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CHAIR OF PNEUMOPHTHISIOLOGY

Evelina LESNIC, Alina MALIC

UPDATES IN DIAGNOSIS AND DETECTION OF TUBERCULOSIS

Methodical recommendations for practical works, seminars, and individual work

CHISINAU, 2022

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Authors:

Lesnic Evelina – Ph.D., associate professor,
Malic Alina – Ph.D., university assistant.

Reviews:

Popa Vasile – Ph.D., associated professor,
Bortă Vasile – Ph.D., associated professor.

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Authors' note

The methodical recommendations for practical works, seminars and individual work were developed according to the actual guide on the methodological scientific publications elaborated by the ,,Nicolae Testemitanu" State University of Medicine and Pharmacy. This methodological guidance paper was developed to present the information used in the teaching-learning-evaluation process described in the curriculum of the discipline of pneumophthisiology and indicates how to transfer knowledge by applying the procedures established in the analytical program of the discipline.

The target group is composed of students attending university studies in the specialty of pneumophthisiology. The proposed topics represent the basic notions about the detection and diagnosis of tuberculosis developed in the context of the international guidelines. At the level of knowledge acquisition, the methodical recommendation aims to develop specific skills in the n detection of tuberculosis suspected cases and to describe the methods of patient examination and diagnosis of tuberculosis. At the application level, the purpose of the paper is to implement into practice all the stages needed for diagnosing tuberculosis, including anamnesis, physical examination, interpreting of objective data, as well as indicating the evaluation of the results of the paraclinical examination to establish the diagnosis.

As theoretical support for the proposed methodological recommendations was used the national clinical standards, the international guidelines, and the scientific-methodological papers published by international and Moldovan scientists with an international profile. Different teaching methods and didactic procedures that had been integrated into the academic process.

The professional skills acquired as a result of the study of the proposed methodological recommendations will include epistemological, investigative, communicative, and metacognitive skills. The compulsory compartments included in the current methodological recommendations are the editorial page, approval page, introductory note, overall aim and specific objectives, informative material, as well as learning methodology written according to the *curriculum* of the specialty pneumophthisiology, self-assessment tests, and bibliographic references.

Authors

INTRODUCTION

The methodical recommendations for practical works, seminars, and individual work were developed according to the updated guide on the methodological scientific publications elaborated by the „Nicolae Testemitanu„ State University of Medicine and Pharmacy. This methodological guidance paper on „Detection and diagnosis of tuberculosis" was developed to present the information about detection and diagnosis of tuberculosis, according to the current curriculum of the discipline of pneumophthisiology and indicates how to transfer knowledge by applying the procedures established in the analytical program.

At the level of knowledge acquisition, the methodical recommendations aim to develop specific skills on the detection of tuberculosis suspects, describing methods of examination and diagnosing tuberculosis. At the application level, the purpose of the methodical recommendations is to implement into practice all the stages needed for diagnosing tuberculosis, including anamnesis, physical examination, interpreting objecttive data, indicating the evaluation of the results of the paraclinical examination in order to establish the final diagnosis.

The objectives of the methodical recommendations were described as those stated within the *curriculum* of Pneumophthysiology. These are reflected in the materials and methods necessary for performing the practical work. For the students' self-assessment the questions and topics that have to be addressed for the individual preparation based on the presented information are exposed. The sources are indicated in the bibliography for the independent training.

The themes of the proposed methodical recommendations are:

- Detection of tuberculosis;
- Methods for diagnosing the latent tuberculosis infection;
- Methods for diagnosing the active tuberculosis.

The overall aim: Development of specific skills in the detection of tuberculosis, describing methods of examination, and diagnosing tuberculosis.

Objectives:

- To define the ways for detection of the individuals suspected of tuberculosis, including the passive way of detection and algorithm for the initial laboratory diagnosis of individuals with symptoms consistent with pulmonary tuberculosis. To define the active detection way and to apply the criteria for identifying high-risk groups, high-vigilance groups, and endangered groups, as well as their management.
- To know the procedure of completing the TB patient's record form.
- To describe and apply examination methods for diagnosing active tuberculosis and latent tuberculosis infection.
- To acquire information on the new methods for diagnosing active tuberculosis.
- To study other methods used in the investigation of the patient with active tuberculosis

The methodical recommendation comprises the following content units:

- Passive detection method. History of the disease. Clinical examination of the patient. Carrying out and evaluating the laboratory investigation results. Patient's medical record and completeness standard.
- Algorithm for the initial laboratory diagnosis of individuals with symptoms consistent with pulmonary tuberculosis. Classification of tuberculosis according to the International Classification of Diseases 10.
- Tuberculin skin test. Tuberculin. Types of tuberculin. The purpose of the tuberculin skin test. Advantages and disadvantages of the tuberculin skin test. Intradermal reaction Mantoux with 2 UT. The body's reaction to intradermal reaction with tuberculin. Interpretation of skin reaction. Tuberculin conversion. Booster effect.

- Active detection. High-risk groups, high-vigilance groups, and endangered groups. Epidemiological investigation.
- Methods of diagnosing tuberculosis. Microbiological examinations and their importance. Aims and overall principles of collecting, transporting, and storing the clinical samples. Microscopic examination. Bacteriological examination. Cultivation of mycobacteria on solid and liquid media (Lowenstein-Jensen, BACTEC, MB/BacT). Molecular-genetic methods for identification of *M. tuberculosis* complex. Drug susceptibility assay *of M. tuberculosis* to anti-tuberculosis drugs.
- Radiological examination of patients with tuberculosis. Radiological patterns in pulmonary tuberculosis.
- Functional respiratory examinations. Spirography. Bodyplethysmography. Gasometry. Perfusion scintigraphy. Interpretation of the results.

Materials and academic methodology

In the academic methodical process, a wide range of methods and learning procedures for increasing the effectiveness of acquiring knowledge and practical skills was used. The students are asked to attend a compulsory lecture lasting for one academic hour. The academic process continues in a practice room. The students' presence at the practical work is compulsory. The student's absence from practical work implies the non-admission to the exam. The students' activities in the practical lessons involve interactive participation, conversation with the university lecturer, assistant professor, or associated professor with explaining, demonstrating the required information.

The training methods include the main methodologies specific to the interference between the fields of medicine and pedagogy recommended by the module „Psychopedagogy for Teachers" developed by the Department of Management and Psychology. For the application of the problem-based learning method and the clinical case-based reasoning, method was paid particular attention. The case study was used to develop clinical

judgment as a fundamental teaching tool in continuous medical education. The strategic approach to teaching, knowledge acquisition, and evaluation was considered by using the new electronic platforms: Google Meet, Zoom, and Moodle.

The formative assessment is systematically performed during the practical lessons. It involves different methods: oral and written ones, tests, and clinical situations with the physical presence or virtually. During the practical lessons, the students are evaluated based on the evaluation test, clinical activity, assessment of practical knowledge, and the case study.

For the individual training, the references to the available literature were provided at the end of the current paper.

TOPIC 1. TUBERCULOSIS CASE DETECTION

The purpose of the methodical recommendations used for practical activity, seminars, and individual work was to create specific skills in the detection of suspected cases of tuberculosis and patient examination. At the application level, this paper was conducted to carry out all steps for identifying patients from the high-risk groups, high-vigilance groups, and endangered groups, as well as to apply methods of investigation to establish the diagnosis of tuberculosis. The practical activity is based on the implementation of the necessary methods for diagnosing tuberculosis, such as data collection (anamnesis), physical examination, assessment of the results obtained by the clinical examination, indicating, and interpretation of the results of the investigations necessary to establish the diagnosis of tuberculosis, as well.

The teaching methods used are the problem-based learning method and the clinical case-based reasoning method. The case study will be used to develop the student's clinical thinking. The materials and tools used within the practical work are the stethoscope, sphygmomanometer, thermometer, pulse oximeter, wet wipes, gloves, hand sanitizer gel, and tongue depressor for examining the oral cavity. The student will wear a white robe and face mask, a cap, and a pair of disposable protective overshoes.

The course study duration required for the diagnosis of tuberculosis will last one academic hour, whereas six academic hours are used for the practical activity.

Topics for individual learning:

The following relevant topics are included within the individual work of the student:

- Methods for detection of tuberculosis. Passive way of detection and active detection.
- Risk factors for tuberculosis. High-risk groups, high-vigilance groups, endangered groups.
- Examination of the patient with tuberculosis. Anamnesis. Clinical examination.

- Algorithm for the initial laboratory diagnosis of individuals with symptoms consistent with pulmonary tuberculosis.
- Medical record of the patient diagnosed with tuberculosis.
- Epidemiological investigation. Identifying contacts and prioritizing them. Contact management.

Theoretical framework

1.1 Methods for detection of tuberculosis

Tuberculosis is detected by applying medical-sanitary measures, which would identify the suspected cases of tuberculosis for the prompt initiation of anti-tuberculosis treatment.

There are two ways of TB detection - passive and active.

- Passive screening is the examination of patients with symptoms consistent with pulmonary tuberculosis.
- Active screening is the annual examination of certain groups of the population at risk for tuberculosis.

Passive detection. Examination of symptomatic patients.

According to the recommendations, the detection of suspects will be done mainly passively. Passive detection consists of direct addressing the patient to the primary healthcare service. The family doctor performs the primary clinical evaluation, then refers the patient to the department specialized in phthisiopneumology to investigate and establish the diagnosis of tuberculosis.

Passive detection consists in examining the patient with respiratory symptoms: persistent cough for more than 2-3 weeks, mucous or mucopurulent sputum, hemoptysis, progressive dyspnoea, and chest pain. Patients with clinical signs of intoxication syndrome, such as asthenia, weight loss, anorexia, fever or evening low-grade fever, profuse night sweats will also undergo examination. In symptomatic individuals, the objective clinical examination, blood count, HIV test, and microbiological investigations, including sputum microscopy to identify acid-fast bacilli and GeneXpert MTB/RIF genetic molecular test will be done. The clinical

specimen, as sputum, will also be subjected to bacterial culture assay and microbial sensitivity tests for first-line anti-tuberculosis drugs. The patients will be investigated using the posteroanterior and lateral chest X-ray as well. Investigations of other organs and systems will be done according to medical indications.

All patients with symptoms suggestive of tuberculosis will undergo the identification of acid-fast-bacilli (AFB) by sputum microscopy and the GeneXpert MTB/RIF test. Thus, the first sputum sample is collected on the day when the patient is referred to the doctor, whereas the second sample is collected the next morning, also known as the „morning sample". The cases will be notified according to the degree of emission of bacilli in the negative, weakly positive (+), moderately positive (2+), heavy positive (3+) results. All the positive cases confirmed by GeneXpert MTB/RIF test will be subjected to bacteriological examination by conventional cultures (Lowenstein Jensen and BACTEC) from the same sputum sample with subsequent susceptibility assessment to first-line anti-tuberculosis drugs, as well as undergo bronchial fibroscopy, tuberculin skin test in children (age 0-18 years), high-resolution computed tomography, nuclear magnetic resonance, functional respiratory examinations, according to medical indications. The diagnosis of tuberculosis is confirmed exclusively by the phthisiopneumologist.

1.2 Tuberculosis diagnostic algorithm

The persons with symptoms consistent with pulmonary tuberculosis and/or radiological abnormalities suggestive of pulmonary tuberculosis will be examined by the healthcare staff of the primary medical-sanitary institutions from the administrative and territorial reference units. The family doctors will refer the suspects to the phthisiopneumology unit located within the district/municipality for a complex check-up. Clinical and laboratory investigations for the diagnosis of tuberculosis will be indicated during the check-up. The microbiological and microscopic examination, as well as the molecular-genetic method Gene Xpert MTB/RIF,

will be indicated for the patients. These investigations will be carried out within the microbiological laboratory of the territory or department.

At least 2 sputum samples should be collected for microbiological examination from all patients with clinical symptoms suggestive of pulmonary tuberculosis. Both clinical samples will be assessed by the microscopic method, whereas only one sample, preferably in the morning will be examined through the molecular-genetic method Gene Xpert MTB/RIF. The molecular-genetic methods Gene Xpert MTB/RIF and Xpert Ultra are used to diagnose pulmonary tuberculosis and detect rifampicin resistance of *M. tuberculosis* in the following clinical specimens: sputum, feces, nasopharyngeal and gastric samples. Molecular-genetic methods are also used to diagnose extrapulmonary tuberculosis. For the etiological confirmation of tuberculosis, before the initiation of the anti-tuberculosis treatment, all the new cases and relapses will require the collection of clinical samples for the cultivation of *M. tuberculosis* on conventional culture media (Lowenstein Jensen and BACTEC) at the microbiological reference laboratories.

In cases with a positive Gene Xpert MTB/RIF result, RIF = REZ (Rifampicin resistant) - the diagnosis of RR/MDR TB will be established. In cases with a positive Gene Xpert MTB/RIF result, RIF = REZ which requires additional arguments to establish the multidrug resistance, the culture, and the molecular-genetic method (MTBDRplus) of the sputum will be performed to confirm the resistance against isoniazid.

All cases with confirmed RR/MDR TB through the Gene Xpert MTB/RIF will be examined by cultural and molecular-genetic MTBDRsl method, followed by the drug-susceptibility testing for the 2nd-line anti-tuberculosis drugs.

Patients at increased risk for multidrug-resistant tuberculosis (MDR-TB), showing a positive Gene Xpert MTB result, will undergo the molecular-genetic test MTBDRplus and/or cultural methods with isoniazid susceptibility testing.

Symptomatic patients with negative results on both the Gene Xpert/RIF and MTBDRplus, who are at increased risk for multidrug-

resistant tuberculosis and whose clinical symptoms and/or radiological images persist, will undergo the culture method.

Passive detection and diagnosis of tuberculosis are based on:

- Anamnesis collection;
- Clinical examination;
- Microbiological examination of sputum: microscopy at Ziehl-Neelsen or fluorochrome staining, Gene Xpert MTB/RIF assay, culture method followed by drug susceptibility testing for the first-line anti-tuberculosis drugs;
- Postero-anterior and lateral chest X-ray (digital or conventional radiological examination);
- Paraclinical examinations for other organs and systems;
- Counselling and testing for HIV biomarkers.

1.3 Active detection. Identification of tuberculosis cases through systematic screening.

Active screening (systematic screening) for tuberculosis will be performed annually in individuals from:

1. High-risk groups for tuberculosis;
2. Groups who require an increased vigilance for tuberculosis;
3. Endangered groups of the population.

According to the National Clinical Protocol, the predetermined high-risk groups for the annual radiological screening are:

1. Contacts with tuberculosis patients, identified during the epidemiological investigation of the outbreaks;
2. Persons who have had tuberculosis or have post-tuberculosis consequences (sequela);
3. HIV-infected people;
4. Immunocompromised people or those who receive immunosuppressive treatment (corticosteroid treatment, chemotherapy, radiotherapy, biological anti-TNF-α therapy);
5. Diabetic patients;

6. Patients suffering from mental diseases, before hospitalization in specialized institutions;
7. Migrants;
8. People without permanent residence or homeless.
9. Emergency health care staff;
10. Employees of institutions specialised in phthisiopneumology.

Other groups with an increased risk for disease, which prevails in a territory can be adjusted to the previously mentioned groups. It allows the extension of the area of the active detection interventions. Although the groups screened annually have been well defined, it is mandatory to maintain clinical vigilance on all groups with a high risk of disease, which have not been included in the list of high-risk groups.

Groups of the population who require an increased vigilance for tuberculosis include:

- Socially vulnerable people: unemployed and people with low-income;
- People with medical and biological risk factors: patients with chronic respiratory diseases, active smokers, chronic alcohol abusers, intravenous drug users, patients with diabetes, chronic renal failure, gastrointestinal pathologies, pregnant women, and BCG unvaccinated children;
- The employees of specific institutions: asylums, prisons, hospices, placement centers, institutions specialized in phthisiopneumology;
- Students of medical colleges, University of Medicine and Pharmacy, residents, clinical auxiliary staff;
- Healthcare staff;
- Nurses.

Through the active screening will be also checked the staff of endangered contingents, which includes:

A) Employees of the healthcare institutions:

1. Medical staff of the medical-sanitary institutions with pediatric profile;
2. Medical staff employed in rehabilitation and placement institutions for children.

B) Employees of the early childhood education institutions:
1. Staff of the preschool education institutions;
2. Staff of the primary education institutions.

The screening methods applied for active detection comprise:
A. Collection of anamnesis;
B. Clinical examination;
C. Posteroanterior and lateral chest X-ray in adults (digital or conventional), or tuberculin skin test in children (0-18 years).

Since the active case-finding programs have not been sufficiently effective and cost-effective, strategies for out-reach activities in high-risk groups have been developed. There are currently no active detection programs and educational activities for socio-vulnerable groups, such as previously incarcerated individuals, migrants, homeless people, and injecting drug users, leading to a delayed diagnosis of tuberculosis.

1.4 Epidemiological investigation

The outbreak of tuberculosis is the area where the source of *M. tuberculosis* infection is located, including people in that area, who are at a higher risk to be infected and developing active tuberculosis. The epidemiological investigation aims to establish the epidemiological danger of the tuberculosis outbreak.

The criteria for classifying the outbreak follows:
A. Microbiological status of the patient regarding *M. tuberculosis*;
B. Presence of the people, who are at a higher risk to be infected and developing the active disease;
C. Level of the general, hygiene and sanitary culture of the patient and members of the outbreak.

Depending on the epidemiological danger, the outbreaks are classified into 3 groups:

First-group tuberculosis outbreak with high epidemiological risk:

A. Patient is microscopically positive (AFB positive);
B. Children and/or pregnant women are present in the outbreak;
C. Precarious sanitary and hygienic conditions: the residential space does not correspond to the sanitary norms. In this category are included hostels, asylums, orphanages, hospitals, and closed-type institutions. Also, in this category is included the patient who does not show compliance to the medical recommendations;
D) Patient is infected with HIV;
E) Patient has the unknown microbiological report, and the diagnosis was established *post-mortem* (after death);

The second-group tuberculosis outbreak with moderate epidemiological risk:

A. Patient is microscopically positive or negative;
B. Children and/or pregnant women are not identified in the outbreak;
C. Patient has pulmonary tuberculosis with lung destruction, but is microscopically negative;
D. Patient is diagnosed with active tuberculosis, is microscopically negative, and is referred to as the endangered contingent of the population.

The third-group tuberculosis outbreak with minimal epidemiological risk:

A) Patient is diagnosed with active tuberculosis of any localization, which cannot be included in the first and the second group outbreaks;
B) Tuberculosis was diagnosed in domestic animals.

The establishment of the diagnosis of active tuberculosis determines the obligatory initiation of the epidemiological investigation in the outbreak within 72 hours from the patient's detection. The background for the initiation of the epidemiological investigation is the receipt of „Urgent declaration of the detection of infectious diseases, intoxications, food and

occupational poisonings, adverse reactions at the administration of immunobiological drugs" form 058/e.

The epidemiological investigation evaluates the estimated interval during which the infection or disease occurred and lists the persons who encountered the source of infection. Depending on the patient's relationship, the contacts may include family members, co-workers, occasionally met people, etc. An important step of the epidemiological investigation is the examination of all the persons included in the list of contacts. Depending on age, children will be tested by the tuberculin skin test, whereas the adults will undergo the chest X-ray. The evaluation of the results followed by the establishment of conclusions regarding the source of infection and people who were in contact and were infected will be carried out. If the infection was identified in cattle, an analysis of the milk and dairy products will be performed. The last stage of the epidemiological investigation consists of isolation of the patient and initiation of the anti-tuberculosis treatment, as well as chemopreventive treatment in contacts.

