PULMONARY INFECTION DUE TO NON-TUBERCULOSIS MYCOBACTERIUM

Minsk BSMU 2020

МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ РЕСПУБЛИКИ БЕЛАРУСЬ
БЕЛОРУССКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ
КАФЕДРА ФТИЗИОПУЛЬМОНОЛОГИИ

МИКОБАКТЕРИОЗЫ ЛЕГКИХ

PULMONARY INFECTIONS DUE TO NON-TUBERCULOSIS MYCOBACTERIUM

Учебно-методическое пособие

Минск БГМУ 2020

УДК 616.24-002(075.8)-054.6
ББК 55.4я73
М59

Рекомендовано Научно-методическим советом университета в качестве
учебно-методического пособия 20.03.2020 г., протокол № 7

А в т о р ы: д-р мед. наук, доц., зав. каф. фтизиопульмонологии Белорусского государственного медицинского университета Г. Л. Бородина; врач-бактериолог Республиканской референс-лаборатории Республиканского научно-практического центра пульмонологии и фтизиатрии О. М. Залуцкая; канд. мед. наук, доц. каф. фтизиопульмонологии Белорусского государственного медицинского университета П. С. Кривонос; д-р мед. наук, проф. Л. К. Суркова

Р е ц е н з е н т ы: д-р мед. наук, проф., чл.-корр. Национальной академии наук Беларуси, директор Республиканского научно-практического центра пульмонологии и фтизиатрии Г. Л. Гуревич; д-р мед. наук, проф. 1-й каф. внутренних болезней Белорусского государственного медицинского университета А. Э. Макаревич

Микобактериозы легких = Pulmonary infections due to non-tuberculosis mycobacterium : учебно-
М59 методическое пособие / Г. Л. Бородина [и др.]. – Минск : БГМУ, 2020. – 24 с.

ISBN 978-985-21-0665-8.

Изложены современные представления об основных видах нетуберкулезных микобактерий, патогенных для человека, и вызываемых ими заболеваниях. Рассматриваются вопросы классификации микобактерий, биохимические и культуральные методы их идентификации, а также клинико-рентгенологические особенности различных микобактериозов легких. Представлены основные бактериологические и клинические критерии диагностики, а также принципы и схемы лечения микобактериозов на основе согласованных международных рекомендаций.

Предназначено для студентов 4–6-го курсов медицинского факультета иностранных учащихся, обучающихся на английском языке.

УДК 616.24-002(075.8)-054.6
ББК 55.4я73

Учебное издание

Бородина Галина Львовна
Залуцкая Оксана Михайловна
Кривонос Павел Степанович
Суркова Лариса Константиновна

МИКОБАКТЕРИОЗЫ ЛЕГКИХ

PULMONARY INFECTIONS DUE TO NON-TUBERCULOSIS MYCOBACTERIUM

Учебно-методическое пособие

На английском языке

Ответственная за выпуск Г. Л. Бородина
Переводчик О. М. Залуцкая
Компьютерная вёрстка Н. М. Федорцовой

Подписано в печать 16.10.20. Формат 60×84/16. Бумага писчая «Xerox office». Ризография. Гарнитура «Times». Усл. печ. л. 1,39. Уч.-изд. л. 1,36. Тираж 99 экз. Заказ 504.

Издатель и полиграфическое исполнение: учреждение образования
«Белорусский государственный медицинский университет».
Свидетельство о государственной регистрации издателя, изготовителя,
распространителя печатных изданий № 1/187 от 18.02.2014.
Ул. Ленинградская, 6, 220006, Минск.

ISBN 978-985-21-0665-8

MOTIVATIONAL CHARACTERISTIC OF THE TOPIC

Last decade in many countries, especially economically developed, there has been an increase in the number of patients with mycobacteriosis (**Pulmonary infection due to non-tuberculosis mycobacterium — PINM**) and the increase in the proportion of non-tuberculosis mycobacteria among the total number of the isolated cultures of mycobacteria. For example, in 1997–2007, the prevalence of mycobacteriosis in the USA increased more than two and a half times. Moreover, this rate was always higher among female compared to male and was 57 per 100 000 population in 2007 (Fig. 1).

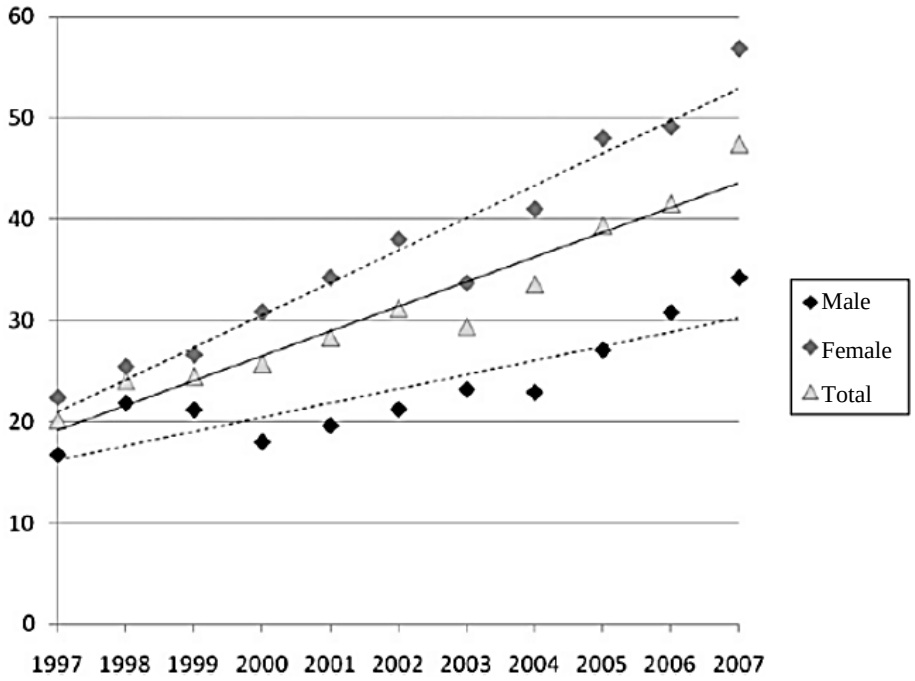

Figure 1. The prevalence of mycobacteriosis in economically developed countries (per 100 000 population) in 1997–2007

While deaths rate from TB in the USA in 1999–2010 have decreased by 41.06 %, the number of deaths related to mycobacteriosis even rose by 29.2 %.

The prevalence and incidence of mycobacteriosis have been increasing rapidly in East Asia, especially in Japan, demonstrating a high vulnerability of the population of that region to non-tuberculous mycobacteria. In South Korea during the decade 2007–2016, the cases of PINM had been observed practically at any age of patients, but the prevalence had increased rapidly with age. Unlike the USA, the prevalence of mycobacteriosis in most age groups were only slightly higher among women, excluding the patients over 70 years old, where men predominated significantly (Fig. 2).

The growth of incidence and prevalence of mycobacteriosis is primarily due to use of new sensitive and specific methods of their isolation and

identification that let considerably accelerate the diagnosis of PINM and increase its efficiency.

