mycobacterial Lung Disease: Clinicians' Perspectives// Tuberc Respir Dis. 2016. № 79. P. 74-84
17. Johnson MM, Odell JA. Nontuberculous mycobacterial pulmonary infections// J Thorac Dis. 2014. Vol. 6. № 3. P. 210-220

**UDC 616.98:579.873.2]-078**

**NONTUBERCULOUS MYCOBACTERIOSES: EPIDEMIOLOGY, CLINIC AND POSSIBILITIES OF LABORATORY DIAGNOSTICS IN MODERN CONDITIONS**

**Shevchenko O.S., Novohatska M.F., Sharapova O.V, Pogorelova O.O.**

**Introduction.** Today there are more than 150 species of nontuberculous mycobacteria (NTMB) known, of which 99 were registered in Europe. Due to the similar clinical picture with tuberculosis, mycobacterioses are difficult to diagnose and often occur with TB as a mixed infection. Also, there are cases of NTMB detection in the sputum of persons previously suffered with tuberculosis, which can lead to a false diagnosis of recurrent disease. Currently, the role of atypical mycobacteria in human pathology is increasing. Atypical mycobacteria are characterized by a wide spectrum of sustainability and potential pathogenicity to humans and animals. It is generally accepted that the reservoir of infection are animals and the environment (water, soil). NTMB presence in water can lead to the erroneous diagnosis of mycobacteriosis due to laboratory contamination of samples with nontuberculous mycobacteria from the outside. Moreover recently it has been found the possibility of transferring NTMB from person to person on the background of existing lung disease. For many types of infections reservoir still has not been found. **Aim** of this work was to study identified in the Kharkiv region cases of non-tuberculous mycobacterioses, features of their clinical manifestations and laboratory diagnostic options.
**Materials and Methods.** We examined 32 patients (25 men and 7 women), residents of Kharkiv and Kharkiv region, Ukraine, who were diagnosed with "non-tuberculous mycobacteriosis of lungs" during 2014-2016. Patients were examined with routine diagnostic algorithm for TB. Nontuberculous mycobacteriosis was diagnosed on the basis of NTMB growth in BACTEC system, after which the diagnosis was verified by the following criteria: 1. Smear: the absence of formation of Cord-factor (in the smear NTMB are located scattering); 2. Negative immunoassay (ID-test); 3. Negative GeneXpert MTB/RIF Then non-tuberculous mycobacteria were identificated by investigation on solid media. They were divided on groups of susceptible causative agents according to Runnyon classification and the modified classification, approved by the Order Ministry of Health of Ukraine №45 from 06.02.2002, which takes into account the NTMB growth rate, character and color of colonies.
**Results and discussion.** In all 32 reported cases infiltrative changes were identified radiographically. In 13 patients (40.6%) cavities were found. In addition in smear of 10 patients (31.2%) was found scant bacterial excretion, 2 (6.2%) - moderate, and 1 (3.1%) - massive. According to the results of culture NTMB were identified. Chromogenic slow-growing non-tuberculous mycobecteria prevailed (65,4%). Another causative agents belonged to M. avium complex. The heaviest lesions were caused by chromogenic slow-growing non-tuberculous mycobecteria, as 41,1% of these patients had extensive lung damage, and 64,7%% - destruction of lung tissue. Radiographic changes correlated with the severity of clinical manifestations. Also in this group most often was found detectable by smear bacterial excretion, including moderate and massive. In our patients prevailed cavernous form which is is typical for smoking middle-aged men, often develops on the background of previous lung disease - cystic fibrosis (M. abscessus), COPD, bronchoectatic disease, tuberculosis in the past, aspergillosis (M. xenopi), as well as on the background of HIV infection and drug treatment that suppress the immune system (corticosteroids, TNF-alpha inhibitors). Clinical and radiographic this form is almost indistinguishable from pulmonary tuberculosis (infiltrates in upper lobes with formation of cavities), more severe, often with a syndrome of intoxication, cough, shortness of breath, sometimes hemoptysis. Without treatment, the disease progresses rapidly and leads to formation of large cavities in the lungs and respiratory failure. The most common pathogens that cause such clinical and radiological picture are M. kansasii, M. xenopi and Mycobacterium avium complex. **Conclusions.** In Kharkiv region there is an increase of non-tuberculous mycobacterioses, but their diagnostics is difficult due to the lack of diagnostic capabilities: it is impossible to perform CT examination in all patients, no possibility of reliable identification of the type of pathogen (no DNA strip technology, tablet technology, DNA sequencing, high-performance liquid chromatography). Among the identified cases of mycobacterioses prevailed chromogenic slow-growing NTMB. Clinical and radiographic pattern was characterized by severe thoracic (cough, shortness of breath) and intoxication (fever, weight loss, weakness) complaints and extensive lesions of pulmonary system. Also in this group was often found detectable by smear bacterial excretion, including massive one. It was noted that often mycobacterioses developed on the background of existing pulmonary disease. Failure to conduct complete laboratory diagnostics, identification of pathogen and determination of its individual sensitivity to drugs significantly complicates prescribing of adequate chemotherapy regimens. In this regard, we consider a priority the development of standards for diagnosis and treatment of non-tuberculous mycobacterioses and further strengthening the capacity of laboratory diagnostics.

**Keywords:** non-tuberculous mycobacteria, epidemiology, diagnostics