Measures to be taken in the infectious outbreak:

A) Elimination of the outbreak by isolating the patient and initiating the anti-tuberculosis treatment as soon as possible;
B) Disinfection (absolute or current) in the outbreak;
C) Examination of the contacts;
D) Diagnosis and treatment of all cases of tuberculosis detected during the epidemiological investigation;
E) Administration of the chemopreventive treatment with isoniazid 10 mg/kg body weight daily, for 6 months, to contacts aged between 0 and 18 years;
F) Provide information to the patient and contacts using discussions, brochures, leaflets.

The medical surveillance of the contacts is performed during the whole period of the anti-tuberculosis treatment and after the patient's recovery, till the end of the anti-tuberculosis treatment, the death, or the patient's departure from the outbreak. The tuberculosis outbreak data are

removed from the Public Health Centers records for group 1 - over 12 months, in cases with drug resistance over 24 months; group 2 - over 6 months; group 3 - over 6 months after:

A) Patient's treatment was completed;

B) Patient's departure from the permanent residence on another administrative territory;

C) Patient's death.

1.5 Medical record of the tuberculosis patient. Instructions for completion.

The hospital medical record is an official document, completed by the medical staff, which provides information on the disease diagnosis, evolution, and dynamics, as well as the applied treatment. It has clinical, epidemiological, instructive-educational, judicial, administrative, and scientific significance and represents an informative basis for research. The completion of the medical record file is carried out based on the following official documents: A) identity card; B) healthcare insurance policy; C) birth certificate for children.

The registration number of the medical record is made after the entry-exit register.

The personal identity number is completed based on the identity card or birth certificate.

The patient's gender is recorded using the letter M (male) or F (female).

Birthdate is encoded within the specific boxes with 01-31 (day), 01-12 (month), and further on for the following calendar year.

Residence: urban/rural, street, and the number are copied from the identity card, or „no permanent residence" is encoded.

Weight is encoded in the specific box after checking the patient's weight.

The occupation and the job, the patient's insured status are documented by an insurance policy.

Blood group type, Rh, and allergic status are filled based on the patient's medical records.

The type of hospitalization is introduced in the box next to the code of the referral type. The date of the hospitalization and discharge, as well as the duration of the hhospitalization is encoded in the specific box.

Referral diagnosis is made by the doctor who sent the patient for hospitalization. It is written by the doctor based on the referral note and is not coded. The final diagnosis of hospitalization is established by the doctor who admitted the patient and is coded according to the International Classification of Diseases, version 10. The main diagnosis is followed by a secondary diagnosis of complications or comorbidities and can be listed maximum 6 associated diseases along with the main diagnosis.

The patient's state at discharge, the type of discharge, and death are filled and coded by the responsible physician in the respective boxes.

If the patient died, the diagnosis is completed and coded by the responsible physician simultaneously with the death certificate, according to the International Classification of Diseases, version 10.

Anatomopathological diagnosis is completed based on the histopathological examination performed by the anatomopathological specialist.

The functional respiratory examinations and radiological investigations are completed by the responsible physician based on the results of the functional and radiological examinations.

Reasons for hospitalization

The patient's complaints that caused the hospitalization are summarized. The pulmonary localization of tuberculosis determines a wide range of clinical manifestations. Clinical signs are included in two major syndromes: intoxication and bronchopulmonary syndromes. Intoxication syndrome includes the clinical signs: asthenia, weight loss, underweight, loss of appetite, fever or low-grade fever, and profuse night sweats. Broncho-pulmonary syndrome suggestive of pulmonary tuberculosis includes: persistent cough for more than 2-3 weeks, mucosal or mucopurulent sputum, hemoptysis, progressive dyspnoea, and chest pain.

The anamnesis of the disease (*anamnesis morbi*) aims to identify the onset of the disease, the patient's complaints, and establish the risk factors for tuberculosis (comorbidities, surgery interventions, harmful habits: smoking, alcoholism, and drug addiction). Particular attention is given to the results of the previous consultations and hospitalizations, treatment methods, therapeutic compliance, and their effectiveness. The source from which the information was collected (patient's family, medical records) must be obligatory specified.

The onset of tuberculosis can be imperceptible, insidious, subacute, and acute. The clinical signs of the onset can be erroneously attributed to other diseases: pneumonia, influenza, or pleural disease.

Most of the detected cases have a slow progressive onset, and the clinical symptoms are dominated by the clinical signs of intoxication syndrome and broncho-pulmonary syndrome. An important component of the life history (*anamnesis vitae*) is the identification of factors with high risk such as:

A) social and economic risk factors: unemployment, homelessness, chronic or abusive alcohol consumption, active smoking, intravenous drug use.

B) epidemiological risk factors: tuberculosis exposure and high-risk factors (detention, migration, living in shelters for elders, people with mental disorders, homeless).

C) medical-biological risk factors with potential immuno-suppressive impact: HIV infection, diabetes, gastrointestinal ulcer, chronic renal failure, neoplastic diseases and immunosuppressive treatment, immunotherapy, and underweight (<10% of ideal weight).

The epidemiological history should be compulsorily collected. The infectious contact with the patients with tuberculosis and especially the presence of children, pregnant women is recorded, as well.

The allergy history is written on the title page of the medical record file and includes information about drug intolerance.

The objective examination of the patient with tuberculosis is an essential component of the medical sheet, where all the changes observed in the patient are recorded.

The general inspection is initiated and then performed consecutively on organs and systems. It starts with a general inspection, establishing the overall condition (satisfactory, medium severity, severe, very severe), followed by consciousness (having perceptions, coma, delirium, etc.), position (active, passive, forced), facial expression, constitutional type (normostenic, asthenic, hypersthenic), the condition of the skin and their elasticity, the color of the skin and mucous membranes, the degree of nutrition of the patient (body mass index is calculated), the palpability of peripheral lymph nodes, the state of the osteoarticular and muscular system. Changes may occur regarding the general appearance, height, weight, constitution, position, movements), the body segments (face, head, chest, abdomen, extremities), the skin (color, eruptions, general appearance), and mucous membranes (mouth, nostrils, ears). An objective examination of the organs and systems reflects the results of inspection, palpation, percussion, and auscultation.

Inspection of the respiratory system and chest

The general inspection consists in identifying the elements of skin semiology and soft tissues. Frequently found changes are intercostal neuralgia, venectasia at the base of the chest, localized interscapular venectasia in children, thoracic scars, chest contusion, or edema.

The specific inspection assesses changes in the chest caused by diseases of the respiratory system. Therefore, an inspection of changes in the chest shape (morphological) and inspection of respiratory movements are carried out. The morphological inspection can identify the main changes:

- kyphotic thorax with accentuation of physiological kyphosis, accentuation of the posterior curvature with convexity towards the posterior, and increase of the anteroposterior diameter of the thorax.

- gibbosity chest („hump") with the presence of angulation of the spine and posterior prominence.
- kyphoscoliosis consists of a prominent curvature in the antero-posterior plane and lateral plane.
- flat chest does not show dorsal physiological kyphosis, and the spine is rectilinear.
- infundibuliform („shoemaker") thorax is characterized by a deformation of the sternum, which has a more or less deep concavity in the lower third segment.
- thorax „in the hull" represents the morphological change shaped like a bird's sternum.
- emphysematous thorax is a thoracic deformity caused by the development of pulmonary emphysema.
- paralytic thorax is flattened with vertical ribs and narrowed intercostal spaces.
- conoid thorax is a change in the shape of the thorax secondary to a significant increase in abdominal volume (pregnancy, ascites, giant abdominal tumors).

The thorax cage is modified in tuberculosis only in severe forms: reduced costal enlargements (in extensive infiltrative processes), asymmetric participation of the rib cage in breathing (in massive pleural effusions), and involvement of accessory respiratory muscles (in respiratory failure).

The dynamic inspection follows the respiratory movements of the chest and their frequency. Physiological breathing is abdominal. In women, chest-type breathing is common, with more extensive movements of the chest wall. The respiratory rate is 14-18 breaths per minute. The inspiratory time is shorter and longer than the expiratory one. The inspection may change the breathing rhythm. Tachypnoea represents the increase of respiratory rate above 18 breaths per minute, polypnea is the increase of the respiratory amplitude and bradypnea means the decrease of the respiratory rate below 14 breaths per minute.

Cardiovascular inspection

1) Inspection of the anterior cervical region provides important data on the pathologies of the cardiovascular system. The jugular veins, which reflect pressure from the right atrium and carotid arteries, are clinically examined.

a. Jugular veins

b. The examination of deep and superficial jugular veins is carried out with the patient having the thorax raised at 30-40 degrees horizontally. If the jugular veins appear turgid, full, it is a sign of increased venous pressure in heart failure. Asymmetric turgidity means unilateral compression.

c. Carotid arteries

The carotid arteries are not visible, but their ample, systolic pulsations can be observed.

2) Anterior chest inspection

a) Morphological changes of the anterior thorax, such as bulging of the thoracic region, can be established in cardiomegaly.

b) Abnormal movements in the precordial region, such as apex shock, which is a projection of the tip of the left ventricle. In adults, it is located normally in the left intercostal space V, whereas in children, it is localized with one or two spaces above.

3) Inspection of the arteries

On inspection, the synchronous pulsations of the heart and the large superficial arteries can be observed. These pulsations are of small amplitude, easily palpable, determined by the volume-beat, but also by the artery location and the thickness of the adipose tissue.

4) Vein inspection identifies the filling of normal veins, the development of an abnormal venous network, pathological veins, venous pulse.

Inspection of abdominal organs

The inspection of the abdomen is carried out from the standard right lateral position, following the general appearance, shape, appearance of the skin, umbilical scar, respiratory movements, and peristaltic movements.

a) General aspect and the shape of the abdomen:

- symmetrical deformities, prominent abdomen, increased volume in obesity, ascites, large tumors, abdominal flatulence.
- excavated abdomen (scaphoid abdomen or „in a boat") is found in consumptive diseases.
- localized deformities (asymmetric): the presence of an enlarged organ or a tumor formation.

b) Skin, scars, stretch marks, collateral circulation, rashes, ecchymosis, etc. are recorded.

c) Respiratory movements.

Palpation of the respiratory system - palpation of the thorax

a) Identification of areas or painful points. The areas where the pain is described will be palpated. Pleural or pericardial pain may be exacerbated on palpation.

b) Characteristics of formations observed on inspection regarding consistency, mobility, dimensions, and sensitivity.

c) Assessment of the thorax size and symmetry during the respiratory act.

d) Assessment of the amplitude of respiratory movements or the amplitude of respiration.

e) Palpation of vocal vibrations and pectoral tremor.

Vocal vibrations or pectoral rumbles represent the transmission to the chest wall of laryngeal vibrations caused by phonation. To perceive them, the subject must speak loudly, and the vibrations are more intense when pronouncing consonants such as „R". Therefore, the patient is asked to pronounce „thirty-three" aloud. Vocal vibrations must be transmitted symmetrically. Vocal vibrations can be accentuated, diminished, or abolished globally or locally.

Palpation of the cardiovascular system

On palpation, the apex shock and other movements of the precordial area are assessed.

Palpation of the cardiovascular system, the palpation of the arteries.

Palpation allows the evaluation of the qualities of the arterial wall and the pulsating wave. The normal heart rate is 60-100 beats per minute. The most common pathological changes are extrasystoles and atrial fibrillation.

On palpation, the condition of the rib cage and the abdominal wall are assessed, by highlighting the painful points, as well as the condition of the intra-abdominal or retroperitoneal parenchymal organs, the presence of pathological formations, and their characteristics are revealed.

Respiratory percussion - chest percussion

Chest percussion can be immediate and mediated. Immediate percussion consists of direct percussion of the thorax. It starts with the percussion of the back and the lateral thorax walls. The armpit is taped on the middle axillary line, the patient holding the upper limb high, with his hand on his head. In the patient lying on his back, the anterior side of the chest is taped. The percussion of the posterior side of the thorax is always systemic and symmetrical. It is recommended to tap the entire hemithorax and then symmetrically each area of both hemithorax. It starts with hemithorax supposed to be healthy. The percussion of the posterior side of the thorax begins with the percussion of the supraspinatus areas, starting from the base of the neck to up and lateral. Therefore, it delimits two areas about 5 cm wide (Kronig bands), corresponding to the lung apex. After delimiting the area which corresponds to the lung apex, the interscapulovertebral areas are percussed, and then the pulmonary bases, internally and externally. The percussion of the anterior side begins with the percussion of the supraclavicular fossae. The percussion of the anterior side is continued by percussion of each intercostal space on the midclavicular line. On the right side, at the level of the 6^{th} rib, the liver dullness is established. On the left side, at the level of the 7^{th} and 8^{th} rib, on the midclavicular line in Traube's space, a tympanic sound is established. On the left, the cardiac dullness is delimited.

Changes in normal lung sound: tympanic sound over the entire lung surface, with lowering of the posterior and anterior pulmonary boundaries in the pulmonary emphysema; or located in the presence of an underlying cavity (cavern or lung abscess) or dullness. In pulmonary tuberculosis, the percussion of the thoracic cage demonstrates a diminished lung sonority (in extensive infiltrates) or absent (in pleural effusion), pronounced lung sonority (extensive pulmonary destruction, compensatory pulmonary emphysema, or pneumothorax).

Percussion of the cardiovascular system

The percussion of the heart is done with the patient placed in the supine position, after identifying by palpation the apex shock. The upper edge of the liver is delimited, percussing from the top to the bottom, on the anterior axillary line. The upper edge of the liver is normally in the 4^{th} intercostal space. Then the right edge of the cardiac dullness is determined, percussing each intercostal space, from the lateral side to the right sternal edge. Cardiac dullness does not exceed the edge of the sternum and the angle between the edge of the sternum and the upper limit of liver dullness. The determination of the apex (tip) of the heart follows, percussing radiating convergent to the place where the apex shock was palpated. The position of the apex shock corresponds to the extreme left limit of cardiac dullness. The distance between the cardiohepatic angle to the tip of the heart forms the lower diameter of the cardiac dullness and is 12 cm. Subsequently, the left edge of the cardiac dullness is delimited, oblique from the top to the 2^{nd} left parasternal intercostal space. The percussion ends with the delimitation in the 2^{nd} intercostal space. The delimited cardiac dullness includes a triangular area, located parasternal to the left and a peripheral area of dullness, where between the heart and the thoracic wall, the lung is interposed.

Percussion of the abdominal organs aims to delimitate and assess the size of the parenchymal organs, to establish the fluid in the peritoneal cavity, as well as the content of palpable formations. The abdomen is

tapped radially, from the umbilicus in all directions. If ascites are suspected, the patient will undergo percussion from the epigastrium in all directions. Then the percussion of the liver and spleen follows. The lower edge of the hepatic dullness is delimited by percussing superficially from the bottom to the top. The spleen is percussed with the patient in left lateral decubitus, with the right lower limb in extension and the left one in semi-flexion. The percussion is performed from top to bottom. If the spleen reaches the medial axillary line, the presence of splenomegaly can be established.

Auscultation involves determining the sound produced by the functioning of the internal organs. It can be done immediately, by placing the ear directly on the target surface, using a protective napkin, or mediated, with the help of a stethoscope. The auscultation will be done symmetrically, starting from the supraspinous fossa, then in the interscapulovertebral and basal areas. The armpits must be auscultated from the top to the base on the middle axillary line. The anterior side is auscultated supra- and subclavicularly on the midclavicular line.

Auscultation of the respiratory system allows to evaluate the airflow, which passes through the tracheobronchial tree. Auscultation characterrizes the normal respiratory sounds, the pathological transmission of the normal sounds, pathological sound, and cough. Chest auscultation frequently demonstrates a divergence between the lack of auscultator data and the diversity of radiological signs in pulmonary tuberculosis. The abnormal sound may indicate the type and localization of the pathology. Different calibers rales may be determined, the most often are supcrepitant rales, found in the upper thoracic region (supra-, subclavicular, interscapular), diffuse crackling rales (an indicator of pulmonary congestion), snoring rales, amphoric murmur in a chronic process associated with a giant caverna, tubal murmurs (in the pleural effusion). The cavitary syndrome includes audible rales, amphoric breathing which is characteristic of giant cavities (caverna). Regardless of the type of auscultating anomalies, their location in the upper segments and the located character „in the focus„ are indicators of tuberculosis.

The auscultation of the cardiovascular system is performed in a quiet room, the whole pericardial area is auscultated, with the patient lying on his back, lateral left bent forward, during normal breathing, and in post-inspiratory or post-expiratory apnoea. It starts by identifying normal heart sounds and then moves on to auscultation of systolic and diastolic beats. The following area of auscultation are described: mitral area - at the tip of the heart, where the apex shock is palpated; tricuspid area - parasternal right lower or in the lower third of the sternum, at the level of the 4th and 5th intercostal space; aortic area - in the right 2nd intercostal space, parasternal; pulmonary area - in the 2nd left intercostal space. Auscultation of the arteries causes shortness of breath in the presence of partial arterial obstruction.

Auscultation of the abdominal organs determines intestinal sounds. It is recommended to be performed before percussion of the patient because the maneuvers can stimulate intestinal activity. The preferred focus of auscultation is the right subumbilical site. Normally, isolated hydro aerial sounds are heard, called gargoyles, with a frequency of 5-30 per minute and occasionally prolonged sounds („squeaks").

The objective examination at hospitalization should comprise the diagnosis at admission, the investigation plan, and the treatment regimen. The patient is informed about the regulations of the clinical institution. The patient's informed consent is obtained for the proposed medical services with the signature of the patient or the legal representative (for children or unconscious adults). In case of refusal expressed by the patient or the legal representative, the possible consequences are explained. The refusal is recorded in the medical documentation with the indication of the consequences. It is signed by the patient or the legal representative, as well. The final patient's refusal, or when the patient cannot express the will, the medical staff has the right to make the decision based on the patient's interests.

Dynamic monitoring of the patient is recorded within the daily ward rounds. The information must fully reflect the particularities of the evolution of the patient's overall condition (especially of the vital signs), the

results of clinical and paraclinical examinations, changes in the diagnosis or treatment, and the specific healthcare handlings. Registrations must start with the date of the first visit. Patients in a good general state are checked at least 3 times a week, those with a moderately modified state - daily, and those with severe modified state - several times a day, mentioning the time. Patients in critical condition will be monitored continuously, the registrations will be recorded every 15-30 minutes. It is not allowed to abbreviate and ignore information about the objective state of patients and local or special evolutionary changes. Surgically treated patients are monitored several times daily for the first three days after surgery and will be examined daily after that. In case of transferring the patient to another ward, a transferring note is carried out, which includes information on the patient's general condition at the time of transfer, the treatment and diagnostic-curative measures which were performed, the dynamic evolution of the pathological process, and the reasons for the transfer. The results of all complementary investigations will be attached following the time of the investigations. Confirmation of the drug administration is performed by the nurse, who is in charge of appropriate procedures. Blood transfusions are performed by the nurse, under the supervision of the consulting clinician. The therapeutic drugs prescribed to the patient are recorded by the attending clinician in the indication file, mentioning the date of the prescription and drug cessation. The nurse strictly follows the doctor's instructions and confirms the administration of the drugs by signing the record file. The patient's body temperature record chart is completed by the nurse twice a day. When the patient is discharged, the attending physician completes the discharge epicrisis. The epicrisis is the synthesis of the reasons for admission, including the results of clinical and paraclinical investigations, the final clinical diagnosis, the diagnostic-curative measures, the evolution of the clinical data, and the recommendations referred to the patient. The discharge data will also be attached to the epicrisis (statistical form no. 027/e) that is given to the patient to be presented to the family doctor and to be inserted in

the patient's outpatient medical file. The epicrisis is signed by the physician and the head of the department, and the rectangular and triangular stamps of the medical institution is applied.