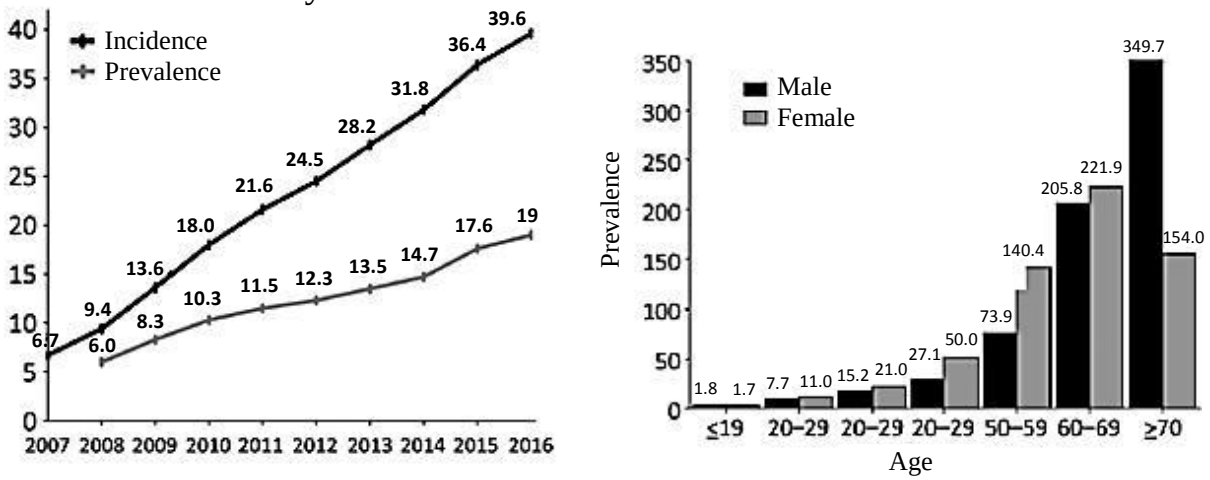

Figure 2. The incidence and prevalence of mycobacteriosis (per 100 000 population) in South Korea

In the Republic of Belarus during the last years, there is an increase in the number of patients with mycobacteriosis and the growth of in the proportion of non-tuberculous mycobacteria (NTM) among the total number of isolated cultures of mycobacteria.

Mycobacteriosis problem has become more significant because of spread of HIV infection, as HIV-positive persons (especially those at the stage of AIDS) often have pulmonary mycobacteriosis, potentially leading to their death.

In economically developed countries of the world, mycobacteriosis is the third most frequent opportunistic infection (following pneumocystis pneumonia and candidiasis) the proportion of which exceeds TB. The incidence of mycobacteriosis is 0.11–0.17 per 100 patients/year in the general cohort of HIV-positive, and 1.4–5.3 per 100 patients/year with CD4 < 50 cells/ml, moreover, the median survival rate for generalized mycobacteriosis is 3.6 months after diagnosis.

The clinical significance of mycobacteriosis is not limited only by HIV-positive persons and other categories of immunocompromised individuals, but the wide range of patients with different lung diseases. Pulmonary mycobacteriosis are diagnosed mainly among the people over 50 years having prior destructive or obstructive lung damage: chronic bronchitis, emphysema, bronchiectasis, pneumoconiosis, and also among the patients cured of chronic diseases, such as TB and mycosis. Despite the obvious importance of pulmonary mycobacteriosis as a clinical and epidemiological problem, concerted international and national recommendations for diagnosis and treatment of this pathology have not been developed yet. Notably that the problem of pulmonary mycobacteriosis is largely ignored in existing textbooks and teaching aids for medical students.

The purpose of the practical lesson: to study clinical features, methods of laboratory diagnostics and principles of pulmonary mycobacteriosis treatment.

Lesson objectives:

– to study classification of mycobacteria;

– to study main species of NTM pathogenic for humans;

– to study biochemical and cultural methods of mycobacteria identification and the algorithm of bacteriological diagnostics of pulmonary mycobacteriosis;

– to learn how to interpret correctly the results of microscopic, cultural and molecular studies on mycobacteria;

– to study the main clinical manifestations of pulmonary mycobacteriosis;

– to understand the basic principles of pulmonary mycobacteriosis treatment depending on etiology, form and severity of disease.

Baseline knowledge requirements. Repeat:

from the course of microbiology with virology and immunology:

– general characteristics of non-tuberculous mycobacteria;

– taxonomic position of non-tuberculous mycobacteria;

from the pharmacology course:

– pharmacological characteristics of anti-tuberculous drugs;

– pharmacological characteristics of antibiotics (fluoroquinolones, macrolides, aminoglycosides), their possible side effects;

from the phthisiopulmonology course:

– pathomorphological manifestations of TB;

– X-ray syndromes in lung pathology;

– clinical forms of pulmonary TB;

– principles and treatment regimens of TB chemotherapy;

– cultural study on MTB.

Control Questions from Related Disciplines:

1. Microscopic and cultural study on TB, the drug susceptibility testing of M. tuberculosis on solid media and automated system: techniques, interpretation of results.

2. Molecular methods used for detection of M. tuberculosis and drug susceptibility testing: principle, interpretation of results.

3. Treatment of TB including drug resistant TB.

Control Questions on the Topic:

1. Classification of non-tuberculous mycobacteria (by Runyon). Grouping of mycobacteria by pathogenicity for human. Characterization of certain types of NTM pathogenic for humans.

2. Epidemiology of mycobacteriosis, risk groups among the population.

3. Cultural diagnostics of mycobacteriosis, interpretation of results.

4. Diagnostic criteria for mycobacteriosis.

5. General principles and treatment regimens of the various pulmonary mycobacteriosis.

GENERAL CHARACTERISTICS AND CLASSIFICATION OF MYCOBACTERIA

In most economically developed countries, there has been a steady increase in the number of lung diseases caused by non-tuberculosis mycobacteria (NTM). This is primarily due to the use of new sensitive and specific methods for their isolation and identification, which have significantly accelerated the diagnosis of pulmonary infection due to non-tuberculosis mycobacterium (PINM) and improved its effectiveness. The problem of NTM has acquired considerable significance as a result of the spread of HIV infection, since HIV-infected individuals (especially those at the stage of AIDS) often develop PINM potentially leading to their death.

Recent years, in the Republic of Belarus, there has also been an increase in the number of patients with PINM and an increase in the proportion of NTMs among the total number of isolated cultures of mycobacteria. Despite the obvious importance of PINM as a clinical and epidemiological problem, up to now there have been no concerted international and national recommendations for the diagnosis and treatment of this pathology.

Currently the Mycobacterium genus includes more than 100 species of bacteria and their number continues to grow. Several pathogenic species of mycobacteria are included in the so-called M. tuberculosis complex, namely *M. tuberculosis (MTB)*, *M. bovis*, *M. africanum*, *M. microti*, *M. canettii*, *M. caprae* and *M. pinnipedii*. The rest of the mycobacteria excluding M. leprae belong to opportunistic and non-pathogenic mycobacteria. They are called NTM (non-tuberculosis mycobacteria) or MOTT (Mycobacterium other than tuberculosis). They live in an environment and can be found everywhere in environmental reservoirs of various domestic and wild animals, in the soil, etc. Based on the analysis of the DNA and serological methods of investigation, it was established that NTMs are independent species and not the mutants of Mycobacterium tuberculosis.

According to the classification of Runyon (A. Timple, E. H. Ranyon, 1954), NTM for growth rate, morphology of colonies and the ability to form pigments are divided into 4 main groups presented in Table 1.

Recent years, this classification has been supplemented by the NTM grouping in terms of the degree of pathogenicity for humans (Table 2).

NTMs are ubiquitous in the environment. In urban conditions, tap water and swimming pools, they can serve as sources of infection. NTMs are able to grow even in chlorinated tap water and distilled water (at a temperature of 45 °C and above) and resist the effects of disinfectants.

Modes of transmission of the infection have not yet been reliably established. NTMs most often enter the human body through inhaled air containing aerosols formed over natural water bodies, swamps and soil.

Infection through contaminated food is also possible. Currently it is believed that patients with PINM do not pose a danger to others, so they don't need to be isolated.

Table 1

Runyon classification of non-tuberculosis mycobacteria

Group I Photochromogenic (not pigmented when grown in the dark but acquiring pigmentation after exposure to light)	**Group II** Scotochromogenic (forming a pigment in the dark)	Group III Non-chromogenic (not forming a pigment or having a pale yellow color that is not enhanced by light)		**Group IV** Fast-growing
M. asiaticum	*M. gordonae*	*M. avium-*	*M. haemo-*	*M. abscessus*
M. intermedium	*M. interjectum*	*intracellulare*	*philum*	*M. chelonae*
M. kansasii	*M. lentiflavum*	*M. braderi*	*M. malmoense*	*M. fortuitum*
M. marinum	*M. scrofulaceum*	*M. celatum*	*M. shimoidei*	*M. mucogenicum*
M. simae	*M. szulgai*	*M. gastri*	*M. terrae*	*M. peregrinum*
	M. xenopi	*M. genavense*	*M. ulcerans*	*M. phlei*

Table 2

NTM grouping by degree of pathogenicity for humans

Potentially pathogenic (capable to cause human diseases under certain conditions)	*M. avium–M. intracellulare (MAC), M. abscessus, M. chelonae, M. fortuitum, M. kansasii, M. malmoense, M. scrofulaceum, M. ulcerans, M. xenopi*
Saprophytes (found in the environment and as a rule not dangerous to humans)	*M. gastri, M. gordonae, M. flavescens, M. phlei, M. terrae, M. triviale*

The epidemiology of human mycobacteriosis is currently being studied very intensely, but the incidence of pathology caused by NTM is very different in different countries and even in different regions of the same country. Mycobacteriosis is much more often diagnosed in warm regions of the world.

The most vulnerable group for pulmonary mycobacteriosis are patients with local and systemic immunity disorders (HIV infection), with the use of immunosuppressive drugs that are used in the treatment of cancer and autoimmune diseases, after transplantation of organs and stem cells. Respiratory diseases such as COPD, alveolitis, sarcoidosis, pneumoconiosis, bronchiectasis, cystic fibrosis, bronchial asthma, TB may be background for the development of mycobacteriosis. There is evidence that the administration of drugs with anti-TNF-α effects (monoclonal antibodies to TNF-α) used in the treatment of systemic connective tissue diseases, Crohn's disease, sarcoidosis, malignant tumors may lead to the development of mycobacteriosis in 2–4 months from the beginning of treatment. In addition, patients with impaired γ-IFN or IL-12 production are also predisposed to severe mycobacterial infections.

NTM SPECIES PATHOGENIC FOR HUMANS

M. abscessus is often associated with nosocomial infections (most often affects the skin and lungs). Infection with *M. abscessus* can be a serious complication in the recipients of the transplanted lung.

M. avium–M. intracellulare (*M. avium complex*, MAC). The pathology caused by MAC in many countries is at the first place among all diseases caused by NTM. The clinical picture of the disease is similar to pulmonary TB. The damage of the skin, muscle tissue and bone skeleton including osteomyelitis of the spine associated with MAC infection are also described. MAC is one of causative agents of opportunistic infections that complicate HIV infection. It is established that infection of MAC in HIV-infected patients usually develops after the amount of CD4 lymphocytes decreases below 50 cells/ml and is usually manifested as disseminated lung injury involving the lymph nodes, gastrointestinal tract and bones.

M. chelonae most often causes mycobacteriosis in patients with chronic immunosuppression, as well as those on renal dialysis, after severe injuries, surgeries, prolonged use of catheters.

M. fortuitum is most often associated with post-traumatic, postoperative and post-injection infections of the skin and soft tissues. *M. chelonae* and *M. fortuitum* are a common cause of nosocomial infections.

M. kansasii causes mycobacteriosis in patients with chronic pulmonary diseases and in immunosuppressed individuals.

M. marinum causes aquarium granuloma. *M. marinum* is ubiquitously present in the aquatic environment, including both fresh and salt water. As a rule, lesions occur on the most traumatized areas — hands, feet, elbows and knees. At the site of the injury there is an inflammatory node with a varicose or hyperkeratotic surface which can reach 3–4 cm in diameter.

M. scrofulaceum predominantly causes lymphadenitis of the cervical and submandibular lymph nodes in children.

CULTURAL STUDY ON PINM

The cultural study on NTM is conducted similarly to the MTB study using standard methods for diagnosing TB.

Identification of the isolated culture of mycobacteria includes several stages:

– evaluation of colonies morphology and growth rate of MB culture;

– microscopic examination of the isolated culture according to Ziehl–Neelsen (ZN) for evaluation of cell morphology and the presence of cord-factor;

– differentiation of NTM from M. tuberculosis complex;

– species identification of NTM.

Despite the fact that the morphology of colonies, the presence of pigment and growth characteristics gives some idea of the isolated culture of mycobacteria, identification using special laboratory methods is mandatory.

At present, immunochromatographic, molecular, cultural and biochemical methods of mycobacteria identification are used in practical laboratories.

MICROSCOPIC CHARACTERISTICS OF MYCOBACTERIA

Mycobacterium cells are straight or slightly curved, 0.2–0.7 × 1.0–10 μm size. Virulent strains of MTB have a so-called cord factor which results in M. tuberculosis cells sticking together like the strands of cord that make up a rope. NTM strains do not have a cord factor.

The cell wall of all mycobacteria contains a significant amount of lipids and mycolic acids, therefore mycobacteria are acid-fast. Acid-fast bacilli (AFB) is stained with fuchsin (ZN) in bright red and do not lose this color after discoloration with alcohol or acids.

ZN microscopic examination of sputum smears is a non-specific method, therefore, a positive study result (AFB+) may indicate presence of both MTB or NTM in the sample. The differentiation of MTB and NTM is carried out during further investigation.

IMMUNOCHROMATOGRAPHIC IDENTIFICATION OF M. TUBERCULOSIS COMPLEX

Unlike NTM, M. tuberculosis complex produces MPB64 protein. Thus, the detection of MPB64 in the sample indicates that the test strain belongs to the M. tuberculosis complex. Immunochromatography belongs to the group of reactions with labeled antibodies. Test kits based on this principle allows to detect MPB64 protein and therefore to identify M. tuberculosis complex and has high specificity.

MOLECULAR IDENTIFICATION OF MYCOBACTERIA

The identification of NTM most frequently isolated from the diagnostic samples can be carried out using molecular methods, in particular, GenoType Mycobacterium CM/AS (Hain Lifescience, Germany).

The procedure of the test consists of three stages: isolation of DNA from cultures on solid or liquid medium, multiplex amplification (PCR) with biotinylated primers, reverse hybridization of the DNA of the test strain with specific mycobacteria DNA probes immobilized on a nitrocellulose strip. The use of molecular methods allows identify mycobacteria within 1–2 working days.

BIOCHEMICAL AND CULTURAL METHODS FOR IDENTIFICATION OF MYCOBACTERIA

A set of biochemical and culture tests has been developed for the identification of NTM. The combination of specific morphological features with the results of biochemical and culture tests makes it possible to identify the mycobacteria with high accuracy.