If the patient's clinical death was established, in the medical record must be indicated the exact date and time, the resuscitation measures performed and their results, signs of the clinical death (absence of the central nervous system, cardiovascular, and respiratory activity). At the end of the medical file, the epicrisis of death is attached in which the physician or resuscitator briefly indicates the reasons for hospitalization, the clinical evolution of the disease in dynamics, the diagnostic and curative measures performed, the causes of death, and the final clinical diagnosis.

Some standardized medical records need to be filled in before the initiation of the anti-tuberculosis treatment:

- TB01 – Tuberculosis patient treatment form is filled by the phthisiopneumologist with the registration of the patient's data, treatment schedule and regimen, data on bacteriological monitoring, clinical and evaluation of treatment results.
- TB03 – The register for recording the tuberculosis cases is filled in by the phthisiopneumologist from the institution where the patient is registered, including clinical, bacteriological, and paraclinical monitoring data, according to the periodicity of the examination.
- TB03 RR/MDR TB – The register for recording drug-resistant tuberculosis cases is filled in by the phthisiopneumologist from the institution where the patient is registered and contains clinical, bacteriological, and paraclinical monitoring data, according to the periodicity of the evaluation of the case diagnosed with RR/MDR TB.
- TB04 – Register about the evidence for laboratory bacteriological investigations.
- TB05 – The form recording microscopy and GeneXpert MTB/RIF exams for the diagnosis of tuberculosis.
- TB06 – The form for requesting microbiological investigations for the diagnosis of tuberculosis.

- TB09 - Tuberculosis patient referring or transfer form.
- F089/1-e – The form on tuberculosis notification and treatment results. The document includes 3 parts: A, B, and C. Part A will be completed when the case of tuberculosis is confirmed (within 72 hours of detection). Part B consists of 3 parts to be completed for new cases: at the end of the 2nd or 3rd month of treatment, at the end of the 5th month, 6th month, or the end of treatment, and for cases included in the re-treatment (relapses, after lost to follow-up and failure): at the end of the 3rd month, 4th month, 5th month, 6th month and at the end of treatment. Part C describes the initiation of treatment and its results. It should be filled in when the patient is withdrawn from the active surveillance register of the specialized institution
- F090-RR/MDR tuberculosis declaration and evidence form will be filled by the phthisiopneumologist, who diagnosed the patient with RR/MDR tuberculosis. It contains 3 parts: A1A2, A3, and B. Part A1A2 will be completed when confirming the TB RR/MDR case. Part A3 will be completed by the phthisiopneumologist when is confirmed the case of TB RR/MDR. Part B (treatment monitoring for patients undergoing TB RR/MDR treatment) will be processed after a certain periodicity during the patient's evaluation.
- Register of the anti-tuberculosis drugs.

Summary of the first topic

- The detection of tuberculosis consists of the application of medical-sanitary measures, which would detect the suspect for tuberculosis, for the prompt initiation of anti-tuberculosis treatment.
- Passive screening consists of examination of patients with symptoms suggestive of tuberculosis: persistent coughing for more than 2-3 weeks, mucoid or mucopurulent sputum, hemoptysis, progressive dyspnoea, chest pain, and clinical signs of intoxication syndrome, such as as thenia, weight loss, loss of appetite, fever or evening low-grade fever, profuse night sweats.

- Symptomatic patients will undergo the objective examination, as well as complete blood count, HIV testing, microscopic examination of the sputum to identify acid-fast-bacilli and GeneXpert MTB/RIF genetic molecular test, bacteriological examination with drug-susceptibility testing to first-line anti-tuberculosis drugs, chest X-ray, investigations of other organs and systems according to the recommendations.
- Active detection consists of the annual screening of people from high-risk groups, high-vigilance groups, and those from endangered contingents based on the anamnesis, clinical examination, chest X-ray in adults, and tuberculin skin test in children (0-18 years).
- Detection of the patient with active tuberculosis determines the initiation of the epidemiological investigation in the tuberculosis outbreak after receipt of the form „Urgent declaration on detection of infectious diseases, intoxications, food and occupational poisonings, adverse drug reactions to the administration of immunobiological drugs".
- The tuberculosis outbreak is the area where the source of *M. tuberculosis* infection is located and the people in that area, who are at a greater risk for infection and developing the disease.
- Depending on the epidemiological danger, outbreaks are classified into 3 groups: high, medium, and low-risk groups.
- The measures to be carried out in the outbreak are liquidation of the outbreak through the isolation of the patient and early initiation of anti-tuberculosis treatment, disinfection (absolute or current) in the outbreak, examination of the contact persons, diagnosis and treatment of all associated cases; chemoprevention with isoniazid 10 mg/kg body weight daily, for 6 months, in contact persons aged between 0 to 18 years; informing the patient and contact persons about tuberculosis.
- The medical record is an official document, issued by the medical staff, which contains information regarding the diagnosis, the evolution of the disease, applied treatment and has clinical, epidemiological, instructive-educational, judicial, administrative, and scientific purposes.

TOPIC II. METHODS OF DIAGNOSING LATENT TUBERCULOSIS INFECTION

The purpose of the practical work and the seminar on this topic is to develop specific skills regarding the methods for diagnosing latent tuberculosis infection. At the application level, it aims to perform the tuberculin skin test, collect and evaluate the results, and establish the conclusion. The practical activity is based on performing the tuberculin skin test with 2 tuberculin units (UT) of tuberculin type PPD-L, followed by reading and evaluating the results to establish the diagnosis of tuberculous infection. The training methods included the problem-based learning method and the clinical case-based reasoning method. The case study will be used to develop the student's clinical thinking. The materials required for the practical training are a needle syringe adapted for intradermal injection, cotton wool kit, sterile compresses, transparent plastic ruler, stethoscope, sphygmomanometer, thermometer, pulse oximeter, wet wipes, gloves, and hand sanitizer. The student will wear a white robe and a face mask, a cap, and a pair of disposable overshoes. The course study duration on the diagnosis of tuberculosis is one academic hour, whereas the practical activity will last 6 academic hours.

Topics for individual study

The following topics are proposed for the individual work of the student, based on the presented material:

- Evolution of the infection with *Mycobacterium tuberculosis* in humans. The role of risk factors in the development of active tuberculosis.
- Pathogenesis of the infection caused by *Mycobacterium tuberculosis*.
- Tuberculin skin test. Purpose of testing, advantages, and disadvantages of tuberculin skin test.
- The evolution of the body's reaction to tuberculin. Interpretation of tuberculin skin test results. Tuberculin conversion and booster effect.
- Differential diagnosis of latent tuberculosis infection by active tuberculosis.

- Interferon Gamma Release Assays for establishing the tuberculosis infection.

Theoretical framework

2.1 Pathogenesis of tuberculosis

The human immune response to mycobacterial infection is effective against *Mycobacterium tuberculosis*. Successive epidemic waves in Europe have contributed to the selection of genetically resistant individuals, eliminating susceptible people. The different evolution of epidemiological indicators in different regions worldwide is explained by the genetic susceptibility of the black and indigenous people of the Americas to the tuberculous infection.

The body's reactivity to mycobacterial infection is explained by the Koch phenomenon described by Robert Koch in 1891. In guinea pigs, the subcutaneous inoculation of a virulent dose of bacilli causes the appearance of a nodule, which after 2 weeks progresses into ulceration with the elimination of a caseous mass. The guinea pig shows all the clinical signs of active tuberculosis: loss of appetite, weight loss, apathy, fever, tachypnoea, and cachexia. If a new inoculation will be performed at least 8 weeks later on another body site, the body reactions will be different compared with the first inoculation. In 24-48 hours, a necrotic nodule occurs that will eliminate the caseum. The local ulcer will heal spontaneously. Differences in the evolution of the lesions demonstrate a delayed hypersensitivity reaction, which will determine the immune resistance against a new infection. This hypersensitivity causes a rapid and harsh response to the pathogen, followed by acute tissue necrosis, whereas immune resistance contributes to the blocking of infection at the site of inoculation. The morphological substrate of the Koch phenomenon represents a cell-mediated response. The effector immune cells involved in the Koch phenomenon are the circulating monocytes that migrated into the inoculated tissue and were transformed into macrophages, epithelioid cells, giant Langhans cells, as well as T lymphocytes (CD4+ and CD8+ phe-

notype). The cockade placement of these cells is characteristic of tuberculous granuloma. The center of the granuloma consists of caseous necrosis, surrounded by a crown of macrophages, epithelioid cells, giant Langhans cells, and lymphocytes. Macrophages located in the granuloma differentiate into epithelioid cells, giant Langhans cells, and foamy macrophages. At the cross-section, the caseous necrosis is surrounded by a crown of giant Langhans cells, surrounded by macrophages, epithelioid cells, lymphocytes, and a crown of the B lymphocytes and neutrophil leukocytes at the peripheral parts. The center of caseous necrosis is a necrotic lesion of the tissue with a low concentration of oxygen. Hypoxia from caseous necrosis forms an unfavorable environment for the growth and multiplication of mycobacteria. Foamy macrophages are located at the delimitating line of caseous necrosis from the cellular component of the granuloma. The induction of foamy macrophage is carried out by mycolic acids from the mycobacterial wall. After the mycobacteria phagocytosis, foamy macrophages lose their capacity for phagocytosis and enzymatic degradation, however, these are secretors of *tumor necrosis factors* (TNF-α). By secreting cytokines, foamy macrophages stimulate tissue necrosis. Under the action of the hypoxic intracellular environment, the mycobacteria which are present in the foamy macrophages are induced in the dormant state, also called the latent form (L-form) of *Mycobacterium tuberculosis*. Under certain conditions, foamy macrophages lose their ability to keep the infection dormant and allow the spread of active bacilli in the body. The lymphocyte complex of tuberculosis granuloma consists of CD4+, CD8+ lymphocytes, and B lymphocytes. At the periphery of the granuloma, there is an increased number of fibroblasts, responsible for the synthesis of collagen with the formation of a capsule. Within 5-10 years the granuloma is completely replaced by a connective tissue impregnated with calcium salts. In calcinates, mycobacteria turn into the latent form L, which keeps the tuberculosis infection in a dormant state – latent form. Under the influence of the immunosuppressive factors, *M. tuberculosis* can switch into an active form from the latent and the clinical signs of tuberculosis appear.

Infections and diseases in humans have a cyclical nature. The penetration of *M. tuberculosis* from the air into the lung alveoli in an uninfected person causes the development of a lesion within 3 to 8 weeks, which is called the primary affect or the primary focus. The lymphatic vessels are affected and lymphangitis with intrathoracic lymphadenopathy develops. At the same time, the organism acquires the IVth type hypersensitivity, which is also known as delayed-type hypersensitivity. The method that determines the delayed hypersensitivity to mycobacterial antigens is the tuberculin skin test. The positive or hyperergic result of the tuberculin skin test identifies the infection, without differentiating the latent infection from active disease.

Latent tuberculosis infection (LTBI) is a condition of the body in which mycobacteria are maintained in latent form (dormant) and the person is conventionally healthy. Indicators of the presence of LTBI are the positive or hyperergic result of the tuberculin skin test, the tuberculin skin test conversion, the positive result of the *in vitro* interferon-γ release test (IGRAs assays).

Infected people are not infectious and contagious, but under the influence of complex risk factors, they can reactivate the infection and develop active disease. Several criteria for differentiating LTBI from active tuberculosis have been formulated: patient's clinical manifestations, results of the laboratory tests (microbiological, radiological, immunelogical), as well as the requirement for the anti-tuberculosis treatment.

The infected person is asymptomatic, the results of laboratory tests are negative, the radiological examination is normal, immunological tests (tuberculin skin test, IGRAs test) show positive results. Children who were in contact with a patient with tuberculosis and have a positive or hyperergic tuberculin skin test, and active tuberculosis is excluded, will receive the chemoprevention with isoniazid 10 mg/kg bodyweight for 6 months.

The patient with pulmonary tuberculosis complains the intoxication syndrome - asthenia, fatigue, anorexia, weight loss, low body weight, fever or low-grade fever, profuse sweating, and bronchopulmonary syn-

drome - persisting cough for more than 3 weeks, expectoration and hemoptysis, rarely dyspnoea and chest pain. Laboratory microbiological and molecular genetic tests can identify the etiological agent; the radiological examination shows the radiological pattern; the immunological tests (tuberculin skin test, and IGRAs test) are positive. In severe forms associated with immune suppression, the tuberculin skin test may be false negative. The patient requires the treatment that should be administrated based on the case type and the spectrum of drug-susceptibility of *M. tuberculosis*. Lack of adequate treatment determines the death of the patient within two years *(Table 2.1).*

Table 2.1

Criteria for differentiation of the latent tuberculosis infection from active tuberculosis

Criteria	Latent tuberculosis infection	Active tuberculosis
Epidemiological anamnesis	TB contact present or absent	TB contact established
Clinical symptomatology	Asymptomatic	Clinical signs of the intoxication syndrome and bronchopulmonary syndrome
Tuberculin skin test with 2 UT	Positive or hyperergic	Negative (false) or positive or hyperergic
Radiological examination	No pathological changes or post tuberculosis consequences	Infiltrative opacities, nodules, cavities in „alarm zones", enlarged hilum, pleural effusion, etc.
Microbiological examination	AFB negative, culture-negative, GeneXpert MTB/RIF negative	AFB positive, culture-positive, GeneXpert MTB/RIF positive
Treatment	Chemoprevention	Anti-tuberculosis treatment according to the case type and sensitivity spectrum

An estimated one-third of the human population contains latent infection. About 10% of infected people will get sick, including 5% in the first two years after infection and 5% later on. Tuberculosis develops in certain susceptible individuals, whose predisposition is determined by factors with an increased risk of disease. HIV infection is the most

important risk factor for tuberculosis. It has been found that people living with HIV, are at a greater risk to die of tuberculosis. The annual risk for tuberculosis in co-infected people is 7-10%. Patients with diabetes mellitus, chronic end-stage renal disease, neoplastic pathologies, and patients treated with immunosuppressive drugs (corticosteroids, chemotherapy, immunomodulatory therapy, radiation therapy) are at a high risk of developing tuberculosis. Non-specific respiratory disorders reduce mucociliary clearance and non-specific respiratory resistance. As a result, there is an increased risk for both the infection and the disease. Gastrointestinal tract disorders, associated with maldigestion and malabsorption, also lead to an increased risk of tuberculosis. The causes of gastrointestinal maldigestion are gastrectomy, gastroenteroanastomosis, and Zollinger Ellison syndrome. The biliary causes of maldigestion are chronic liver disease and chronic biliary obstruction. The pancreatic causes of maldigestion are chronic pancreatitis and cystic fibrosis (cystic fibrosis of the pancreas). Intestinal enzyme deficiency, celiac disease, Crohn's disease, short bowel syndrome (post-surgical, enterocolitis fistulas, and intestinal bypass) are the intestinal causes of maldigestion.

2.2 Methods for diagnosing latent tuberculosis infection

Immune tests are the basic methods for identification of the infection with *Mycobacterium tuberculosis* in children and adolescents. For the diagnosis of tuberculosis infection in the Republic of Moldova, the tuberculin skin test with 2 tuberculin units (TU) of PPD-L is used. The tuberculin skin test (TST) is the oldest, cheapest, and simplest test for assessing the body's delayed-type hypersensitivity reaction to mycobacterial antigens. Tuberculin was discovered by Robert Koch in 1890. The scientist concentrated a mycobacterial culture filtrate, degraded by exposure to heat (*Alt Tuberculin, Old Tuberculin*). Subsequently, the tuberculin was purified using purified protein derivatives *(PPT-protein purified derivatives*), some of which were declared as international standard products. The technique of the intradermal reaction and the results were described in 1908 by Charles Mantoux (1877-1947). It's based on the

principle of injecting 2 tuberculin units intradermally on the anterior surface of the middle third of the left forearm and then measuring after 72 hours the size of the papule occurring at the injection site.

The tuberculin test is performed in:

- Children who were in contact with tuberculosis patients;
- Children with suspected tuberculosis;
- Children at high risk to get the infection;
- Children before admission in foster care, ancillary schools, or other institutions at risk.

Contraindications to the tuberculin skin test have not been established. Certain conditions allow rescheduling the test, such as infections and overheating of chronic diseases, allergies, and cutaneous rashes.

Injection of tuberculin into allergised organism causes the following reactions:

- Local response- edema, cell infiltration, blisters, and tissue necrosis;
- Focal response - edema, congestion, and local bleeding.
- General response - fever, hypotension, and vascular collapse;

The result of the tuberculin test is recorded in Form 112/e. Local reactions to tuberculin injection can be classified into several categories.

The negative reaction is confirmed by**:**

- A red point at the injection site;
- Hyperemia at the injection site;
- Papule less than 4 mm in unvaccinated and less than 9 mm in BCG-vaccinated individuals;

The negative tuberculin skin test indicates the absence of the skin's allergy to tuberculin. It can be identified in people who have not been infected either naturally or artificially by BCG vaccination. Exceptionally, occurs in people who have sterilized the infection. The negative test result does not exclude tuberculosis in the following situations:

- During the pre-allergic period up to 12 weeks after the primary infection;
- Severe forms of tuberculosis with the suppressed immune response;

- Neoplasms, lymphogranulomatosis, and other diseases associated with suppressed immune response.

False-negative results occur in immune suppression during acute infectious diseases (measles, influenza, whooping cough, etc.), cachexia, trauma, surgery, menstruation, pregnancy, irradiation, treatment with immunosuppressive drugs, or immunomodulators.

A positive tuberculin skin test is confirmed by:

Presence of induration of 5 mm in unvaccinated and 10 mm in BCG vaccinated individuals, up to 16 mm in children, and 20 mm in adults. The positive result cannot differentiate latent tuberculosis infection from active tuberculosis.

Hyperergic tuberculin skin test is confirmed by:

- Presence of an induration above 17 mm in children and 21 mm in adults;
- Bladder-necrotic reaction;
- Lymphangitis and regional lymphadenopathy.

The tuberculin conversion is established by performing two tuberculin tests during a year, the first showing a negative and the second a positive result. The tuberculin conversion indicates the occurrence of the primary infection.

The booster effect is the increased results of the tuberculin skin test, performed at short intervals.

The false-negative result can be determined by the following conditions:

- Factors contributing to immunosuppression: fever, cachexia, viral and bacterial infections, recent vaccination, neoplastic diseases, vulnerable age groups (newborns and elders), treatment with immunosuppressive drugs;
- Technical factors: administration of the expired tuberculin, incorrect tuberculin dilutions, biochemical denaturation, subcutaneous or intramuscular injection, massaging of the injection site, as well as reading errors of the results.

The false-positive result is caused by infection with nontuberculous mycobacteria. The tuberculin reaction reading errors and the booster effect contribute to the false-positive interpretation.

Several criteria were established to differentiate the positive tuberculin skin test which can occur during the natural infection and after the BCG vaccination: tuberculosis contact, size, color and evolution of the induration, history of the BCG vaccination, post-vaccination scar, and paraspecific reactions *(Table 2.2)*

Table 2.2

Differences between natural infection and post-vaccination allergy

Indicators	Natural infection	Post-vaccination allergy
TB contact	Present	Absent
Size and color of the induration	Positive, hyperergic result, red or violet color of the induration	Mild, positive, Rose-pale color of the induration
Evolution of the induration	Increasing	Decreasing
BCG vaccination	Over 5 years ago	Recently vaccinated
Paraspecific reactions	Present	Absent

2.3 Serological methods for diagnosing the latent tuberculosis infection

The low sensitivity and low specificity of the skin test for tuberculin contributed to the development of serological methods of diagnosis. Serological tests are attractive, fast, and simple methods for the diagnosis of tuberculosis, however not recommended by the WHO. The sensitivity of serological tests varies from 1% to 60% and the specificity from 53% to 98.7%. Tests with high specificity (>53%) have a low sensitivity(1-21%) for the diagnosis of tuberculosis. In vitro tests based on the production of interferon-gamma (IFN-γ) measure the amount of IFN-γ released by T lymphocytes stimulated with mycobacterial antigens. The amount of IFN-γ is detected by the ELISA method (*enzyme-linked immu-*

nosorbent assay) or by the ELISPOT method (enzyme-linked immunospot assay). The Quantiferon-TB Gold Assay uses ESAT6 and CFP 10 mycobacterial antigens.

Summary of the second topic

- Tuberculosis is a disease with lymphatic tropism because it affects the cells involved in the cellular immune response such as the lymphocytes and plasmatic cells.
- The morphological substrate of mycobacterial inflammation is the tuberculous granuloma, described by Robert Koch in 1882.
- The immune response mainly involved in delayed hypersensitivity, also known as the 4th type of immune reaction.
- The cellular elements of tuberculous granuloma are the circulating monocytes, migrating into the inoculated tissue and changing into macrophages, epithelioid cells, and Langhans giant cells, as well as T lymphocytes (CD4+ and CD8+ phenotype).
- At the cross-section, the tuberculous granuloma showed the caseous necrosis, surrounded by a crown of giant Langhans cells and macrophages; epithelioid cells and lymphocytes towards the periphery; B lymphocytes and neutrophil leukocytes on the margins.
- The center of the caseous necrosis is a necrotic lesion of the tissue, which differentiates the tuberculous granuloma from the tuberculoid granuloma. The tuberculoid granuloma does not show caseous necrosis.
- One-third of the global population is infected with *Mycobacterium tuberculosis* and the latent infection is confirmed by the positive or hyperergic result of the tuberculin skin test and IGRAs.
- The criteria for differentiation of latent tuberculosis infection from active tuberculosis include epidemiological anamnesis, patient's symptoms, laboratory tests (microbiological and immunological findings), and radiological abnormalities in the respiratory system.
- The symptomatic patient complains of clinical signs of tuberculosis intoxication syndrome - asthenia, fatigue, anorexia, weight loss,

hypoponderability, fever or low-grade fever, profuse sweating, broncho-pulmonary syndrome, coughing for more than 2 weeks, hemoptysis, dyspnoea, thoracic pain.

- For the diagnosis of infection the tuberculin skin test with 2 tuberculin units and IGRAs are used. Interferon-gamma release assays (IGRAs) are in vitro tests based on the production of IFN-γ released by T lymphocytes stimulated by the mycobacterial antigens.
- IGRAs tests have a higher sensitivity and specificity compared to conventional tuberculin skin tests, showing low correlation with non-tuberculosis mycobacterial infection, lower cross-reactivity with BCG vaccine, and a higher potential to detect tuberculosis infection.
- Lack of a standard value and evidence of the sensitivity of IGRAs in HIV-infected and immunocompromised individuals, children, extrapulmonary tuberculosis, and infection with drug-resistant mycobacteria, did not recommend their use in countries with high tuberculosis incidence and limited resources.

TOPIC III. DIAGNOSIS OF TUBERCULOSIS

At the level of knowledge acquisition, the purpose of the practical works and seminars on this topic is to develop specific skills in diagnosing active tuberculosis. At the application level, it is proposed to perform practical procedures and methods of diagnosing active tuberculosis with the interpretation of the results and formulation of the final diagnosis. The type of practical activity is based on performing the collection procedures, using methods for diagnosing pulmonary tuberculosis, and other methods necessary to investigate patients with tuberculosis. The training methods include the problem-based learning method and the clinical case-based reasoning method. The case study will be used to develop the student's clinical thinking. The materials needed to carry out the practical work are the stethoscope, sphygmomanometer, thermometer, pulse oximeter, wet wipes, gloves, hand sanitizer gel, tongue depressor for examining the oral cavity, and peak flow meter. The student will wear a white robe and face mask, cap, and a pair of disposable overshoes.

The duration of the course study on the diagnosis of tuberculosis lasts one academic hour and the practical activity will last for six academic hours.

Topics for individual study

The following topics are proposed for the individual study of the student, based on the accumulated material:

- Principles of collecting, transporting, and storing the clinical samples for the diagnosis of tuberculosis.
- Microscopic examination of the sputum to identify acid-fast bacilli.
- Bacteriological examination of clinical specimens to identify *Mycobacterium tuberculosis*. And drug- susceptibility testing.
- Genetic molecular tests: GeneXpert MTB/RIF, GenoType®MT-BDRplus, BD ProbeTec ™, and evaluation of the results.
- Clinical classification of tuberculosis. Principles of classification and diagnostic criteria of tuberculosis.

- Tuberculous pleurisy. Pathogenesis, symptomatology, evolution, radiological aspects, differential diagnosis, and treatment. Thoracocentesis, indications, and methods of extraction of pleural fluid. The microbiological, biochemical, and cytological examination of the pleural fluid.
- Tuberculous meningitis. Pathogenesis, symptomatology, evolution, differential diagnosis, treatment, and prognosis. Biochemical, microbiological, and cytological examination of cerebrospinal fluid for the diagnosis of tuberculous meningitis.
- Other investigations were used in the examination of the patient with tuberculosis.

Theoretical framework

3.1 The patient's medical history and clinical examination

The anamnesis of the disease (*anamnesis morbi*) aims to elucidate the onset of the disease, the patient's complaints, as well as to establish the risk factors for tuberculosis. The onset of tuberculosis may be unobserved and the patient is asymptomatic. The diagnosis is occasionally established by radiological examination of the thorax. In one-third of cases, the onset of the disease may be acute and may clinically resemble pneumonia, influenza, or pleural disease. The acute onset commonly contributes to diagnostic errors and delay of the anti-tuberculosis treatment. However, most of the detected cases have a slow progressive onset. The clinical symptoms are characterized by clinical signs of intoxication syndrome and broncho-pulmonary syndrome.

An important component of the life history (*anamnesis vitae*) is the identification of factors with an increased risk for tuberculosis, such as:

- Unfavorable socioeconomic factors: unemployment, homelessness, chronic/abusive alcohol consumption, active smoking, intravenous drug use, etc.

- Epidemiological factors: tuberculosis contact and epidemiological features with high risk such as the history of imprisonment, migration, living in shelters for the elderly or people with mental disorders, or homeless people.
- Medico-biological factors include diseases or conditions that suppress immune resistance: HIV infection, diabetes, gastrointestinal ulcer, chronic renal failure, neoplastic diseases and immunosuppressive treatment, biological anti-TNF-α therapy, malnutrition, or underweight (<10% of ideal weight).

The clinical manifestations of tuberculosis depend on the association of diverse contributing factors: age groups, person's immune resistance, associated diseases, the virulence of mycobacteria, etc.

Pulmonary tuberculosis causes a wide spectrum of clinical manifestations. It was established the dependence between the extension of tuberculosis and the severity of clinical signs. However, there are cases with severe clinical manifestations of an underlying localized tuberculous process, as well as mild symptoms associated with an extensive tuberculous process.

Clinical signs are included in two major syndromes: intoxication and bronchopulmonary. The most common clinical signs of the intoxication syndrome are asthenia, weight loss, malnutrition/underweight, loss of appetite, fever or low-grade fever in the evening, and profuse night sweats. Broncho-pulmonary syndrome suggesting pulmonary tuberculosis includes persistent cough for more than 2-3 weeks, mucosal or mucopurulent sputum, hemoptysis, progressive dyspnoea, and chest pain. Clinical manifestations vary in patients with tuberculosis. Patients detected through the prophylactic radiological examination (active screening) are more frequently asymptomatic or oligosymptomatic. The most common clinical manifestations are asthenia, decreased or loss of appetite, low-grade fever, profuse sweating, weight loss, and tachycardia.

Most tuberculosis patients detected by the examination of symptomatic suspects report persistent cough for more than 2-3 weeks, mucopu-

rulent sputum, dyspnoea, and chest pain. The onset of the cough is insidious, without sputum, of low intensity in limited forms of tuberculosis. It develops in a continuous cough with mucoid then mucopurulent sputum, which is frequently complicated by hemoptysis. Intense convulsive coughing can be established in tuberculosis of the intrathoracic lymph nodes, due to ganglion-bronchial compression. Tachypnoea, shortness of breath, long conversations can cause painful coughing in patients with severe forms of tuberculosis. The sputum amount is usually insignificant in limited forms of pulmonary tuberculosis. The cough intensity, sputum amount, and color typically change in tuberculosis on an underlying chronic smoking-related bronchitis. Mucopurulent sputum over 50 ml per day is characteristic for tuberculosis associated with chronic smoking-related bronchitis. A greater sputum amount with a „full mouth" indicates the formation of a broncho-pleural fistula and the evacuation of the pleural effusion. Intense mucopurulent sputum demonstrates the evacuation of a pulmonary caverna. Haemoptysis is sputum with streaks of fresh blood. Recurrent hemoptysis is common in recent infiltrative processes and exacerbation of chronic forms of tuberculosis. Cases with limited forms of tuberculosis, with the appearance of hemoptysis in an apparent healthy state, were also noticed.

Dyspnoea has a diminished intensity, even in extensive tuberculosis, and most often is perceived as an increased respiratory effort. Inspiratory dyspnoea demonstrates the presence of restrictive respiratory disorders due to the reduction of the alveolar-capillary bed surface. Dysphonia, dysphagia, and swallowing disorders indicate tuberculosis of the larynx or upper respiratory tract.

Chest pain is caused by: damage to the pleura, diaphragm, or ribs, involvement of the main respiratory airways (trachea and bronchi) in the tuberculosis process, arteriolar vasoconstriction in the small cycle, and pulmonary artery thromboembolism, displacement of the mediastinum by pleural effusion, pulmonary fibrosis, or retraction of the mediastinal organs. The most common causes of chest pain are the inflammation of pleura and pleurisy. In dry pleurisy and small volume pleurisy, intense

chest pain with acute onset is localized. The sudden onset of chest pain with dyspnoea suggests the onset of compressive pneumothorax.

Fever above 38°C is uncommon. However, low-grade fever (temperature rise to 38°C) can be assessed in 40% of cases. In extended processes, the temperature curve changes constantly with an oscillating tendency. In generalized forms, the temperature can exceed 39-40°C, followed by chills.

Weight loss is a specific clinical sign of tuberculosis. Cachexia offered the disease its Greek name *phtysis*, translated into emaciation, and the specialty was renamed the „phthisiology". In the scientific literature, were described as „tuberculosis masks". These are uncharacteristic clinical signs that contribute to a misdiagnosis of tuberculosis. The most frequent masks include the pneumonic, hemoptoic, digestive, cardiac, neuro-endocrine, etc.

The clinical examination is performed to identify objective data suggestive of tuberculosis. The inspection confirms the malnutrition of the patient, the pallor of the skin, and *habitus phtysicus* in severe cases. The overall appearance was described by Hippocrates in 425 B.C. as „a weak body, retracted like the lens, with blue eyes, leukophlegmatic shoulder-blades projecting like wings".

Particular attention will be paid to the inspection of the chest. The rib cage reveals signs suggestive of tuberculosis only in severe processes: reduced intercostal spaces (in extensive infiltrative processes), asymmetric participation of the thorax cage in respiration (in massive pleural effusions), involvement of accessory respiratory muscles (in respiratory failure). The percussion of the thoracic cage shows a decreased (in extensive infiltrates), absent (in pleural effusion), or pronounced lung sonority (extensive pulmonary destructions, compensatory pulmonary emphysema, and pneumothorax). Frequent auscultation of the rib cage demonstrates a divergence between the low number of obtained auscultative data and the wide range of radiological signs. The abnormal sound may indicate the type and location of the pathology. The most frequently are perceived the supcrepitant rales in the upper thoracic region (supra- and

subclavicular, interscapular), diffuse crackling rales (an indicator of pulmonary congestion), rhonchi sounds, amphoric breathing (in a chronic process associated with the giant cavern), and tubal sound (in the pleural effusion). A cavity syndrome that includes rales and amphoric breathing is characteristic of giant cavities. Regardless of the type of auscultator abnormalities, their location in the vulnerable area of the lungs, i.e. in the upper segments and the circumscribed character „in the focus" are indicators of tuberculosis.

3.2 Assessment of the paraclinical examination results

The paraclinical examination according to the current national clinical protocol includes:

- Radiological examination of the rib cage, which frequently reveals a polymorphic appearance, composed of a complex of proliferative, exudative, necrotic lesions, and fibrous changes.
- The complete blood count shows anemia, or iron deficiency, with different clinical severity; leucocytosis with a deviation of the leukocyte formula to the left in extensive processes with long evolution; lymphocytosis in limited forms of tuberculosis, and then progress to lymphopenia in severe forms; systemic inflammatory syndrome assessed by an increase in blood sedimentation rate, increase in acute phase proteins: C-reactive protein, ceruloplasmin; an increased erythrocyte sedimentation rate.
- Biochemical analysis of the blood may reveal hepatocytolytic syndrome, nitrogen retention, disturbances of hydroelectrolytic balance depending on the associated comorbidities.
- The coagulogram is important in establishing the associated coagulopathies.
- Urine analysis is performed to identify cellular elements within the urine: erythrocytes, leukocytes, as well as other biochemical compounds (urates, phosphates)
- The Elisa test for HIV infection screening is mandatory for all patients diagnosed with TB; and in case of a positive result the

confirmation of the result via the Western-Blot technique is required.

- Microbiological examination of sputum or other biological products depending on the clinical context comprises the microscopic examination of the smear stained by the Ziehl-Neelson or fluorochrome method, sputum assessment by the XpertMTB/RIF genetic molecular method, and cultural methods.
- Other examinations are also recommended: bronchial fibroscopy; biopsy and histological examination of the collected clinical sample, ultrasound examination of the abdominal organs.

3. 3 Microscopic examination. Modalities and principles of the harvesting, transportation, and storage of the clinical specimens

Microscopic examination of the smear stained using the Ziehl-Neelsen method allows identifying of acid-fast-bacilli (AFB) if their number exceeds 100.000/ml of sputum. It is the cheapest, fastest, and most affordable way to identify the acid-fast organisms, mainly Mycobacteria. The sensitivity of the Ziehl-Neelsen method is low for liquefied sputum smears and reaches 20-40%. The positive result is due to the destruction of the lung parenchyma and confers an advanced epidemiological danger. The identification of AFB does not confirm *M. tuberculosis*, since the atypical mycobacteria have the same aspects. The sensitivity of the microscopic method depends substantially on the quality of the collected clinical sample. The sputum, broncho-alveolar lavage, pleural fluid, gastric aspirate, urine, biopsy material can serve as clinical specimens for examination, but the most effective is the sputum. The storage of the biological material must not exceed 3 days and the storage temperature from +4 to +8 ^{o}C. Due to the low sensitivity in limited forms of tuberculosis, several methods for inducing sputum can be used. Inhalation with saline 0,9% solution for nebulization or muco-secretory drugs is preferred. Two sputum samples are collected on two consecutive days. The first sample of the sputum is collected during the first clinical examination. The second sample is collected early morning at the patient's home

the next day, before breakfast, and after the patient has rinsed his mouth. The patient should be asked not to smoke for at least two hours before the sputum collection. The medical staff receiving the container with the sputum must inspect the quality. Qualitative sputum is viscous, mucoid, with fragments of necrotic tissue, which can be deposited as sediment in colors from white to matte green. The TB06 form, requesting the microbiological investigations for diagnosis of tuberculosis should be filled. One hundred microscopic fields should be compulsorily examined and for giving a negative result - 300. Microscopic examination of the sputum smear by Ziehl-Neelson or fluorochrome methods will be done for both samples, and by genetic method GeneXpert MBT/RIF only one, preferably in the early-morning sample, which is considered more qualitative. The reporting and recording of the results of the microscopy are done using the TB05 form. The results of the examination must correspond to the grading scale, presented in *table 3.1.*

Table 3.1

Grading distribution by Ziehl-Neelsen staining

Number of the AFB	Result	Gradation
0 AFB in 100 microscopic fields	Negative	-
1 - 9 AFB in 100 microscopic fields	Positive	Recorded the number of the AFB
10 – 99 AFB in 100 microscopic fields	Positive	1+ (mild positive)
1 – 10 AFB in 100 microscopic fields	Positive	2 + (moderate positive)
> 10 AFB in 1 microscopic field	Positive	3+ (heavy positive)

Fluorochrome staining increased the sensitivity of the microscopic examination by 10-20%. The main disadvantage is the increase in the investigation costs as more laborious methods and trained technicians are involved. Fluorescent microscopy is based on the detection of mycobacteria using fluorochrome dyes: auramine, orange acridine, rhodamine, and coryphosphin. Mycolic acids in the cell wall have an affinity for fluorochrome staining (auramine-phenol) which allows the dye to be retained

after decolorization with acid-alcohols. On examination with a fluorescent lamp, bacilli capable of fluorescence appear yellow on a black background.

The identification of *M. tuberculosis* by culture is essential for the diagnosis of tuberculosis. A positive result is obtained if at least 50 viable mycobacteria are in 1 ml of sputum. After identifying the colonies of mycobacteria, it is mandatory to test the drug susceptibility to first-line anti-tuberculosis drugs: isoniazid, rifampicin, ethambutol, and streptomycin. Cultivation requires at least 4-6 weeks, and the results are obtained in 120 days. In all cases with positive microscopic examination, molecular-genetic and radiological abnormalities, the collected sputum will be examined by culturing on conventional media (Lowenstein Jensen and MGIT BACTEC) followed by a drug-susceptibility test. In all cases with the resistance obtained at GeneXpert MTB/RIF test, the sputum of the positive sample will be examined by culturing on MGIT BACTEC, followed by the testing of the susceptibility to first-line (isoniazid, rifampicin, ethambutol, and streptomycin) and second-line anti-TB drugs (amikacin, capreomycin, kanamycin, levofloxacin, ofloxacin, PAS). Depending on the laboratory equipment, the sputum will be examined by the genetic molecular method MTBDRsl. Patients at high risk for drug-resistant tuberculosis with a preserved sensitivity to rifampicin will be further examined by the MTBDRplus version 2 method, which is a molecular-genetic method that tests the susceptibility to isoniazid and rifampicin. All patients with a negative sputum result on microscopy and the molecular-genetic method, but with an increased risk for drug-resistant tuberculosis and with symptoms suggestive of tuberculosis will be examined by the genetic molecular method MTBDRsl.

Microscopic examination of sputum by Ziehl-Neelson staining is an investigation that does not provide high sensitivity and specificity, however, demonstrated its epidemiological role in the detection of contagious patients.