Cultural characteristics of mycobacteria. Mycobacteria multiply slowly: colonies of fast-growing NTMs on egg media usually appear in 4–7 days, slowly growing in 21–56 days. Typical M. tuberculosis cultures grown on solid media are usually dry, wrinkled R-colonies (rough), not pigmented (cream or ivory). NTMs usually form moist and smooth S-colonies, which can be creamy, white, bright yellow or orange.

Niacin test. Niacin test is the basic test used to identify *M. tuberculosis*. All mycobacteria produce niacin (a nicotinic acid derivative), but in M. tuberculosis, nicotinic acid accumulates in amounts many times greater compared to other mycobacteria.

Nitratreductase test. The principle of the method is to determine the activity of the nitrate reductase enzyme by the amount of nitrite formed from nitrate which is accompanied by a color reaction. In M. tuberculosis, nitrate reductase activity is high, unlike most non-tuberculosis mycobacteria, in which this enzyme is absent. But several species of NTM (*M. kansasii, M. terrae, M. fortuitum*) have nitrate reductase activity.

PNB test. Para-nitrobenzoic acid (PNB) has an inhibitory effect on MTB, but not NTM growth, so they may be identified.

CLINICAL CHARACTERISTICS AND DIAGNOSTICS OF PULMONARY INFECTION DUE TO NON-TUBERCULOSIS MYCOBACTERIUM

Among all pathologies caused by NTM, pulmonary diseases predominate in adults. The most common pathogens causing pulmonary diseases among NTM are *M. avium complex*, *M. kansasii*, *M. abscessus*, *M. fortuitum*, *M. malmoense, M. chelonae*.

Despite the fact that PINM cases are registered in persons without concomitant pathology, the majority of patients with progressive course of the disease have a history of destructive and obstructive respiratory diseases, which provide favorable conditions for the colonization of the respiratory tract and the development of PINM.

Histological study of the affected lung tissue finds granulomatous changes of varying severity, similar to lesions caused by MTB. Among them, lymphocytes, clusters of epithelioid cells and caseous tubercles similar to those

of TB are observed. Most often, the morphological picture of PINM is not distinguished from TB.

The clinical symptoms of PINM are polymorphic, nonspecific and similar to those of pulmonary TB. Most patients complain of prolonged productive cough with a small amount of sputum, shortness of breath, episodes of hemoptysis, chest pain, weakness, fever, weight loss, loss of appetite, night sweats. PINM is often accompanied by clinical signs associated with preceding lung diseases (pneumoconiosis, COPD, bronchiectasis or a tumor process). One of the typical signs of PINM is a long prodromal period, averaging about 2 years.

The X-ray picture of PINM is diverse and also does not allow to uniquely differentiate PINM and pulmonary TB. At the same time, some X-ray signs allowing suspect PINM are described: single giant caverns with a thin wall, the absence of bronchogenic dissemination and insignificant infiltration around the cavity.

In PINM caused by MAC, lesions in the form of focal and interstitial dissemination in combination with multiple bronchiectasis of small size are detected. These changes are visualized most clearly by high resolution CT.

X-ray manifestations of PINM caused by *M. kansasii* are traditionally considered to be the most similar with TB lesions. The X-ray picture can be identical to infiltrative TB in the phase of infiltration and decay with dissemination.

In PINM, gross pleural changes according to the lesion localization are noted. Exudation to the pleural cavity is rare.

According to the international classification, there are 3 main forms of PINM:

– focal changes in the lungs on the background of bronchiectasis;

– fibrotic cavity form of PINM;

– PINM on the background of other lung diseases.

It is known that one of the main classic variants of PINM in the form of focal changes in the lungs on the background of bronchiectasis was called the syndrome of Lady Windermere, named after one of the heroines of Oscar Wilde's "Lady Windermere's Fan". It is believed that tall, pale, slender women older than 60 suffer from it. He is found in non-smoking women with chest deformity (funnel chest-shaped thorax or scoliosis) without severe heart disease, but with mitral valve prolapse and bronchiectasis in the middle lobe and reed segments of the lungs.

The fibrotic cavity form of mycobacteriosis is typical for the patients with long-term smoking experience. The clinical picture is most similar to TB (weakness, fever, weight loss, loss of appetite, night sweats, cough, chest pain, hemoptysis). In X-ray study, a large cavity with fibrous walls was usually found in such cases, which required differential diagnosis, primarily with lung cancer, TB or pneumonia.

PINM on the background of other lung diseases is usually the most difficult to diagnose. This clinical form has long been hidden under the mask of background disease, which is usually chronic obstructive pulmonary disease.

Thus, the difficulty in PINM diagnostics lies in the fact that the clinical, X-ray and histological characteristics are similar to those of TB. The main criterion in the diagnosis is cultural study, namely the isolation of the mycobacteria cultures and their identification.

The American Thoracic Society alongside the American Society for Infectious Diseases published a manual in 2007 on the diseases caused by NTM and developed the main diagnostic criteria for PINM (Table 3).

Table 3

Diagnostic Criteria of Non-tuberculosis Mycobacterial Lung Disease (ATS/IDSA Statement, 2007)

Clinical criteria	Pulmonary symptoms Nodular or cavitary opacities on chest X-ray or an high-resolution computer tomography (HRCT) scan that shows multifocal bronchiectasis with multiple small nodules Appropriate exclusion of other diagnoses
Microbiological criteria	Positive culture results from at least two separate expectorated sputum samples (If the results from the initial sputum samples are nondiagnostic, consider repeat sputum AFB smears and cultures) **or** Positive culture results from at least one bronchial wash or lavage **or** Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or AFB) and positive culture for NTM **or** biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washings that are culture positive for NTM

These criteria are not absolute. Normally two positive sputum cultures are required on NTM. In patients with AIDS, even a single isolation of NTM culture should be regarded as clinically significant. The fact that NTM is isolated from the patient requires a thorough clinical analysis from the doctor to determine the etiological significance of the isolated culture and conduct a differential diagnosis of the disease.

There is great practical interest in the possibility of using tuberculin skin test, other tests for latent TB and species-specific antigens for intradermal tests for differential diagnosis of TB and PINM. Unfortunately, a large number of cross reactions are observed between different mycobacterial antigens while the tuberculin skin test with 2 TE does not allow differential diagnosis between TB, PINM and complications of BCG vaccination (since the strain of *M. bovis BCG* was used as a vaccine). Previously proposed sensitins (for example

M. avium sensitin, tuberculin-type preparation derived from NTM) have not found wide application in the clinic because of insufficient specificity and low effectiveness.

Currently, a new test for the diagnosis of latent TB infection, namely diaskintest (tubercular recombinant allergen, protein CPF10-ESAT6) is used in addition to the tuberculin skin test. Virulent strains of M. tuberculosis and *M. bovis* have a region of RD1 in their genome, coding the synthesis of specific proteins CFP10 (the protein of the culture filtrate) and ESAT6 (the early marker of TB infection); however, a RD1 region is absent in the genome of all strains of *M. bovis BCG* and non-tuberculosis mycobacteria.

Due to the use of the CPF10-ESAT6 protein, the diaskintest has a higher specificity compared to the Mantoux test. A positive test result indicates the activity of a TB infection and can be used for differential diagnosis of TB and PINM, while a positive tuberculin skin test determines only the presence of an organism's infection with mycobacteria.