According to international guidelines, the detection of the majority of patients is performed in a passive way through the microscopic examination of the sputum. The main disadvantage of smear microscopy is a

low sensitivity (20 - 80%). In the Republic of Moldova, only 37% of new cases are microscopically positive and multiple factors are involved: the method of sputum collection, storing, transporting, staining, and the experience of the technician. Fluorochrome staining was implemented to increase the sensitivity of the microscopy, which raised by 10%. Decontamination methods associated with sputum centrifugation increased the sensitivity of the microscopy by 11-26% as well. Passive sedimentation from 30 minutes to several hours, followed by decontamination, increased the sensitivity of the microscopy insignificantly.

The accuracy of sputum sampling, storage, transport, processing, and smear examination is reflected in the rate of positive results of the microbiological examination (microscopic and in culture). According to the clinical protocol, 2 sputum samples are collected, defined as the first sample on the request (taken at the time of the patient's visit to the medical institution, most frequently at the primary care facility) and the second sample, called the „early-morning" sample, brought next morning. The second is considered with better quality if the patient was properly trained and correctly executed the instructions of the sampling operation, as well as due to the accumulation of tracheobronchial secretions during the night. Due to the rich saprophytic flora of the tracheobronchial tree, most specimens of the bronchial tree (sputum, induced sputum, tracheobronchial lavage, and bronchoalveolar lavage) are contaminated with nonspecific flora, which under the same cultivation conditions grow faster and show erroneous results. The amount taken is important both for microscopy and culture, as well as for the genetic molecular method. The sample amount greater than 5 ml provides a higher sensitivity, especially for the microscopic examination and molecular genetic assay.

Sputum samples used for the microbiological examination are:

- Spontaneous sputum;
- Induced sputum with hypertonic saline nebulization, irritation of the pharynx with a sterile tampon or bronchoalveolar lavage;
- Tracheobronchial secretions;
- Gastric content in children.

The transportation of the samples should be carried out according to the biosecurity standards, in closed containers. After collection, the sputum sample should be processed by a sequence of techniques: digestion, homogenization, decontamination, and concentration. Chemical decontamination with N- acetyl -L-cysteine- NaOH is the most common method of chemical decontamination and reduces the method's sensitivity. The concentration by centrifugation increases the sensitivity of the microscopic examination because the supernatant contains a high number of bacilli. A greater advantage of molecular-genetic investigations compared to conventional microbiological methods is no need for storing and decontaminating the samples. The result is positive by identifying specific DNA sequences whether the bacilli are viable or dead, whether the sample is contaminated or not.

3.4 Bacteriological examination. Cultivation of mycobacteria on solid and liquid media (Lowenstein-Jensen, BACTEC, MB/BacT). Methods of the drug-susceptibility testing of M. tuberculosis to first-line anti-tuberculosis drugs.

The „gold standard" in the diagnosis of tuberculosis is culture, using both solid (Lowenstein-Jensen, Ogawa) and liquid (Middlebrook 7H) media. The culture is much more sensitive than microscopy and allows drug susceptibility testing. While a positive microscopic result requires a concentration of about 5.000-10.000 AFB/ml of the sputum, the culture can detect from 10 to 100 viable bacteria/ml. Detection of *M. tuberculosis* growth on solid media is possible in 12 weeks compared to 3-8 weeks on liquid media. There are different types of automatic devices for the culture of mycobacteria on selective liquid media: BACTEC TB 460, BACTEC MGIT 960, BacT/Alert 3D.

BACTEC 460 TB system is a semi-automatic radiometric detection system that uses a liquid medium with ^{14}C-marked palmitic acid. Mycobacteria catabolize palmitic acid and release $^{14}CO_2$, which is automatically quantified by the system. Radioactive carbon dioxide CO_2 is measured quantitatively. The rate of radioactive CO_2 production is directly

proportional to the rate of *M. tuberculosis* multiplication. A positive result for *M. tuberculosis* occurs in 4-25 days. The disadvantage of the method is the impossibility of identifying the type of mycobacteria.

BACTEC MGIT 960 system is based on the colorimetric principle to automatically detect the fluorescence of a fluorochrome embedded in tubes with liquid culture medium Middlebrook 7H12. The tubes in the system are read every 60 minutes. Initially, fluorescence is inhibited by O_2 present in the culture medium. The growth of mycobacteria decreases the level of O_2 in the environment and the property to stop fluorescence. At the moment the fluorescence is captured by the sensors. The tube is marked positively when fluorescence is detected. A positive result for *M. tuberculosis* occurs in 3-21 days (averaging 14 days), and for non-tuberculous mycobacteria in 7 days.

The MB/BacT-Alert method uses the liquid medium Middlebrook 7H9, based on the colorimetric principle that monitors the multiplication of mycobacteria through the CO_2 accumulation. The reflectometric device detects the light every 10 minutes at the level of the detector built into the bottom of the culture tube (the color changing from dark green to yellow) and reacts when *M. tuberculosis* density is 10^6-10^7 colony formingunits/ml medium.

There are various methods to test the susceptibility to anti-tuberculosis drugs. The direct method in patients with a positive result to the microscopic examination involves the cultivation of a direct sputum inoculum on solid or liquid media with and without anti-tuberculosis drugs after decontamination. In indirect methods, the susceptibility test is performed after the pure culture is isolated from the clinical specimen.

Standard methods used to detect the resistance of *M. tuberculosis* against the anti-tuberculosis drugs are:

1. Using the Löwenstein-Jensen solid medium:

- Absolute concentrations method (Meissner) is an indirect method, which uses previously isolated strains. The bacterial suspension is inoculated on drug-free media and the media containing a range of concentrations of the drugs. The minimum inhibitory concentration is the

lowest drug concentration that inhibits the culture growth. This method is time-consuming in evaluating the results.

- The Canetti proportion method determines the percentage of the increase (number of colonies) of inoculum on a reference media with no anti-tuberculosis drug compared to the number of colonies on culture media containing the critical concentration (or range of concentrations) of the tested anti-tuberculosis drug.
- Resistance coefficient method (Mitchison) is the assessment of the growth of the patient's strain and the sensitive reference strain, performed in 5 tubes with different concentrations of anti-tuberculosis drug per tube. Resistance is expressed as the ratio between the minimum inhibitory concentration of the tested strain and the reference strain, respecttively.

2. Using the liquid medium:

- The BACTEC 460 radiometric method allows for assessing of the sensitivity to the first and second-line anti-tuberculosis drugs.
- The BACTEC MGIT 960 colorimetric method is recommended for testing the susceptibility to the first and second-line anti-tuberculosis drugs.
- MB BacT/Alert or VersaTrek allows testing the susceptibility to the first-line anti-tuberculosis drugs.

3. Rapid phenotypic methods:

- The nitrate reductase method (Griess) can be used as a direct or indirect method. It is a liquid or solid medium technique that measures the reduction of nitrogen by *M. tuberculosis*, which indicates the growth and resistance to isoniazid and rifampicin. The medium is supplemented with potassium or sodium nitrate, which will be reduced to nitrite by the active growth of *M. tuberculosis.* It is colored in pink-purple when a detection reagent (Griess reagent) is added into the tube with *M. tuberculosis*.
- Direct microscopic observation method is a manual technique based on microscopic observation of string formation in the liquid medium (Middlebrook 7H9 or 7H12) on plastic well plates. It is used for

rapid detection of susceptibility to the first and second-line anti-tuberculosis drugs.

- The thin-layer agar technique uses light microscopy to simultaneously detect the growth of *M. tuberculosis* colonies and resistance to isoniazid and rifampicin directly from processed sputum specimens. Plates with a thin layer of supplemented solid medium (Middlebrook 7H10, 7H11) with and without antibiotics are inoculated with sputum specimens, incubated, and examined under a microscope. Microcolonies of *M. tuberculosis* can be detected in less than 7 days, the results of the drug susceptibility are revealed within 10-15 days.

3.5 Molecular-genetic methods for the identification of M. tuberculosis: Gene Xpert MTB/RIF, GenoType®MTBDRplus, BD ProbeTec™

Molecular-genetic methods for amplifying the nucleic acids have been introduced in the current use of reference laboratories in mycobacterial microbiology. Currently, the following methods are used to identify mycobacterial nucleic acids: Amplicor PCR (Roche Molecular Systems), transcription-mediated amplification (MTD amplification method), GenoType Mycobacteria Direct Assay method (Hain Lifescience), displacement chain amplification method BD ProbeTec™), the loop-mediated isothermal amplification method (LAMP; Eiken Chemical Co.).

The Xpert MTB/RIF method is a molecular-genetic method that detects the mycobacterial DNA and mutations in the *rpoB* gene responsible for rifampicin resistance. It was implemented at the national level in the Republic of Moldova in 2014. GeneXpert Cepheid equipment provided by Sunnyvale is required. The equipment includes a tool for inserting 1-16 cartridges, a computer, a program for reading the results, and a scanner for reading the barcode. Reagent cartridges are used to perform polymerization chain reactions. The polymerization reaction takes place inside the reagents. The test results are interpreted via the GeneXpert DX system by measuring fluorescence signals. The obtained results are as follows:

1. MTB detected - DNA of the *Mycobacterium tuberculosis* complex was identified; MTB detected and rifampicin resistance detected - mutation of the *rpoB* gene responsible for rifampicin resistance was identified; MTB detected and the *rpoB* gene mutation was undetermined;
2. Undetected MTB - no DNA of the *Mycobacterium tuberculosis* complex was identified;
3. Invalid - the presence or absence of DNA of the *Mycobacterium tuberculosis* complex cannot be determined;
4. Error - lack of result;

The result depends on the number of mycobacteria, sputum collection methods, handling, storage, and transportation. The positive result does not necessarily indicate the presence of viable microorganisms. It can be obtained by evaluating patients who have been treated and further eliminating bacilli after the treatment completion. For the reasons listed, evaluation of the patient by the GeneXpert MTB/RIF during the antituberculosis treatment and after its completion is not recommended. The test is simple and non-laborious, has a short duration, the result is obtainned in 2 hours, and does not require biosecurity conditions. It is used in regional laboratories and primary care institutions. The main disadvantages involve the costly maintenance, reagents, and cartridges.

3.6 Criteria for diagnosis of tuberculosis. Classification of tuberculosis according to the International Classification of Diseases, version 10

The presumptive diagnosis of tuberculosis is based on the clinical signs of intoxication and bronchopulmonary syndrome, the epidemiological context (tuberculous contact), and radiological abnormalities on the chest -X ray. The confirmation of the clinical diagnosis can be obtained after the isolation and identification of *M. tuberculosis* through the microbiological methods on conventional culture media or molecular-genetic methods.

The diagnosis of tuberculosis is confirmed by the following methods:

1. Microscopic examination:
    - Sputum smear examination using light microscopy and Ziehl-Neelsen staining;
    - Sputum smear examination using light microscopy and auramine-rhodamine staining.
2. Bacteriological examination:
    - Cultures on Lowenstein-Jensen solid medium;
    - Cultures on MGIT BACTEC, MB/BacT liquid medium.
3. Molecular-genetic examination: GeneXpert MTB/RIF, GenoType MTBDRPlus, GenoType MTBDRsl, BD ProbeTec™;
4. Histological examination of clinical samples obtained from biopsies of the organs or surgical excisions.
5. Clinical-radiological evaluation.

Classification of tuberculosis

The clinical classification of tuberculosis allows the clinician to obtain an overview of the disease, prognosis, and therapeutic management. The first classification of tuberculosis was attributed to Turbanov-Gerhard who differentiated the 3 stages of tuberculosis depending on the localization. The 1st stage included apical localization. The 2nd stage was identified in the median lung segments, and the 3rd stage included generalized tuberculosis. Ludwig Aschoff in 1924 also classified tuberculosis, based on the phases. This classification allowed the staging only after the necropsy. Ranke's classification defined primary tuberculosis, which follows the primary infection and secondary tuberculosis, resulting from the reactivation of latent infection, as well as tuberculosis of the localized in other organs. In 1923, Abricosov A.I. (1875-1955) defined a new classification based on the clinical-radiological features of the phases, as well as on the microbiological findings of the sputum samples. This classification is currently used throughout the Commonwealth of Independent States.

The classification of tuberculosis according to the current protocol includes several compartments:

1. Clinical-radiological form of tuberculosis;
2. Location of the disease;
3. The phase (progressive, regressive, stabilizing, and stationary);
4. The microbiological and molecular-genetic results;
5. Complications;
6. Case-type depending on the history of anti-tuberculosis treatment.

Thus, the components of the clinical diagnosis of tuberculosis are the clinical-radiological form of tuberculosis, topographic location, phase of the evolution, validated microbiological and molecular-genetic investigations, complications, the case type according to the treatment history, and associated diseases.

Classification of tuberculosis according to the International Classification of the Diseases Version 10th

Clinical forms of pulmonary tuberculosis:

A 15.7; A16.7 Primary tuberculosis complex;
A 19.0 Pulmonary disseminated tuberculosis (miliary);
A 15.0.1.2.3; A 16.0.1.2 Pulmonary nodular tuberculosis;
A 15.0.1.2.3; A 16.0.1.2 Pulmonary infiltrative tuberculosis;
A 15.0.1.2.3; A 16.0.1.2 Pulmonary fibro-cavitary tuberculosis;
A 15.5; A 16.4 Laryngeal and tracheobronchial tuberculosis;

Clinical forms of extrapulmonary tuberculosis:

A 15.6; A16.5 Pleurisy (empyema);
A 15.4; A16.3 Tuberculosis of the intrathoracic lymph nodes;
A 15.8; A16.8 other forms of extrapulmonary tuberculosis;
A 17.0.1.8.9 Tuberculosis of the central nervous system;
A 19.1 Generalized tuberculosis (polyserositis, miliary with multiple localizations);
A 18.0 Tuberculosis of bones and joints;
A 18.1 Urogenital tuberculosis;
A 18.2 Peripheral tuberculous adenopathy;
A 18.3 Tuberculosis of peritoneum, intestine, and mesenteric lymph nodes;

A 18.4 Tuberculosis of the skin and subcutaneous tissue;
A 18.5 Tuberculosis of the eye;
A 18.6 Tuberculosis of the ear;
A 18.7 Tuberculosis of adrenal glands;
A 18.8 Tuberculosis of the other organs.

Sequelae of tuberculosis

B 90.0 Sequelae of the central nervous system tuberculosis;
B 90.1 Sequelae of the urogenital tuberculosis;
B 90.2 Sequelae of bones and joints tuberculosis;
B 90.8 Sequelae of other organs tuberculosis;
B 90.9 Sequelae of the respiratory system tuberculosis.

The characteristics of the tuberculosis process are assessed through the:

- Microbiological method: sputum microscopy, molecular-genetic method (Xpert MTB/Rif, etc.), culture methods;
- Histological method;
- Clinical-radiological investigations.

Location and extension of the tuberculous process:

- Limited tuberculosis is localized within 1-2 lung segments;
- Extensive tuberculosis affects 3 or more lung segments;

The phase of the tuberculosis process:

- Progressive (infiltration, destruction, dissemination);
- Regressive (resorption, induration);
- Stationary (with no radiological dynamics);
- Stabilization.

The phase allows for to define of the activity of tuberculosis and the evolution in dynamics.

Complications:

- Haemoptysis;
- Spontaneous pneumothorax;
- Respiratory failure;
- Chronic pulmonary heart disease;
- Atelectasis;

- Amyloidosis;
- Organ failure;
- Fistulas, etc.

The case-type registration according to the anti-tuberculosis treatment anamnesis:

New case – the patient who has never received anti-tuberculosis treatment or received less than 1 month;

Relapse (recurrence) – the patient who received anti-tuberculosis treatment and completed the most recent treatment with the result „cured" or „treatment completed" and is currently diagnosed with a new episode of tuberculosis.

Treatment after failure – the patient who restarted the anti-tuberculosis treatment after being assessed as „failure" for the previous treatment.

Treatment after lost to follow-up – the patient who started again the anti-tuberculous treatment after being assessed as „lost to follow-up".

Others – the patient who initiated the treatment abroad and continued in the Republic of Moldova (documented).

Case of TB detected post mortem – patient diagnosed with TB post-mortem (after death).

Characteristics according to the type of drug-resistant tuberculosis:

Mono-drug resistant TB – the patient with tuberculosis with the drug susceptibility test that confirmed the resistance to a single first-line anti-tuberculous drug, with the exception of rifampicin.

Poly-drug resistant TB – the patient with tuberculosis with the drug susceptibility test that confirmed the resistance against two or more first-line anti-tuberculosis drugs, except the combination of isoniazid and rifampicin.

Multidrug-resistant TB (MDR-TB) – the patient with tuberculosis with the drug susceptibility test that confirmed the resistance against isoniazid and rifampicin, with or without resistance to other anti-tuberculosis drugs.

Extensively drug-resistance (TB-XDR) – the patient with tuberculosis with the drug susceptibility test that confirmed the resistance to isoniazid and rifampicin (TB-MDR) with additional resistance to any of fluoroquinolone (ciprofloxacin, levofloxacin, ofloxacin) and to at least to one of the injectable anti-tuberculosis drugs (capreomycin, kanamycin, amikacin).

Case of TB with rifampicin resistance (RR) – the patient with tuberculosis with the drug susceptibility test that confirmed the resistance to rifampicin or the rifampicin resistance was determined by the genotypic methods with or without associated resistance to other anti-tuberculosis drugs.

Characteristic of the type of the case that initiates the treatment for RR/MDR-TB:

New case – the patient who has never received the anti-tuberculosis treatment or received less than 1 month;

Relapse (recurrence) – the patient who received anti-tuberculosis treatment and completed the most recent treatment with the result "cured" or „completed treatment" and is currently being diagnosed with a new episode of tuberculosis.

Treatment after failure – the patient who during the anti-tuberculosis treatment was evaluated as „failure".

Treatment after loss to follow-up – the patient who during the anti-tuberculous treatment was assessed as „lost to follow-up"

Other – the patient who initiated the treatment abroad and returned to continue it in the Republic of Moldova (documented).

3.7 Other methods for the investigation of patients with tuberculosis

Radiological investigations

The radiological investigations of patients with pulmonary tuberculosis are the standard techniques including radiography, radioscopy, and conventional tomography. In individual cases, high-resolution computed tomography (HRCT) and nuclear magnetic resonance imaging are recommended. The general purpose of the radiological investigation is to

diagnose the disease, to evaluate the extension, complications, and evolution under the treatment. The radiological semiology of pulmonary tuberculosis integrates the following patterns:

1. Infiltrative (alveolar) opacities with a systematized or unsystematized character, having different sizes and different intensities (homogeneous or heterogeneous), with unclear contour, single or multiple, preferably located in the lung segments S1, S2, S6 and S10 in adults and S4, S5 and S8 in children. Typical radiological features of infiltrative opacity are greater than 1 cm in size, of medium intensity, heterogeneous, with blurred contour and apical caudal extension, the parenchymal destruction being visible as hypertransparencies. The changes are atypical in TB/HIV co-infection. Depending on the number of affected lung segments, infiltrates may be limited (1-2 segments) and extended (≥ 3 segments). Infiltrates are most commonly located in one lung, but can affect both lungs.