QuantiFERON® test (in vitro) in which the release of interferon-gamma is evaluated also offers highly specific results for the presence of latent TB. This test is approved for the diagnosis of TB and can be used for differential diagnosis of the disease by WHO. QuantiFERON® is a highly sensitive test and is performed within one day.

Thus, the isolation and identification of NTM are the most clinically significant methods of diagnosis of PINM. If establishing the diagnosis of PINM using microbiological and radiological methods is impossible, bronchoscopy with pulmonary tissue biopsy or video-assisted thoracoscopy with a histological examination of the biopsy specimen is recommended.

The International Statistical Classification of Diseases and Related Health Problems of the Tenth Revision (ICD-10) includes NTM-related infections under A31 “Infections caused by other mycobacteria”. Until now, a unified clinical classification of PINM has not been developed. It is strongly recommended to use the clinical classification of TB for the diagnosis, replacing the term “TB” with “PINM” and to specify the species of non-tuberculosis mycobacteria isolated from the patient instead of abbreviation “MTB+”. For example, PINM, *M. avium,* disseminated form.

TREATMENT OF PULMONARY INFECTIONS DUE TO NON-TUBERCULOSIS MYCOBACTERIUM

Establishing diagnosis of pulmonary mycobacteriosis in itself does not entail mandatory treatment. Indications for therapy are determined individually based on the ratio of possible risks and benefits for each individual patient.

Treatment of mycobacteriosis was initially based on the use of anti-TB drugs. However, it became clear very soon that treatment using anti-TB drugs is

ineffective in some cases of PINM. NTMs are susceptible *in vitro* only to anti-TB drugs in high concentrations. Today, the results of many studies indicate that the treatment of PINM is an even greater problem than the treatment of TB. The duration of chemotherapy depends on the form and severity of the lesions. Treatment of PINM is prolonged, often lasting up to 24 months with severe side effects and low effectiveness of therapy. This is largely grounded in the fact that unified methods for testing drug sensitivity of NTM have not been developed.

Standard protocols for PINM treatment have not been developed yet. The American and British Thoracic Societies reported about drugs effective against different NTM species *in vitro* and used in the treatment of PINM. However, there is no absolute parallel between the effect of drugs *in vitro* and the effect of chemotherapy in real clinical practice.

TREATMENT OF PULMONARY INFECTIONS DUE TO *M. AVIUM* COMPLEX

According to the ATS/IDSA Statement, 2007, the primary drugs in the treatment of PINM caused by MAC are macrolides (clarithromycin and azithromycin) and ethambutol (Table 4).

Table 4

Therapy of PINM – Recommendations according to the disease status or/and severity

Initial Therapy for Nodular/Bronchiectatic Disease	Initial Therapy for Cavitary Disease	Advanced (Severe) or Previously Treated Disease
Clarithromycin 1000 mg TTW or azithromycin 500–600 mg TIW	Clarithromycin 500–1000 mg/d or azithromycin 250–300 mg/d	Clarithromycin 500–1000 mg/d or azithromycin 250–300 mg/d
Ethambutol 25 mg/kg TTW	Ethambutol 15 mg/kg/d	Ethambutol 15 mg/kg/d
Rifampicin 600 mg TTW	Rifampicin 450–600 mg/d	Rifampicin 450–600 mg/d
	Amikacin or none	Amikacin or streptomycin 25 mg/kg three times a week during the initial 3 months of therapy

Definition of abbreviations: TTW = three times a week.

Comparative studies of the efficacy of treatment regimens containing clarithromycin or azithromycin have not been conducted. It is believed that a certain additional positive effect of therapeutic regimes containing macrolides may be due to their immunomodulatory effects.

Macrolides are combined with rifampicin and in some cases with injectable aminoglycosides (amikacin), although the advantage of regular administration of amikacin in the early stages of treatment has not been proven. In general, treatment regimens consisting of only two drugs are not recommended because of the likelihood of the development of resistance to macrolides, although no

major studies have evaluated the efficacy of two- or three-component treatment regimens. Previous unsuccessful therapy of diseases caused by the MAC reduces the chances of successful subsequent treatment, even if there is MAC sensitivity to macrolides.

Most patients who do not need an intensive treatment strategy (focal changes in the lungs in light of bronchiectasis) or patients with a fibrous-cavity form of the disease with the presence of side effects which make it impossible to take drugs daily, intermittent therapy (3 times a week) are recommended. Treatment is continued until sputum is negative. The total duration of the therapy is at least 1 year. Daily drug intake is recommended for patients with fibrous-cavity forms or a severe progressive course of the disease. The daily dose of clarithromycin can be divided into two doses (500 mg twice a day) to improve tolerability. Additionally, patients with low body weight (less than 50 kg) or at the age over 70 should also reduce the daily dose of clarithromycin (up to 500 mg/day or 250 mg twice a day) in a case of gastrointestinal side effects. In some cases of a severe course of the disease and intolerance to oral drugs, injections are possible. The collective clinical experience also supports the use of the intermittent aminoglycoside therapy (amikacin or streptomycin at 25 mg/kg three times a week during the initial 3 months of therapy) in extensive or drug resistant MAC infection. For extensive disease, at least 2 months of intermittent (twice or three times a week) streptomycin or amikacin is recommended, although longer parenteral therapy may be eligible in patients who do not tolerate other drugs.

TREATMENT OF PULMONARY INFECTIONS DUE TO *M. AVIUM* COMPLEX IN HIV-INFECTED PATIENTS

The development of disseminated forms of PINM is typical for HIV-infected patients. The therapy regimens of the disseminated process caused by MAC is presented in Table 5.

Table 5

Recommendations for Treatment and Prevention of PINM caused by *M. avium complex* in HIV-infected patients

Preferred	Alternative
Treatment	
Clarithromycin 500 mg orally twice a day	Azithromycin 500 mg a day
+	
Ethambutol 15 mg/kg orally a day	Ethambutol 15 mg/kg a day
±	
Rifampicin 450–600 mg orally a day	Rifampicin 450–600 mg orally a day
Prevention	
Azithromycin 1200 mg orally a week	Clarithromycin 500 mg orally twice a day
	Rifampicin 450–600 mg orally a day

All patients should receive clarithromycin (1000 mg a day or 500 mg twice a day) or alternatively azithromycin at a dose of 500 mg a day. Etambutol should be administered at a dose of 15 mg/kg a day.

In the presence of resistance to macrolides, treatment becomes less effective. Aminoglycosides (amikacin) or fluoroquinolones (moxifloxycin) may be included in the treatment regimen in such cases. Clofazimine is not used in treatment, since its use is associated with an increased risk of mortality. The effectiveness of intermittent therapy has not been studied at present.

Treatment of PINM in patients with AIDS is complicated by adverse reaction and interaction of drugs. Combinations of clarithromycin and rifampicin may increase serum rifampicin level and are associated with arthralgia, uveitis, neutropenia, and impaired hepatic function. Rifampicin should be used at a lower dose or even discontinue if these adverse effects develop. Clarithromycin should not be used at doses higher than 500 mg twice a day, as this increases the risk of death of the patient. It is important to consider that rifampicin is an inducer of cytochrome P-450 isoenzymes and therefore interferes with the metabolism of many protease inhibitors and reverse transcriptase inhibitors used in the treatment of HIV infection.

Preventive therapy recommended for persons with $CD4^+ < 50$ cells/ml; may stop if > 100 cells/ml. Azithromycin is mostly used in the dose of 1200 mg or clarithromycin 1000 mg a day for the prevention. It is possible to use rifampicin 450–600 mg or rifabutin 300 mg a day, but its tolerance is lower.

TREATMENT OF PULMONARY INFECTION DUE TO OTHER MYCOBACTERIA

M. kansasii. The daily regimen of isoniazid (300 mg a day), rifampin (600 mg a day) and ethambutol (15 mg/kg a day) is recommended. Therapy lasts for 1 year (until sputum negativation).

M. abscessus. A treatment regimen with proven efficacy has not been developed. A variety of regimens are recommended, including clarithromycin 1000 mg a day, which cause an improvement of the condition. It is recommended to combine chemotherapy and surgical treatment.

SURGICAL TREATMENT OF PULMONARY INFECTIONS DUE TO NON-TUBERCULOSIS MYCOBACTERIUM

Surgical treatment is often used in PINM, although the indications for it are developed much less in detail than for TB.

Indications for surgical treatment of pulmonary infection due to non-tuberculosis mycobacterium:

– prolonged hemoptysis;

– progression of pulmonary lesions detected by X-ray examination.

Principles of surgical treatment of pulmonary infection due to non-tuberculosis mycobacterium:

– do not wait too long for the process progression when any intervention is already ineffective;

– it is necessary to combine surgical treatment with chemotherapy.

In some cases, surgical treatment is very successful. For example, the efficiency of surgical treatment of cervical lymphadenitis caused by NTM exceeds 90 %.

TASKS FOR INDEPENDENT WORK OF STUDENTS

1. Estimate morphology and growth of NTM colonies on egg medium.
2. Analyze the results of the cultural study for curated patients.
3. Analyze the results of cultural and molecular methods for the patients with PINM.
4. Plan the treatment of the patient with pulmonary infection due to MAC.
5. Plan the treatment of a HIV-infected patient with pulmonary infection due to MAC.
6. Determine the required minimum of examinations for a patient with suspected PINM.

SELF-MONITORING TEST TASKS

You must select 1 or more answers.

1. Species of mycobacteria included in the M. tuberculosis complex:
   a) *M. tuberculosis*; b) *M. bovis*; c) *M. avium*; d) *M. africanum*.
2. Which NTMs belong to the group of scotochromotogenic mycobacteria according to Runyon classification:
   a) not pigmented in the dark but acquired pigmentation after exposure to light;
   b) forming a pigment in the dark;
   c) not forming a pigment or having a pale yellow color, which does not increase on the light;
   d) fast growing.
3. Which mycobacteria are obligate pathogens for humans?
   a) *M. tuberculosis*; b) *M. intracellulare*; c) *M. avium*; d) *M. leprae*.
4. Colonies of the fast-growing NTM appears on egg media:
   a) in 4–7 days; b) in 14–17 days; c) in 24–27 days.
5. Select the correct statement for the cord-factor:
   a) the MTB virulence factor; b) the NTM virulence factor.

6. Choose the most reliable diagnostic criterion for pulmonary mycobacteriosis:
   a) symptoms of lung pathology;
   b) the presence of focal cavity or infiltrative changes on X-ray picture;
   c) NTM culture isolation on solid or liquid media from 2 sputum samples;
   d) detection of AFB in sputum.
7. Identify the most common X-ray signs of mycobacteriosis caused by MAC:
   a) single focal lesions in the lungs;
   b) cavity formations with thick fibrous walls in the upper lobes of both lungs;
   c) focal and interstitial dissemination in combination with multiple bronchiectasis in both lungs;
   d) left pleural effusion.
8. Choose the most effective antibiotics for the treatment of mycobacteriosis caused by *M. kansasii*:
   a) rifampicin; d) cefepime;
   b) levofloxacin; e) clarithromycin.
   c) amoxicillin;
9. Determine the patients with which diseases are at risk group for pulmonary mycobacteriosis:
   a) diabetes mellitus; d) bronchial asthma;
   b) HIV infection; e) long-term administration of glucocorticosteroids.
   c) COPD;

SITUATIONAL CLINICAL TASKS

Task 1

Patient K., 74 years old, a retired person. Changes in the lungs were revealed when consult with the therapist about shortness of breath increasing during physical activity, weakness, cough with mucous sputum, and body weight loss. The patient considers himself sick for about 4 months. No contacts with TB patients were revealed. Last X-ray examination was 2 years ago. The experience of smoking is more than 40 years. He was hospitalized with a preliminary diagnosis of infiltrative TB in the left lung, decay phase, MTB−. On admission: The general condition is satisfactory. The left half of the chest lags behind when breathing. On the left, there is a shortening of the percussion sound, bronchial breathing, single multi-caliber wet and scattered dry crepitation. Hemogram: L 12.2 × 10^9/L, ESR 53 mm/h. There are moderate violations of the lung functional test by obstructive type. AFB 1+ were detected in sputum three times. The culture of mycobacteria without a cord factor isolated twice using BACTEC MGIT 960 and solid media and identified as *M. malmoense* by LPA.

Survey X-ray. A system of cavity formations is located on the background of fibrosis in the volume-reduced upper lobe of the left lung. In the upper lobe of the right lung fibrous foci. The mediastinum is shifted to the left (Fig. 3).

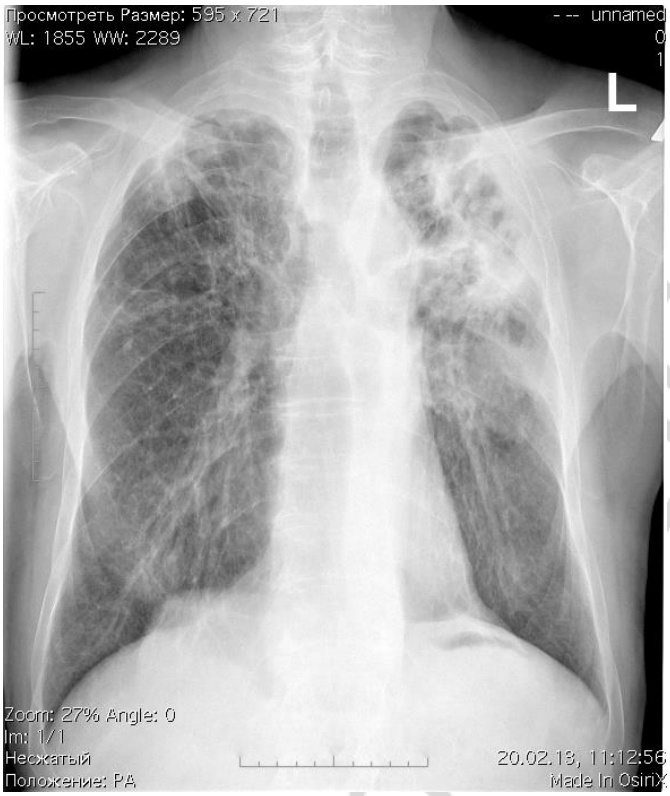

Figure 3. Survey X-ray of patient K. Direct projection

Task:
1. Diagnose the underlying disease.
2. Identify the background disease.
3. Assign the most rational treatment regimen.

Task 2

Patient M., 47 years old, an engineer. He smoked for 25 years, hasn't been smoking for 6 years. He suffers from rheumatoid arthritis, 2 years constantly takes methylprednisolone. He undergoes an X-ray examination annually. He denies a contact with patients with TB. He went to the clinic with complaints of cough with sputum, general weakness, temperature increase up to 37.5 °C. Objective examination: underweight patient, there are single dry wheezes in the lungs. Heart tones are clear, rhythmic, pulse 78 per min, AP 130/85. General and biochemical blood tests are without pathology. In X-ray examination, changes in the lungs were detected (Fig. 4). The course of treatment with amoxicillin and azithromycin for 14 days did not led to positive X-ray dynamics. Preliminary diagnosis: infiltrative TB in the upper lobe of the right lung. Pre-examination in the anti-TB dispensary: **chest CT** (Fig. 4); sputum

examination. AFB 1+ has been detected twice. Treatment: isoniazid, rifampicin, pyrazinamide, ethambutol.