2. The sizes of nodular opacities may be:

A) Small, less than 1 cm located in the apical segments, which is characteristic of nodular pulmonary tuberculosis *(figure 3.1)*

B) Micronodules, less than 2 mm in size, symmetrically localized in both lungs, are characteristic of acute disseminated pulmonary tuberculosis *(figure 3.2).*

C) Nodular opacities have a size of more than 1 cm. Are located in segments S1, S2, S6, and S10 and is characteristic of infiltrative pulmonary tuberculosis *(Figure 3.3).*

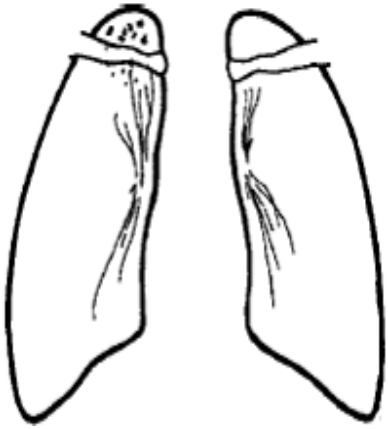

Figure 3.1. Nodular opacities

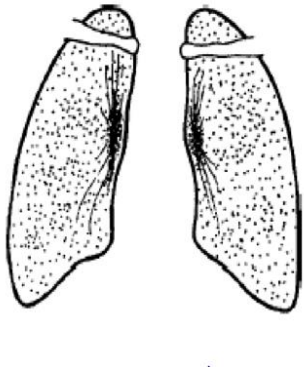

Figure 3.2. Micronodular opacities

Dissemination is the most common way through which tuberculosis evolves. The dissemination occurs through bronchial, lymphatic, and hematogenous ways. Lymphatic dissemination is involved in the pathogenesis of primary tuberculosis. The bronchogenic dissemination pathway contributes to the progression of post-primary (secondary) tuberculosis. The hematogenous dissemination is responsible for the appearance of 2-4 mm micronodular opacities, showing a low intensity and unclear

contour, then having a higher intensity and well-defined contour, disseminated on both lungs, harmoniously defined as in „the mirror" and its characteristics for acute disseminated tuberculosis.

It begins from the reactivation of the apical nodular lesions (Simon nodules) with the apical-caudal extension of the parenchymal infiltrate *(schemes 1 and 2).* Due to bronchogenic spread, new localisations appear in both lungs, evolving with necrosis and parenchymal destruction *(scheme 3).*

The caseous content is eliminated by the drainage through the bronchus and hypertransparencies with thick walls, irregular internal contour (caverna), with the deviation of the mediastinum towards the affected part *(scheme 4).*

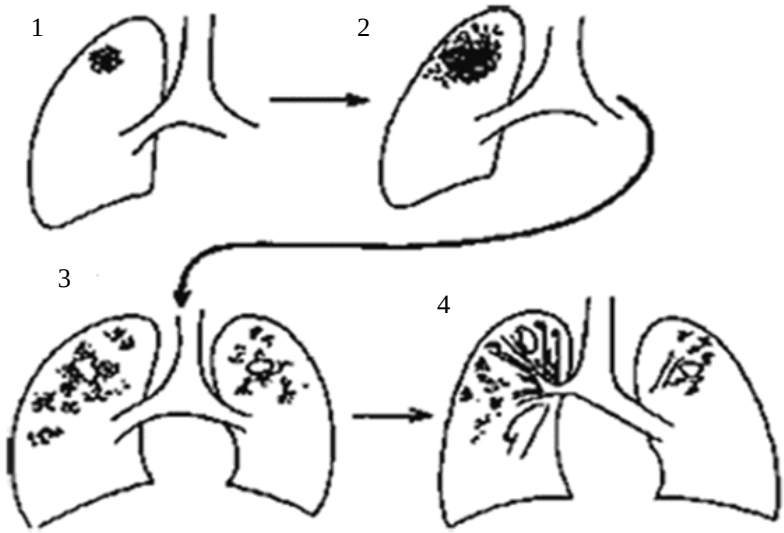

Figure 3.3. Evolution of nodular opacity

In adult patients, one or more infiltrative opacities are most frequently identified. Infiltrative opacity in pulmonary tuberculosis is located in the lung segments S1, S2, S6, and S 10 in adults and segments S4, S5, and S8 in children. Infiltrative opacity in pulmonary tuberculosis reveals the following characteristics: more than 1 cm in size, extended on the

entire lung. Depending on the number of involved segments, the infiltrates are differentiated in limited (1-2 segments) and extended (≥ 3 segments) opacities. The localization is unilateral, however, the bilateral one is more frequently detected in advanced tuberculosis. Depending on the geometric shape, the infiltrative opacity can be round, oval, or triangular. The radiological features of infiltrative opacity in tuberculosis are medium-intensity, heterogeneous, with unclear borders. The features of progressive tuberculosis include parenchymal destruction (radiologically assessed by areas of hypertransparency) and dissemination (lymphatic - in primary tuberculosis, bronchogenic - in secondary tuberculosis, hematogenous - in disseminated or generalized tuberculosis). The lung lesions are typically located in the posterior segments of the upper lobe and the upper segments of the lower lobe. The radiological changes are atypical in the TB/HIV co-infected patients.

D) Nodular opacities of different sizes located in both lungs are characteristic of subacute disseminated pulmonary tuberculosis *(figure 3.4).*

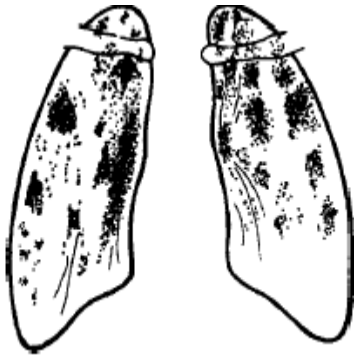

Figure 3.4. Nodular opacities of different sizes (subacute disseminated pulmonary tuberculosis)

1. Cavitary or ring-shaped images with pericavitary inflammatory crown are characteristic for recent caverna *(Figure 3.5., Scheme 1)*, empty caverna and the drainage bronchus *(Figure 3.5., Scheme 2)*, fibrous caverna localized on the underlying pulmonary fibrosis with mediastinal deviation

(Figure 3.5., Scheme 3). The walls of caverna can be thin in subacute disseminated pulmonary tuberculosis or thick in fibrocavitary pulmonary tuberculosis. Usually, there is one caverna. Multiple caverna might be detected in chronic forms.

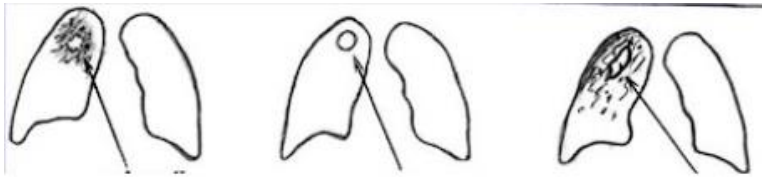

Figure 3.5. Cavity images
1 - recent caverna; *2* - elastic caverna; *3* - fibrous caverna.

2. Fibrous lesions in pulmonary tuberculosis are unsystematized and retractable. The most affected areas are the dorsal and apical segments of the upper lobes and the apical segments of the lower lobes.

3. Enlarged hilum syndrome, established in children is characteristic of tuberculosis of intrathoracic lymph nodes *(figure 3.6)*.

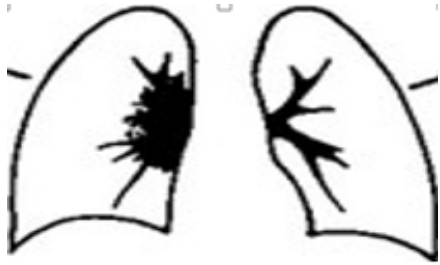

Figure 3.5. Enlarged hilum syndrome

4. The reticulonodular syndrome is described as the thickening of the pulmonary interstitium, followed by the appearance of micronodular opacities (milliary, smaller than 2 mm). It is characteristic of acute disseminated pulmonary tuberculosis.

5. Pleural syndrome is characterized by the appearance and continuous accumulation of pleural exudative fluid, assessed radiologically as massive opacity, of increased intensity, with the upper concave oblique line (Damoiseau) oriented towards the armpit *(figure 3.7).* The radiological features are shown in *figure 3.6:*

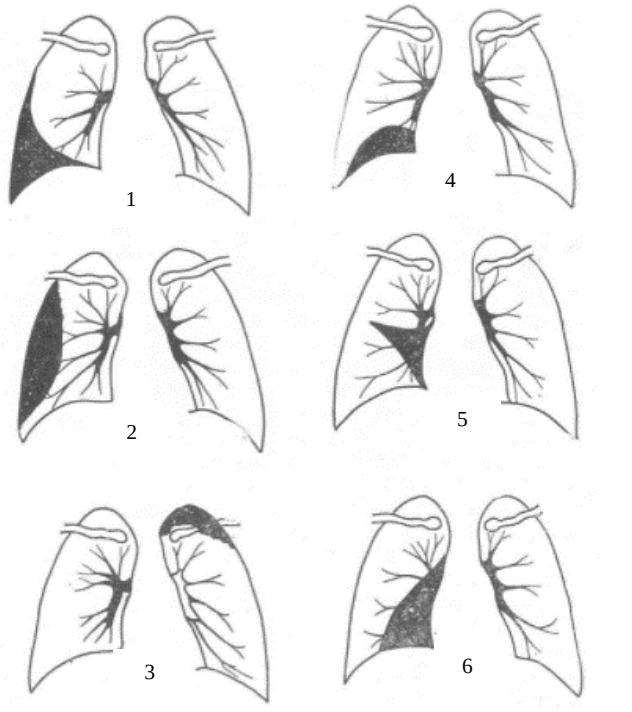

Figure 3.7. Radiological tuberculous pleurisy.

Description:

1. Triangular opacity with upper concave borderline (Damoiseau line).
2. Costal pleurisy.
3. Apical pleurisy.
4. Diaphragmatic pleurisy.
5. Interlobar pleurisy.
6. Mediastinal pleurisy.

Chest X-ray plays a major role in patient screening. If it is normal and no pathological changes occur, pulmonary tuberculosis is excluded. If it shows pathological changes in the alarming areas, as in the posterior segments of the upper lobe and the upper segments of the lower lobe, then the following investigations should be carried out:

- Microbiological examination of the clinical sample: sputum smear microscopy on Ziehl-Neelsen or fluorochrome staining, Gene XpertMTB/RIF, cultural methods with drug susceptibility testing for the 1st line anti-tuberculosis drugs;
- Paraclinical examinations for other organs and systems;
- Counselling and testing for HIV biomarkers.

If the microbiological examination of the clinical specimens is positive, the diagnosis of tuberculosis is confirmed. Negative results do not exclude tuberculosis and further examinations will be performed.

3.8 Methods for diagnosis of extrapulmonary tuberculosis

Tuberculosis pleurisy

Tuberculosis pleurisy is a reversible complication of pulmonary tuberculosis, commonly diagnosed in adolescents and young adults. It is a type of hypersensitivity reaction of the pleura to mycobacterial antigens. Commonly, it has a unilateral location and rarely is bilateral. The diagnosis is established on the basis of clinical, epidemiological, and paraclinical data. The onset is acute, characterized by thorax pain that worsens during coughing and breathing. On objective examination, the affected hemithorax is increased in volume, immobile or shows reduced costal movements, with enlarged intercostal spaces, the mediastinum being pushed to the healthy side. The percussion reveals dullness. On auscultation, the diminution or absence of the vesicular murmur is heard. Pleural effusion becomes radiologically visible on a posteroanterior incidence radiograph in orthostatic position if the amount of fluid is at least 250 ml. Pleurisy with a small amount of fluid located in the costodiaphragmatic sinus can be established on profile radiography or lateral decubitus radiography. In the Trendelenburg position, the small amount

of fluid at the base moves between the pleural sheets and opacities in the apical segments of the lung.

Pleurisy of the medium volume on the lung radiography is highlyghted as a pleural opacity, of increased intensity, occupying the costodiaphragmatic sinus and the lower third of the hemithorax. Opacity does not allow visualization of the lung tissue. Pleurisy with medium-amount fluid, 1000 ml, completely opacifies the base of the lung, tends to rise anteriorly, laterally and posteriorly. In posteroanterior incidence, it is visualized as a triangular opacity, with one side on the diaphragm, and another on the axillary and the third thoracic wall towards the pulmonary transparency, showing an upward concavity, called the Damoiseau curve. In massive pleurisy, the Damoiseau curve extends along the lateral wall of the thorax and rises to the collarbone. The fluid opacifies the entire pleural cavity, the hemithorax is enlarged and the mediastinum is pushed to the healthy side. Other radiological investigations that may differentiate pleural effusions include chest ultrasound and computed tomography. Ultrasound highlights the volume of the effusion and guides thoracentesis. Computed tomography shows thickening of the pleura, pulmonary parenchymal infiltrates, areas of atelectasis, hilar lymphadenopathy.

Thoracocentesis with pleural fluid extraction allows microbiological, biochemical, and cytological investigation. The highest sensitivity in the diagnosis of tuberculosis is thoracoscopy with pleural puncture and biopsy. The biopsy shows tuberculous granulomas, spread all over the surface of the pleural serosa. The biochemical examination establishes sero-citrin exudate, with proteins over 3 g/dL, pleural LDH more than 700 IU/L, pleural LDH/serum LDH > 0.6, pleural lysozyme/serum lysozyme rate > 2, adenosine deaminase activity (ADA) > 40 IU/L. Cytological examination of pleural fluid shows 1000-2000 cells/mm^3 and > 80% lymphocytes. The microbiological and genetic examination (Gene XpertMTB/RIF) is rarely positive. By combining pleural fluid examination with cytology and culture, it is possible to establish the diagnosis of tuberculosis in 80% of cases. Complications of thoracentesis include pneumothorax, pleural hemorrhage, subcutaneous emphysema, vagal

syncope, and pleural fluid infections. The differential diagnosis is made with other non-tuberculous infectious causes (bacterial or viral), pleural effusions associated with subdiaphragmatic suppurations, pleurisy from collagen diseases (acute rheumatoid arthritis and systemic lupus erythematosus), and neoplastic pleurisy. The differentiated diagnosis of tuberculosis pleurisy is presented in *Table 3.2.*

Table 3.2

Criteria for the differential diagnosis of tuberculosis pleurisy

Diseases	Cytology	Microbiological	Pleural biopsy	Additional investigations
Tuberculous pleurisy	> 80% lymphocytes	AFB + cultures +, GeneXpert MTB/Rif +	Tuberculous granuloma	ADA > 40 U/L
Parapneumonic	Predominate polymorphonuclear leukocytes	Non-specific flora	Nonrelevant	Bacteriological examination of sputum
Subdiaphragmatic suppuration	Predominate lymphocytes	Sterile liquid	Nonrelevant	Ultrasound Computed tomography
Connective tissue diseases (periarteritis nodosa, dermatomyositis, scleraderma, and disseminated lupus erythematosus)	Noncharacteristic	Sterile liquid	Specific for vasculitis	Positive serology
Neoplastic	Tumor cytology	Sterile liquid	Neoplastic infiltrates	Tumor biomarkers

Tuberculous meningitis

Tuberculous meningitis is an acute inflammation caused by infection with *Mycobacterium tuberculosis* of the meninges, superficial cerebral layers, and choroid plexuses. The incidence of meningitis is an important indicator of the evolution of tuberculosis epidemics. It is considered the most severe form of tuberculosis with unfavourable evolution. It is diagnosed in early childhood (less than 5 years old), in unvaccinated BCG patients, and adults co-infected with TB/HIV. The localization of

the infection is on the meninges and brain is the result of hematogenous dissemination in the first 6 months after the primary infection. Meningeal lesions are multiple, diffuse with embolization of small cortical vessels. Injuries to the choroid plexuses and meninges cause hypertension of the cerebrospinal fluid and dilation of the ventricles. Lesions of the arachnoid plexuses cause disorders in the functionality of the cranial nerves (hearing loss, nystagmus, balance disorders).

The symptoms are nonspecific. It has an insidious onset, characterized by diffuse pulsating headaches of different intensity, exacerbated by the movements of the head or coughing, fever, intracranial hypertension syndrome (nausea and vomiting, fontanelle bulging), constipation or diarrhea, changes in behaviour, back pain, muscle rigidity, sleepiness, loss of appetite, skin hyperaesthesia. The objective examination reveals the meningeal syndrome: rigidity of the neck, Kerning and Brudzinski signs, epileptic seizures, extrapyramidal dyskinesia, mental disorders, coma, vegetative changes, paralysis, and sphincter disorders. Cranial nerves II, VII, and VIII are more frequently involved. Obvious meningeal signs occur. They are manifested by intracranial hypertension, respiratory disorders, drowsiness, and dilated pupils. The muscular rigidity can be established by the following signs: the rigidity of the neck - the attempt to reflect the head on the thorax fails; the positive Brudzinski sign - the attempt to reflect the head on the thorax triggers a reflection of the calves on the thighs, the sign Kerning 1 - the attempt to reflect the trunk on the pelvis determines the reflection of the calves, the sign Kerning 2 - the attempt to reflect on the pelvis the lower limbs in extension determines their flexion.

The cerebrospinal fluid collected by lumbar or suboccipital puncture is as clear as rock water, rarely opalescent with high pressure (>40 drops/minute). The cytological examination shows 200-500 cells/ml, initially with neutrophilic polymorphonuclear cells, then the lymphocytes predominate over 72 hours. The biochemical examination determines the concentration of albumin increased by over 100 mg/mm^3 and the Pandey reaction highly positive.

Pandey's reaction consists of that 10-15 drops of 10% carbolic acid solution are applied in a tapered glass, which is placed on a dark background, and then a drop of cerebrospinal fluid is added. The reaction is very sensitive and is determined by the intensity of the turbidity formed at the point of contact between the liquid and the reagent over 3 minutes. The cerebrospinal fluid is then left in the test tube for 16-24 hours, forming a fine veil. The glucose concentration in the cerebrospinal fluid is low (<0.3 g/L). The concentration of chloride is low (<0.5 g/dL). The faster the cytological and biochemical changes normalize, the less likely the diagnosis of tuberculous meningitis is. The longer it lasts, the more likely it is to be diagnosed with tuberculous meningitis. Microscopic examination and culture are rarely positive. Examination of the eye fundus may reveal choroidal tubercles characteristic of papillary stasis, atrophy, and papillary edema. The tuberculin skin test can be positive or hyperergic or false negative in severe forms. Chest computed tomography and lung radiography show the location of the lesions in the respiratory tract. Computed tomography of the brain shows hydrocephalus. Nuclear magnetic resonance imaging reveals meningeal changes, stroke, cerebral edema, or cerebral tuberculoma. The prognosis depends on the general condition at the hospital admission, delay till the diagnosis, and the patient's age.

Clinical staging is differentiated into:

- Stage I and II – no neurological signs, hydrocephalus, and no changes in sensitivity;
- Stage III - major neurological signs: paraplegia, tetraplegia, convulsions, and impaired sensitivity.

Stage I and II have low mortality and in stage III, the mortality achieves 70% and only 30% of cases can be cured resulting in neurological sequelae.

Bronchial endoscopy

Fibrobronchoscopy is one of the most important investigations of patient with tuberculosis. The purpose of the investigation is to:

a) provide additional elements for the diagnosis of pulmonary, extrapulmonary, or generalized tuberculosis by harvesting the clinical samples for microbiological, cytological, histological examination;

b) perform the differential diagnosis with other pathologies or situations (aspiration atelectasis);
c) establish the need and risks for surgical treatment or complications (hemoptysis, hemorrhage, fibrous stenosis, evacuation of caseum by ganglion bronchial fistula or granulation tissue).

Endobronchial lesions present in tuberculosis of the respiratory tract are inflammation of the bronchial mucosa, extrinsic compression caused by lymphadenopathy, ganglion bronchial fistula, local sequelae (bronchial stenosis, bronchiectasis).