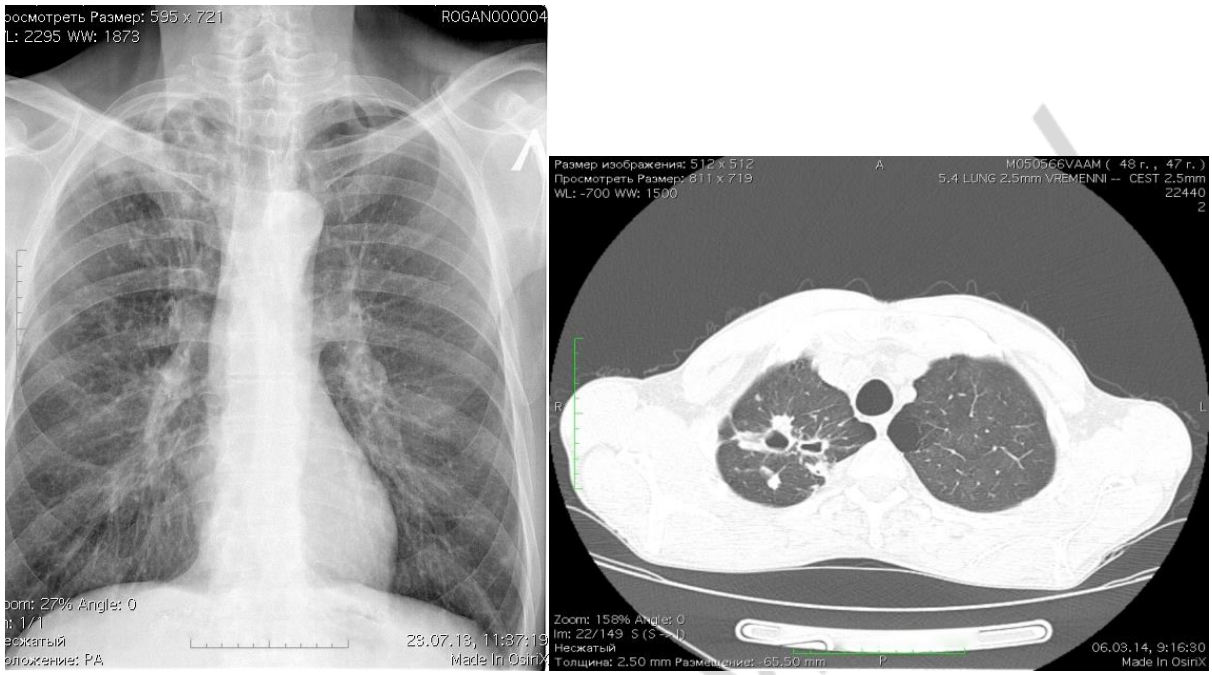

Figure 4. Survey X-ray and CT of patient M.

A culture of mycobacteria without cord factor is isolated in the sputum using BACTEC MGIT 960 and solid media and identified as *M. intracellulare* by LPA.

Task:

1. Describe X-ray changes in lungs.
2. Formulate a clinical diagnosis.
3. Assign a treatment plan to this patient.

Task 3

Patient A., 39 years old, a programmer. Since childhood, he has type 1 diabetes, takes insulin. Changes in the lungs were detected during the regular X-ray examination. He does not smoke and denies contacts with TB patients.

Objective examination at outpatient clinic: breathing is vesicular, heart tones are clear, pulse 80 per minute, AP 120/80. General and biochemical blood tests are without pathology. Patient suffers from a slight cough with mucous-purulent sputum. A course of treatment with ceftriaxone and azithromycin for 10 days was conducted. The patient's condition has improved, the cough reduced, but X-ray changes in the lungs persisted. Focal shadows of small and medium intensity were identified in the upper lobe of the left lung on the **survey X-ray and CT** (Fig. 5).

Bacterial excretion was not found. After a pre-examination in the anti-TB dispensary focal TB of the upper part of the left lung, MTB– was diagnosed.

The result of tuberculin skin test with 2 TE is papule 8 mm, the result of diaskintest is “the injection reaction”. The culture of *M. tuberculosis* susceptible to all anti-TB drugs is isolated on the solid media. Positive clinical and X-ray dynamics of the process were noted during the treatment of TB. However, after the end of the intensive phase of chemotherapy, the NTM culture was isolated once from the sputum and identified as *M. fortuitum* by LPA. The clinical diagnosis has been established: pulmonary mycobacteriosis (*M. fortuitum*); focal TB of the upper part of the left lung in the phase of resorption and compaction. Therapy: isoniazid, rifampicin, levofloxacin, amikacin, doxycycline.

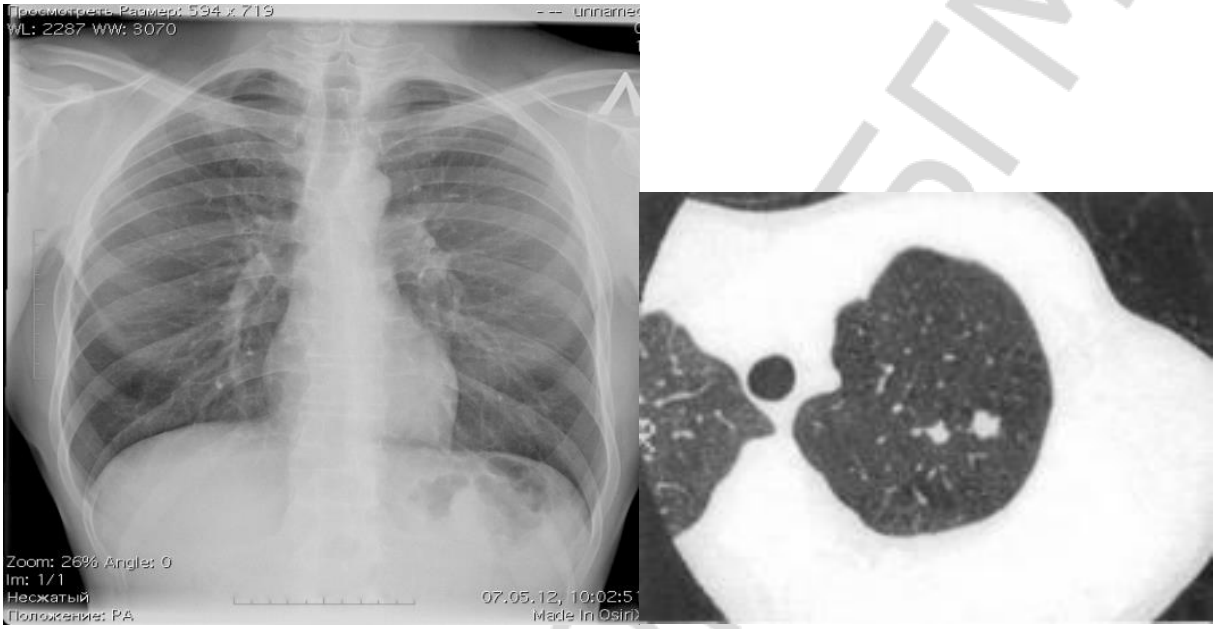

Figure 5. Survey X-ray and CT of patient A.