Fibrobronchoscopy assesses the extent and severity of the inflammatory process of the bronchial mucosa:

Grade I – the bronchial mucosa is pale-pink, covered with mucus, the submucosal layer having a normal vascular pattern;

Grade II – hyperemic and thickened bronchial mucosa, with slight bleeding, the mucosa is covered with mucous or mucopurulent secretions;

Grade III – bronchial mucosa is thickened, purplish, with slight bleeding, covered with mucopurulent secretions.

Fibrobronchoscopy can be complicated by bleeding, damage to the tongue, epiglottis, lesion of the larynx and vocal cords, subglottic edema, or bronchospasm.

Other paraclinical investigations

Changes in complete blood count and the results of biochemical and immunological investigations do not have high specificity for tuberculosis. Laboratory investigations are used to monitor the evolution of the disease, the activity of the inflammatory process, the development of the adverse reactions to anti-tuberculosis drugs and associated diseases.

The inflammatory biomarkers, such as fibrinogen, erythrocyte sedimentation rate (ESR), C-reactive protein allow the evaluation of the inflammatory process. Changes in the blood count show anemia and increased pro-inflammatory indices, leukocytosis with leukocyte deviation to the left, lymphocytosis followed by lymphopenia, and increased ESR.

The dosage of antimycobacterial antibodies has relative value and cannot differentiate latent tuberculosis infection from active tuberculosis. Biochemical examination of the blood is performed to identify comorbidities and monitor the adverse effects of treatment, the degree of hepatotoxicity, and the drug toxicity on other organs. The HIV test is performed in all patients suspected or confirmed with tuberculosis.

Functional exploration of the respiratory system ventilation

For the functional exploration of the ventilation of the respiratory system can be performed: evaluation of the static lung volumes, dynamic lung volumes, maximum instantaneous ventilatory flows, and bronchomotor tests.

For the evaluation of the static lung volumes, spirometry and spirography are used. The spirometer consists of a graduated cylinder, which communicates with the outside through a rubber tube, through which the patient blows the inspired and exhaled air. The graduated cylinder is immersed in a larger cylinder filled with water. Exhaled air causes the cylinder to rise above the water. This movement activates the pen that registers the water level. The result of the recording is the graphic image of the volume of the circulated air. The spirograph uses the same principle but allows the recording of respiratory movements.

The indications of the spirometry:

- To establish the functional disorders in respiratory pathologies;
- To establish the impact of extraspiratory pathologies on lung function;
- To assess the perioperative risk;
- To assess the work capacity and degree of the disability;
- To monitor the therapeutic efficacy.

Contraindications of the spirometry:

- Absolute: acute myocardial infarction and the first month after the infarction ;
- Relative: chest or abdominal pain, pain in the mouth or facial pain exacerbated by the mouthpiece; mental confusion or dementia;

Spirograph recording uses the principle of the spirometer. The spirometer connects to the investigated patient, who breathes through a mouthpiece, and the volumes of inhaled and exhaled air are recorded as a function of time. The technique is performed in the morning. The patient is asked not to eat or drink 6 to 8 hours before the procedure. The patient is not allowed to smoke before the investigation.

The procedure consists in several steps: a) a nasal clip is applied to the patient's nose; b) the patient is connected to the spirometer and allowed to breathe normally for 1 minute; c) the patient is asked to take a maximum breath followed by an exhale as slowly and completely as possible. Normal breathing is required for 15 seconds.

To measure the Forced Expiratory Volume in the first second (FEV1), a maximum inspiration is required, then a 2-second apnea, then a maximum, rapid exhalation. Three similar records are made and the highest value of vital capacity (VC) and FEV1 is assessed. The results are expressed in liters per volume and liters per second or liters per minute for flow rates. Due to the large variability of the ventilatory parameters, the values are expressed as a percentage of the theoretical values, ideal or predicted for the investigated person. The ideal (normal, predicted) value is a theoretical value, calculated based on height, sex, age, and weight.

Bronchomotor tests. In clinical practice, the spirogram is recorded after the administration of drugs in the form of aerosols with broncho-constrictor or bronchodilator action.

According to the figure obtained by the spirograph, it can be calculated the following volumes:

The current volume called the tidal volume (Vt) - is the volume of air that moves in or out of the lungs with each respiratory cycle. The normal value is 500 ml (300-900 ml), decreases in shallow breathing and increases in physical effort, Kussmaul and Cheyne-Stokes breathing, mild respiratory failure.

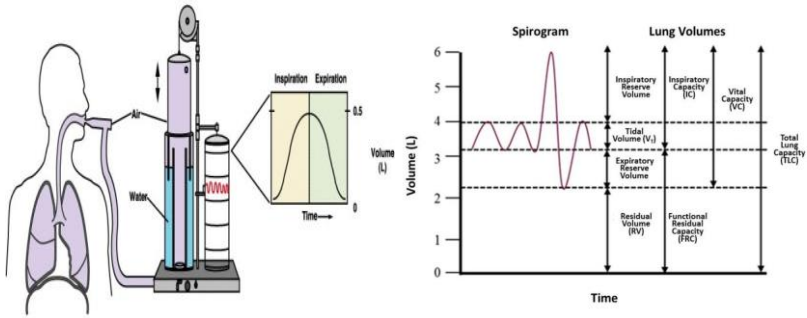

Fig.1 The structure of the spyrometer and registration of the spirography

The expiratory reserve volume (ERV) is the maximum volume of air that can be expired during a forceful breath out. The normal value is 1000-1500 ml, and decreases in pulmonary emphysema, pulmonary stasis, and increases in physical exercise.

Residual volume (RV) is the volume of air that remains in the lungs after fully exhaling and is the difference between the total lung capacity (TLC) and tidal volume. The normal value is 1500 ml - 2000 ml for men and 1000 - 1500 ml for women.

RV = TLC-Vt

Functional residual capacity (FRC) is the volume of air that remainned in the lungs after a normal, passive exhalation. The FRC represents the point of the breathing cycle where the lung tissue elastic recoil and chest wall outward expansion is balanced and equal.

FRC= RV + ERV

The normal value is 3000-3500 ml. FRC decreases in COPD, bronchial obstructions, and lung congestion and increases in pulmonary emphysema, bronchial stenosis, and kyphoscoliosis. FRC can be measured by the helium dilution method and plethysmography.

RV and FRC increase in the following pathologies: pulmonary emphysema (due to decreased elastic recoil), asthma attack (through the mechanism of air-trapping air intake), lung fibrosis (due to decreased elastic recoil), thoracic abnormalities, and decrease in lung stasis.

The vital capacity (VC) is the total volume of air that can be displaced from the lungs by maximal expiratory effort, from the maximum expiratory position to the maximum inspiratory position. The normal value is 3500-5000 ml. It is calculated according to the formula:

VC= Vt + IRV+ERV

Only the highest value of VC, which is obtained after three determinations is taken into account and represents the real value of the patient. Due to the large individual differences, VC is expressed as a percentage of the ideal value. Variations of values ± 20% are allowed for VC and FRC. For RV is allowed + 50%. Estimated volume tidal represents 15% of the VC respectively 500-800 ml, IRV - 60% of the VC which is 1500-2000 ml, and ERV 25% of the VC, which is between 1200 ml and 2500 ml.

The real vital capacity is determined with the spirograph, and the theoretical vital capacity (VCt) is an ideal value, taking into account the particularities of sex, age, height and which can be found in special tables or calculated according to the formulas:

Men: VCt = Height (cm) x 0.052 - age (years) x 0.028 - 3.2
Women: VCt = Height (cm) x 0.049 - age (years) x 0.019 - 3.76

Total lung capacity (TLC) is the maximum volume of air the lungs can accommodate or sum of all volume compartments after maximum inspiration. The normal value is about 6,000mL.

TLC= Vt + IRV+ERV+RV

The normal values of the ventilator flow in the healthy individual are represented in *table 3.2.*

Table 3.2

Normal values of static lung volumes

Indicators	Definition	Values (in L)
Total lung capacity (TLC)	Vt + IRV+ERV+RV	5,97
Vital capacity (CV)	Vt + IRV+ERV	4,78
Volume tidal (Vt)		0,5
Inspiratory reserve volume (IRV)		3,28
Expiratory reserve volume (ERV)		0,98
Residual volume (VR)		1,19
Functional residual capacity (CRF)	RV + ERV	

Restrictive syndrome

External pulmonary ventilation disorders are characterized by the reduced vital capacity (VC) and maintained within normal limits the forced expiratory volume in the first second of expiration (FEV1) and the bronchial permeability index (Tiffeneau Index - TI) define the restrictive syndrome.

Depending on the decrease in vital capacity, the severity of the restrictive syndrome is classified in:

- Mild, which means the reduced VC up to 65 - 80% from the normal VC;
- Moderate – reduced VC up to 50 - 65% from the normal VC;
- Severe – reduced the VC up to 35 - 50% from the normal VC;
- Very severe restrictive syndrome is associated VC reduced less than 35% from the normal VC.

Depending on the type and topographic location of the pathological processes, there are following causes of the restrictive respiratory syndrome:

- Pathological processes of the neuromuscular junction, paresis, paralysis, pathological contractures of the intercostal and diaphragm muscles, *myasthenia gravis*, muscular dystrophies, diaphragm paralysis;

- Diseases that reduce the elastic recoil: pneumonia, idiopathic pulmonary fibrosis, pneumoconiosis, sarcoidosis, diffuse interstitial pneumonia, extrinsic allergic alveolitis.
- Disorders of the thoracic cage (deformities, kyphosis, kyphoscoliosis, calcifications, ankylosing arthritis, pathological rigidity, costal excision) and pleural diseases (pleurisy, massive adhesions, pneumothorax, pleurodesis).

Depending on the location of the pathology, parenchymal or extrapulmonary, certain particularities of the static parameters are described in the *table 3.3.*

Tabel 3.3

Disturbances of static parameters in restrictive ventilatory dysfunctions

Parameters	Parenchymal disease	Extraparenchimal disease
Vital capacity	Decreased	Decreased
Residual volume	Decreased	Normal/increased
Total lung capacity	Decreased	Decreased
Residual volume or total lung capacity	Normal	Decreased

Severe decrease of the total lung capacity, functional residual capacity and of the residual volume occurs in the restrictive syndrome caused by pulmonary parenchymal pathologies such as pneumonia, pneumoconiosis, idiopathic pulmonary fibrosis, extrinsic allergic allergy, diffuse interstitial pneumonia, etc.

In the restrictive syndrome caused by extraparenchymal pathologies, the residual volume is normal or even increased, but there is a decrease of all indicators of inspiration and exhalation, such as vital capacity (VC), total lung capacity (TLC), and the ratio between RV/TLC. The extraparenchymal pathologies that determine this type of restrictive syndrome are the abnormalities of the thoracic cage, pleurisy, and neuromuscular pathologies.

In small airway disease, which results from remodeling, obstruction by mucus and the disappearance of the terminal and transitional bronchioles, the increase of functional residual capacity and residual volume develops on the continuous decrease of the vital capacity, total lung capacity, and of the ratio between RV/TLC. Thus, in the advanced stages, obstructive syndrome is gradually replaced by the restrictive or mixed syndrome.

Evaluation of the dynamic volumes

Lung volumes that depend upon the rate at which air flows out of the lung are defined dynamic lung volumes. To evaluate the dynamic lung volumes is performed the Forced Vital Capacity test and the Maximum Voluntary Ventilation test. The technique using a spirometer consists in instructing the patient to take a maximum breath and then to exhale with all possible force and speed. The recorded graph allows to calculate the following static and dynamic indicators:

Forced vital capacity is the volume of the air that can be exhaled as forcefully and rapidly as possible after a maximal inspiration. Normal values vary depending on gender, age, and height.

The Forced Expiratory Volume in 1 second (FEV_1) is the volume of air exhaled in a second. Normally the percentage of the FVC that can be exhaled during 1 second is around 80% (FEV1/FVC=80%). Decreased FEV_1 is caused by airway obstruction and/or decreased elastic pulmonary recoil.

The real value of FEV_1 is determined using a spirograph, and the theoretical FEV1 is an ideal value for the individual, taking into account the particularities of sex, age, height and which can be found in special tables or calculated according to the formulas:

Men: FEV_1 = Height (cm) x 0.036 - age (years) x 0.031 - 1.41.
Women: FEV_1 = Height (cm) x 0.026 - age (years) x 0.028 - 0.36.

FEV_1 declines physiologically by 50 ml/year of life, but in COPD - 100 ml/year, and in COPD associated with smoking by 150-300 ml/year.

The decline of FEV_1 by more than 20% compared to its theoretical value (predicted) is considered pathological and means bronchial obstruction. The most common ventilatory disorders are the mixed type due to the diminished FEV_1 in association with the specific abnormalities of the restrictive syndrome.

The complex evaluation of bronchoobstructive syndrome, requires the performing of bronchomotor tests, for diagnostic or therapeutic purposes. The test is significant if the FEV_1 increases by more than 12% compared to the value obtained after the first registration. In healthy people, the bronchodilator test increases the FEV1 up to 9-11% reported to the first recording.

For the identification of asthma, bronchoconstrictor tests are used, using parasympathetic agents (acetylcholine, methacholine) or histamine. The test has diagnostic significance if the FEV_1 declines by 15-20% compared to the value of the first registration.

To be more selectively in the degree of ventilatory disorders, the ratio between FEV_1 and vital capacity (CV), also known as the Tiffeneau-Pinelli Index (TI) or bronchial permeability index, is calculated according to the formula:

$$\mathbf{IT = FEV_1 / VC * 100}$$

The Tiffeneau index decreases in bronchial obstruction. The severity of broncho-obstructive syndrome is assessed by comparing with the „normal" value, or "predicted" or estimated characteristic according to the sex, age, and height of the examined individual and is graduated in:

- Mild obstructive syndrome, if IT > 60%;
- Moderate obstructive syndrome, if IT is between 45 and 60%;
- Severe obstructive syndrome, if IT is less than < 45%.

In the initial part of the curve, the FEV_1 value depends more on the muscular effort of the examined person and the muscle development, than on the bronchial resistance. The mild expiratory flow rate between 25-75% of the VC ($FEV_{25-75\%}$) is the average forced expiratory flow rate

over the middle 50 percent of the FVC. It allows the diagnosis of an obstructive ventilatory pattern.

The peak expiratory flow at 50% of forced vital capacity (D Emax50%) is the maximum airflow reached during a complete and forced expiration when 50% of the VC has been removed. The decrease of D Emax50% is determined by the increase of the flow resistance in the peripheral airways, the decrease of the pulmonary elastic recoil, and the decrease of the lung volume.

In clinical practice, the indicator of maximum voluntary ventilation (MVV) is used to assess the maximum level of performance of the respiratory system. This is the largest volume of air that can be breathed in and out of the lungs in one minute. It will be reduced in diseases with an increased in airway resistance or reduced compliance.

Due to the difficulties in obtaining results by direct determination, the maximum indirect ventilation volume (V max ind.) can be determined by the formula:

V max. ind. = FEV1 x 30

Decreased maximum indirect ventilation (Vmax.ind.) more than 20% means reduced the adaptability of the respiratory system to effort. It is estimated that the degree of adaptation to effort can be assessed indirectly based on the decrease in FEV1:

- Mild if FEV1 = 65 - 80% of theoretical FEV1, possible sustained effort of 75 - 100 W.
- Moderate if FEV1 = 50-65% of theoretical FEV1, possible sustained effort of 50 - 75 W.
- Severe if FEV1 = 30 - 50% of theoretical FEV1, possible sustained effort of 25 - 50 W.
- Very severe if FEV1 <30 %% of FEV1 theoretically, no sustained effort is possible.

The normal values of the dynamic ventilation are represented in *table 3.*

Tabel 3.4

Conventional values of dynamic flow rates

Indicators	Normal conventional volumes
Maximum expiratory volume per minute	500 L/minut
Maximum inspiratory rate per minute	300 L/minut
Maximum inspiratory capacity	130 L/minut
Minute-volume of ventilation at rest	4,9 L/minut
Minute-volume of anatomical dead space ventilation	2,1 L/minut

Modification of static and dynamic parameters in obstructive ventilatory dysfunctions

Obstructive ventilatory dysfunction is characterized by the reduction in FEV1 associated with a reduction in the Tiffeneau index while maintaining a vital capacity (VC) within normal limits. The pathologies that evolve with distal bronchial obstruction are chronic obstructive bronchitis, chronic obstructive pulmonary disease, bronchiectasis, stenotic lesions, fibrosis, and bronchial obstructions. Ventilatory disorders are characterized by a vital capacity (VC) and total lung capacity (TLC) within the normal range, residual volume (RV), and the rate between the residual volume to total lung capacity (RV/TLC) increased.

Broncho-obstructive syndrome associated with pulmonary hyperinflation is characterized by decreased FEV1 and Tiffeneau index, a significant increase of the residual volume (RV) and functional residual capacity (FRC), and minor increase of the total lung capacity (TLC). These changes are specific for chronic obstructive pulmonary disease (COPD), pulmonary emphysema, cystic fibrosis, and other lung pathologies that during their evolution develop distal obstruction with hyperinflation (advanced asthma, bronchiolitis, bronchiectasis).

Obstructive ventilatory dysfunction with air trapping is not a clinical diagnosis but a functional feature of broncho-obstructive pathologies such as asthma, COPD, bronchiolitis obliterans and consists of abnormal air retention at the end of the exhalation.

Itt is characterized by a low vital capacity (VC) and normal total lung capacity (TLC), residual volume (RV) and the rate between residual volume and total lung capacity is increased (RV/TLC).

Tabel 3.5

Modification of static and dynamic parameters in obstructive ventilatory dysfunctions

Parameters	Distal obstruction	Obstructive ventilatory dysfunction	
		With hyperinflation	With air trapping
VC	Normal	Normal	Low
FEV1	Low	Low	Low
TI	Low	Low	Low
RV and FRC	Very high	High	Very high
TLC	Normal	Very high	Normal
RV/TLC	Very high	High	Very high

Mixed ventilatory dysfunctions

Mixed ventilatory dysfunctions are characterized by low vital capacity (VC), low maximum expiratory volume per second (FEV1), and a limited or reduced Tiffeneau index. The pathologies that confer restrictive ventilatory dysfunctions are: COPD developed on congestive heart failure, COPD complicated by acute pulmonary edema, COPD complicated by pulmonary artery thromboembolism, pneumonia on COPD background, etc. So, any pathology that causes restrictive ventilatory dysfunction developed against the background of an obstructive ventilatory dysfunction, or *vice versa*, any obstructive ventilatory dysfunction, which develops on a restrictive ventilatory dysfunction, creates mixed ventilatory dysfunction. This ventilatory dysfunction has a much increased fatal potential, due to the appearance of the adjustment of aggravating pathologies on a much reduced functional capacity. For example, pulmonary tuberculosis that develops on a comorbid background of chronic bronchitis or COPD causes mixed ventilatory dysfunction. The general evaluation of the changes of the main spirometric indicators in ventilatory disorders is presented in *table 3.6.*

Table 3.6

Spirometric parameters in different types of respiratory disorders

Parameters	Obstructive disfunction	Distal obstructive disfunction	Restrictive disfunction	Mixt disfunction
VC	Normal	Normal	Low	Low
FEV1	Low	Normal	Low	Low
TI	Low	Low	Normal	Low
Vmax.ind	Low	Normal	Low	Low
DEM 25-75	Low	Low	Normal/ high	Low
TLC	Normal/ high	Normal/ high	Low	Low
RV	Normal/ high	Normal/ high	Low	Low/Normal/ high

Conventional values of the static and dynamic values in the health individual and in pathology is presented in the *table 3.7*

Tabel 3.7

Normal values and the degree of respiratory disturbances

Parameters	Normal	Healthy	Degree of the disrurbances		
			Mild	Medim	Severe
VC from the estimated value (%)	>90	90 - 85	84 - 70	69 - 50	< 50
FEV1 from the estimated value (%)	>85	85-75	74 - 55	54 - 35	< 35
Index Tiffeneau	>65	65-60	59 -50	49 - 40	< 40
TLC % from the estimated value	91-109	110-115	116 -125	126 - 140	>140
		90 - 95	84 - 75	74 - 60	< 60
RV/TLC (%)	< 5	5 - 8	9 - 15	16 – 25	>25
Raw, kPa.	< 2,50	2,51 - 3,00	3,01- 5,00	5,01- 7,00	7,01-10,0
V max, % from the estimated value	>85	95 - 75	74 - 55	54-35	< 35

Note: *VC* – vital capacity, *FEV1* – maximum expiratory volume per second, *TLC* – total lung capacity, *RV* – residual volume, *Raw* – air flow resistance, *V max* – maximum ventilation or maximum respiratory capacity.