Task:

1. Do you agree with the diagnosis?
2. Is the diagnostic tactics defined correctly?
3. Assign an appropriate treatment plan to this patient.

Task 4

Patient T., 41 years old. He has been smoking for more than 20 years, has HIV infection for 16 years (stage IV). He discontinued antiretroviral therapy on his own 2 years ago. During the last year, he treated pneumonia three times. He appealed for medical care with complaints of a cough, weakness, fever up to 37.5 °C, strong sweating, body weight loss for 7 kg. Objective examination: underweight patient, vesicular breathing. Heart tones are clear, rhythmic, pulse 76 per minute, AP 125/85. General blood test: ESR — 52 mm/h. Biochemical blood test is without pathology.

X-ray changes are shown on Fig. 6. AFB were found in the sputum smear. Miliary TB, AFB+ was diagnosed. The culture of mycobacteria without cord

factor was isolated five times using BACTEC MGIT 960 and solid media and identified as *M. avium* by LPA.

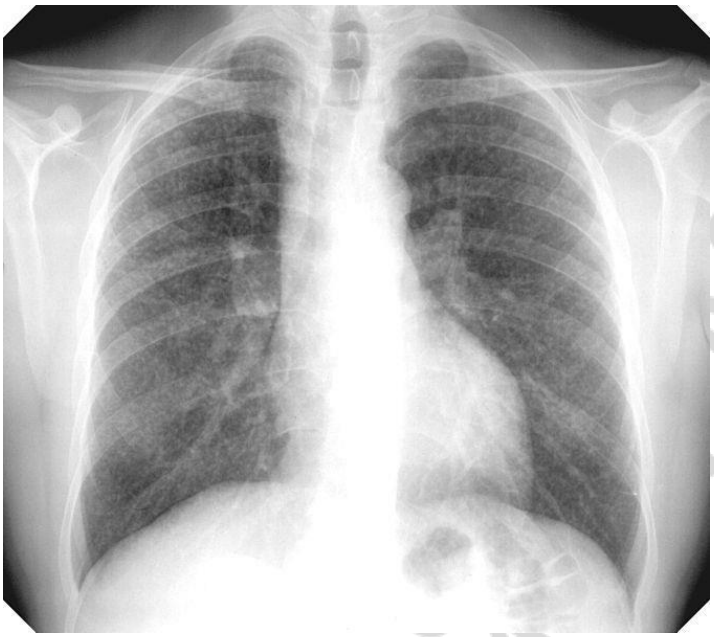

Figure 6. Survey X-ray of patient T.

Task:

1. Describe the changes on X-ray picture.
2. Formulate a clinical diagnosis.
3. Assign a treatment plan to this patient.

Task 5

Patient V., 66 years old, a retired person. He has been smoking for more than 25 years. He complaints about dyspnea increasing during the physical activity, cough with mucous-purulent sputum, temperature increase up to 37.2 °C, body weight loss. He considers himself sick for about 5 years, when dyspnea and cough appeared. He hasn't been suffering from TB and denies contact with TB patients. Objective examination at admission: asthenic physique, low nutrition patient, finger phalanges and nails in the form of "drum sticks and watch glasses", percussion sound over the lungs is shortened, with a tympanic hue, lung breathing is weakened, scattered dry and isolated small-bubble wet rales. The heart tones are muted, the pulse is 92 per minute, AP 140/90. The general blood test: L 15.0×10^9/L, ESR 35 mm/h.

On the survey X-ray, focal and interstitial changes, cellular transformation of the lung pattern are visualized mainly in the upper lobes of both lungs, more on the left (Fig. 7).

AFB 2+ were detected three times in sputum. The culture of mycobacteria without a cord factor was isolated twice using the automated system BACTEC MGIT 960 and solid media and identified as MAC by LPA.

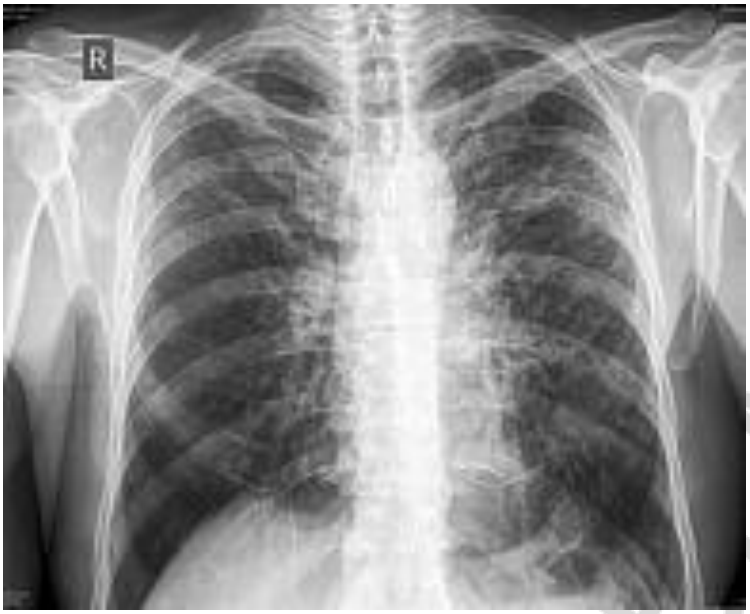

Figure 7. The survey X-ray in the direct projection of patient V.

Task:

1. Make a clinical diagnosis and justify it.
2. Define additional diagnostic methods.
3. Assign a treatment plan to this patient.

LITERATURE

Main

1. *Современная* бактериологическая диагностика туберкулеза : учеб.-метод. пособие / И. И. Дюсьмикеева [и др.]. Минск : БГМУ, 2018. 30 с.

2. *Согласованные* рекомендации Американского фонда кистозного фиброза (муковисцидоза) и Европейского общества кистозного фиброза по лечению микобактериоза у пациентов с кистозным фиброзом / Р. А. Флото [и др.]. Санкт-Петербург : Благотворительный фонд «Острова», 2017. 32 с.

3. *An official* ATS/IDSA statement : diagnosis, treatment, and prevention of non-tuberculosis mycobacterial diseases / D. E. Griffith [et al.] // Am. J. Respir. Crit. Care Med. 2007. Vol. 175. P. 367–416.

Additional

4. *Перельман, М. И.* Фтизиатрия : учеб. / М. И. Перельман, И. В. Богадельникова ; под ред. М. И. Перельмана. 4-е изд., перераб. и доп. Москва : ГЭОТАР-Медиа, 2015. 448 с.

5. *Epidemiology* of Non-tuberculosis Mycobacterial Infection, South Korea, 2007–2016 / Hyewon Lee [et al.] // Emerging Infectious Diseases. 2019. Vol. 25, № 3. www.cdc.gov/eid.

6. *Incidence* of disseminated Mycobacterium avium-complex infection in HIV patients receiving antiretroviral therapy with use of Mycobacterium avium-complex prophylaxis / Y. Jung [et al.] // Int. J. STD AIDS. 2017. Vol. 28, № 14. P. 1426–1432.

7. *Disseminated* Non-tuberculosis Mycobacteria in HIV-Infected Patients, Oregon, USA, 2007–2012 / C. D. Varley [et al.] // Emerg. Infect. Dis. 2017. Vol. 23, № 3. P. 533–535.

TABLE OF CONTENTS

ISBN 978-985-21-0665-8