Bronchodilation tests

Methods that evaluate the bronchospastic component are called bronchodilation tests. Bronchodilation tests are performed using spirometry or body plethysmography before and more than 15-30 minutes after inhalation of a dose of short-acting bronchodilator (salbutamol 400 μg) or a dose of parasympatholytic. In patients with severe obstruction and in children, the nebulisation method or spacer is used to inhale the bronchodilator into the bronchial shaft.

Indications for the bronchodilation test:

- Determining the bronchospastic component of a respiratory condition;
- Initiation of bronchodilator treatment and monitoring its effecttiveness;
- Identification of the bronchospastic component induced by inflammation.

The test is not repeated if the reversibility of the obstruction has not been determined in previous records.

Contraindications to bronchodilation test:

- Known intolerance to short-acting bronchodilators, such as rhythm disturbances, palpitations;
- Thyrotoxicosis;
- Congestive heart failure;
- Treatment with cardiac glycosides;
- Severe and uncontrolled hypertension;
- Diabetes;
- Glaucoma, prostate adenoma;

The test is considered positive if the FEV1 is increased by more than 12% or by more than 200 ml. In some cases, the response is not optimal due to bronchial inflammation. Under these conditions, short-term corticosteroid treatment (7-10 days) with repetition of the bronchodilation test is indicated.

Bronchial challenge test

Highlights the existence of nonspecific bronchial hyperreactivity. Bronchial hyperreactivity is defined by the ability of the smooth muscle in the bronchial wall to contract to different triggers:

- Acetylcholine;
- Histamine;
- Methacholine;

Physical agents: hypertonic or hypotonic solutions, physical exertion, cold air.

Body plethysmography

Body plethysmography is a complex functional test that allows to assess the lung function. The patient is placed in a sealed glass cabinet with a microphone. The volume of the exhaled airflow is recorded with the highest intensity and duration. At least 2 tests are required to obtain a valid result. Contraindications to the method are haemoptysis, intense physical effort 3 hours before testing, and recent acute myocardial infarction. The patient's height and weight will be measured and then the respiratory manoeuvres will be explained. The investigation assesses changes in the air pressure in the cabin atmosphere caused by respiratory movements. Body plethysmography in combination with spirograph allows to assess the lung volumes, lung compliance, and airway resistance.

Gasometry

Gasometry is the method of exploring the acid-base balance, by assessing the nature of the disorders (metabolic, ventilatory, or mixed) in the arterial, venous or capillary blood. When analysing the acid-base balance indicators, the clinical correlation are highly important for the f complete assessment of acid-base balance disorders *(Table 3.8.).* The pH level and blood gases should be determined in the arterial blood. Immediately after collection, blood samples need to be refrigerated. The determination of pH, blood gases and electrolytes must be assessed from the same blood sample. For the correct assessment of the O_2 partial pressure,

this indicator should be adapted to the haemoglobin and haematocrit values. Venous blood erroneously assesses O_2 partial pressure, however the acid-basessed. The overall analysis of gasometry and serum electrolyte indicators is currently performed by the Astrup method.

Table 3.8

Acid-base balance indicators

Indicators	Arterial blood	Venous blood
pH	7,38-7,42	7,26-7,36
$PaCO_2$ (mm Hg)	36-44	46,5-58,0
HCO_3^- (mmol/ l)	22-26	24-28

The assessment of the acid-base balance must be carried out during the disease evolution in order to assess the body's adaptive capacity and to initiate measures to restore blood homeostasis. Although the theoretical notions of pure disorders are defined as respiratory acidosis, respiratory alkalosis, metabolic acidosis, metabolic alkalosis, most commonly the disorders of acid-base balance are of mixed type. Acidosis is an increase in the concentration of H^+ ions above 44 nmol/L and a decrease of pH below 7.36. The most common cause of acidosis in patients with tuberculosis is the increase of the concentration of lactic acid in the blood due to the mitochondrial toxicity of certain anti-tuberculosis drugs, such as linezolid. It is clinically manifested by nausea, vomiting, abdominal pain, deep asthenia, muscle cramps, and tachypnea. Alkalosis is defined by a decrease in the concentration of H^+ ions below 36 nmol/L and an increase in pH above 7.44. Metabolic alkalosis in tuberculosis is very rare resulting from tachypnea or oxygen therapy.

Summary of the third topic

- Identifying the risk factors for tuberculosis is an essential component of anamnesis.
- The clinical examination is mandatory for all patients to identify the objective data suggestive of tuberculosis, whereas in severe cases the appearance of *habitus phtysicus*, described by Hippocrates in 425 B.C. is

determined as severe body weakness, with blue eyes, leukophlegmatic with elevated scapulae like wings.

- Microscopic examination of the sputum staining by the Ziehl-Neelsen method is a cheap, fast, and reasonably priced method of investigation. It allows the detection of acid-fast bacilli (AFB), if their number exceeds 100.000/ml of sputum.
- Microscopic examination does not confirm the diagnosis of tuberculosis, since the atypical mycobacteria are also AFB. The sensitivity makes up 20-40% and the positive result indicates an extended tuberculous process, severe destruction of the lung parenchyma, and an advanced epidemiological danger.
- The „gold standard" in the diagnosis of tuberculosis is culture testing. Conventional media are solid (Löwenstein-Jensen, Ogawa) and liquid (Middlebrook 7H). Sensitivity is higher compared to microscopy and allows to perform the drug susceptibility testing.
- Molecular-genetic methods for amplifying nucleic acids for the identification of mycobacterial nucleic acids use polymerase chain reaction (PCR) and are used in the diagnosis of tuberculosis, such as Amplifier (Roche Molecular Systems), transcription-mediated amplification (BAT amplification method), method GenoType Mycobacteria Direct Assay (Hain Lifescience), displacement chain amplification method (BD Probe Tec method), and loop-mediated isothermal amplification method (LAMP; Eiken Chemical Co).
- The Xpert MTB/RIF method is a molecular-genetic method that identifies mycobacterial DNA and mutations in the *rpoB* gene, which is responsible for rifampicin resistance.
- The diagnosis of tuberculosis is confirmed by microscopic examination of Ziehl-Neelsen staining, bacteriological examination by culture on solid (Löwenstein-Jensen, Ogawa) and liquid media (Middlebrook 7H), molecular-genetic methods, histological method and clinical-radiological method.

- The components of the clinical diagnosis of tuberculosis include data on the clinical-radiological form of tuberculosis, the topographic location, the disease evolution stage, the result of validated microbiological and molecular-genetic examinations, complications, the case type of based on past anti-tuberculosis treatment and associated diseases.

TESTS AND CLINICAL CASES FOR THE EVALUATION OF THE KNOWLEDGE

In the practical classes, the student is evaluated based on control work, the activity at the patient's bed, the practical knowledge on this subject, and the resolution of clinical situations. At the exam, the student is evaluated based on a test and solving the clinical studies. The tests and clinical situations presented below are useful for the individual preparation of students by verifying knowledge and assimilating information logically and visually. Samples for self-assessment of knowledge are randomly distributed in single complement (SC) and multiple complement (MC) response tests. For the simple complement question, there is only one correct answer, and for the multiple complements, there are several correct answers.

1. The ways of detection of tuberculosis are (MC):
   A. Passive;
   B. Mixed;
   C. Active;
   D. Ascending;
   E. Descendant.

2. The main method for detection of tuberculosis is (CS):
   A. Tuberculin skin test;
   B. Sputum microscopy to identify acid-fast bacilli;
   C. Culture on Lowenstein-Jensen and BACTEC medium;
   D. Fluorescent microscopy:
   E. Chest X-ray.

3. The active way of detection includes the following methods (MC):
   A. Chest X-ray of adults from high-riskgroups;
   B. Chest X-ray of the endagered groups;
   C. Occasional chest X-ray;

D. Tuberculin skin test in children;
E. Tuberculin skin test in children from high-risk groups.

4. Respiratory symptoms suggestive for tuberculosis are (MC):
   A. Cough with recent onset;
   B. Cough lasting 2-3 weeks;
   C. Serous sputum;
   D. Mucopurulent sputum;
   E. Hemoptysis.

5. Duration of the cough to be suspected for tuberculosis is (CS):
   A. Not more than a week
   B. Exceeds one week;
   C. Exceeds two weeks;
   D. Exceeds 3 weeks;
   E. Exceeds 4 weeks.

6. In people with symptoms suspected of tuberculosis, it is mandatory to perform (MC):
   A. Objective examination;
   B. Blood count;
   C. Test for HIV markers;
   D. Microscopic examination of the sputum to identify acid-fast bacilli;
   E. Computed tomography.

7. In people with suspected tuberculosis, the following clinical samples must be collected (MC):
   A. Two sputum samples in the same day;
   B. Three sputum samples;
   C. One sputum sample;
   D. One sputum sample on the day of addressing to the general practitioner and the second early-morning;
   E. Blood sample.

8. Clinical samples will be collected before the initiation of the anti-tuberculous treatment for (MC):
   A. To cultivate *M. tuberculosis* on conventional cultures;
   B. Test the susceptibility to anti-TB drugs;
   C. To assess the quality of expectorations;
   D. To assess the risk of infection;
   E. To test the resistance to Rifampicin.

9. Active way of detection will be performed (MC):
   A. Annualy
   B. 2 times per year;
   C. In high risk groups;
   D. In endangered contingents.
   E. In symptomatic patients.

10. Active detection consists in the annual radiological examination of (MC):
   A. Contacts with tuberculosis patients, identified by the epidemiological investigation of filiation;
   B. People who have had tuberculosis or have post-tuberculosis sequelae
   C. People infected with HIV;
   D. People with compromised immunity or who receive immunosuppressive treatment (cortisone treatment, chemotherapy, radiotherapy, biological antiTNFα therapy);
   E. Patients with diabetes;

11. People with an increased vigilance for tuberculosis are (MC):
   A. Socially vulnerable people: unemployed and persons with low-income;
   B. People with high incomes;
   C. People with medical-biological risk factors;
   D. Staff of closed-type institutions;
   E. Students of the medical institutions.

12. The methods used for active detection are (MC):
    A. Anamnesis;
    B. Clinical examination;
    C. Chest X-ray in adults (digital or conventional);
    D. Tuberculine skin test in children (0-18 years);
    E. Microscopic examination of sputum.

13. The outbreak of tuberculosis includes (MC):
    A. Envirnoment where the source of infection in located;
    B. People which are located around the source of infection;
    C. Source of infection;
    D. Pets;
    E. Domestic animals.

14. The epidemiological danger of the tuberculosis outbreak is assessed by (MC):
    A. Degree of elimination of mycobacteria by the patient;
    B. Patient's living conditions;
    C. Level of general and sanitary culture of the patient and of the outbreak members;
    D. Children, pregnant women in the outbreak;
    E. All listed criteria are correct.

15. The epidemiological investigation in the tuberculosis outbreak is initiated (MC):
    A. Mandatory after detection of the patient with tuberculosis;
    B. No later than 72 hours after the detection of the tuberculosis patient;
    C. At any time after the detection of the patient with tuberculosis;
    D. Optional;
    E. To identify the probable interval when infection and illness occurred with the establishment of the contact list.

16. The measures to be tdone in the outbreak are (MC):
    A. Liquidation of the outbreak by isolating the patient and instituting anti-tuberculosis treatment as early as possible;
    B. Disinfection (absolute or current) in the outbreak of tuberculosis;
    C. Examination of contacts;
    D. Diagnosis and treatment of all cases of tuberculosis detected by epidemiological investigation;
    E. Chemoprevenetion with isoniazid 10 mg/kg body weight daily, for 6 months, to contacts between 0 to 18 years old;

17. Latent tuberculosis infection is (MC):
    A. Condition of the organism in which the mycobacteria are kept in dormant state;
    B. Person is healthy;
    C. Person is symptomatic;
    D. Tuberculin skin test is positive or hyperergic;
    E. AFB is positive.

18. The clinical signs of intoxication syndrome in the patient with pulmonary tuberculosis are (MC):
    A. Asthenia;
    B. Anorexia;
    C. Bulemia;
    D. Hyponderability;
    E. Fever or low-grade fever with profuse sweating.

19. Clinical signs of the bronchopulmonary syndrome in patient with pulmonary tuberculosis are (MC):
    A. Cough more than 2-3 weeks;
    B. Hemoptysis;
    C. Chest pain;
    D. Dyspnea;
    E. Rhinorrhea.

20. Persons who must be examined by the tuberculin skin test are (MC):
    A. Children which were in contact with tuberculosis patients;
    B. Children with suspicious signs for tuberculosis;
    C. Malnourished children;
    D. Patients co-infected with TB/HIV;
    E. Children to be admitted to placement centers.

21. What is the morphological character specific for tuberculous inflammation (MC):
    A. Epithelioid cells;
    B. Giant cells;
    C. Caseum in the center of the granuloma;
    D. Macrophages;
    E. Lymphocytes.

22. Hyperergy of the tuberculin skin test (2 UT) in children is defined by an induration larger than (CS):
    A. 5 mm
    B. 10 mm
    C. 15 mm
    D. 17 mm
    E. 21 mm

23. The negative result of the tuberculin test does not exclude tuberculosis infection in (MC):
    A. Pre-allergic period - up to 12 weeks after infection;
    B. Severe forms of tuberculosis;
    C. Neoplasms, lymphogranulomatosis, and other diseases associated with suppression of the immune response;
    D. In healthy, asymptomatic people;
    E. The negative result always excludes tuberculosis infection.

24. Tuberculin is (CS):
    A. Extract from the mycobacterial culture;
    B. Extract from the atypical mycobacterial culture;
    C. A mixture of non-specific allergens;
    D. A vaccine;
    E. A concentrate of mycobacteria.

25. Booster effect consists of (CS):
    A. Increase the result of the tuberculin test when performing the test at short intervals;
    B. The two tuberculin tests performed in one calendar year, the first offering a negative result and the second a positive result;
    C. Lymphangitis and regional lymphadenopathy;
    D. Occurs after BCG vaccination;
    E. Rarely identified.

26. Acid-fast bacilli can be identified in the following specimens, with exception (CS):
    A. Pleural fluid;
    B. Blood;
    C. Tracheo-bronchial lavage;
    D. Sputum;
    E. Gastric lavage.

27. The most important method for detection of tuberculosis (CS):
    A. Sputum smear microscopy;
    B. Cultivations;
    C. Medical radiography;
    D. Computed tomography;
    E. Chest X-ray.

28. First positive result at the tuberculin skin test is (CS):
    A. Hyperergic tuberculin reaction;
    B. Normoergic tuberculin reaction;

C. Tuberculin conversion;
D. Booster effect;
E. Negative tuberculin reaction.

29. The most effective method for detection of tuberculosis is (CS):
    A. Examination of „symptomatic" patients;
    B. Radiological examination of the entire population;
    C. Radiological examination of contacts from the outbreaks of tuberculosis;
    D. Tuberculin skin testing of the entire pediatric population;
    E. Radiological examination performed annually in high risk-groups.

30. The species *Mycobacterium tuberculosis* that causes tuberculosis in humans is (CS):
    A. *Mycobacterium hominis*
    B. Mycobacterium bovis
    C. *Mycobacterium microtti*
    D. *Mycobacterium* caprae
    E. atypical mycobacteria

31. Identify the objective of the passive way of detection (CS)
    A. Diagnosis of pulmonary tuberculosis;
    B. Detection of the symptomatic patient;
    C. Detection of the suspect in the risk group;
    D. Investigation of the contact persons;
    E. Initiation of antituberculosis treatment.

32. Methods used detect by passive way are the symptomatic patient (MC):
    A. Collection of anamnesis and clinical examination
    B. Microbiological examination of sputum - 2 specimens
    C. Radiological examination of the rib cage
    D. Hemoleukogram and biochemical analysis of the blood
    E. High resolution computed tomography

33. Diagnosis of tuberculosis is confirmed by the specialist on the base of (MC):
    A. Tuberculin skin test result;
    B. Examination of sputum and/or other pathological materials for AFB identification;
    C. Radiological examination of the thorax;
    D. IGRAs test;
    E. Clinical examination.

34. Confirmed case of tuberculosis is defined by the criteria (MC):
    A. The case with suggestive symptoms.
    B. Case positive on at least one of the tests: microscopy, GeneXpert MTB/RIF or culture.
    C. Case with positive result in all tests: microscopy, GeneXpert MTB/RIF or culture.
    D. Case with suggestive radiological abnormalities;
    E. Contact case of an index case.

35. The bacteria which causes tuberculosis is (MC)
    A. Acid-fast bacilli;
    B. Identified at the Ziehl-Neelson staining;
    C. Aerobic;
    D. Sporulated;
    E. Anaerobic.

36. Pulmonary tuberculosis is defined (CS):
    A. The patient with lung lesions and bacteriologically confirmed tuberculosis;
    B. The patient with two or more localisations, at least one of which is pulmonary;
    C. The patient with lesions of the lung parenchyma, tracheobronchial tree and larynx;

D. thoracic lymph nodes;
E. The patient with involved abdominal organs, gastrointestinal tract, skin, and mucous membranes, nervous system.

37. Monoresistant tuberculosis is defined as (CS):
A. Resistant tuberculosis confirmed by the GeneXpert MTB/RIF;
B. Bacteriologically confirmed resistance to isoniazid or rifampicin in combination with resistance to other drugs;
C. Bacteriologically confirmed resistance to a single first-line anti-tuberculosis drug;
D. Bacteriologically confirmed resistance to at least one second-line antituberculosis drug;
E. The result after testing for only one of the first-line drugs.

38. Latent tuberculosis infection is established on the base of (MC):
A. Positive result of the tuberculin skin test;
B. Hyperergic result of tuberculin skin test;
C. Positive result on IGRAs (Quantiferon TB-Gold, T-Spot);
D. Positive result at sputum microscopy;
E. Positive result on cultures (Lowenstein-Jensen and BACTEC).

39. Latent tuberculosis infection is (MC):
A. Negative result on sputum microscopy at AFB and at the GeneXpert MTB/RIF ampicin genetic molecular method;
B. Negative result on cultivation on conventional media (Lowenstein-Jensen and BACTEC);
C. Presence of suggestive clinical signs;
D. Absence of suggestive radiological changes;
E. Positive or hyperergic result on tuberculin skin test;

40. Xpert MTB/RIF detects (MC):
A. DNA of the *Mycobacterium tuberculosis;*
B. Resistance to the first-line anti-tuberculosis drugs;
C. Mutation of the *inhA* gene responsible for isoniazid resistance;

D. Mutation of the *rProb* gene responsible for rifampicin resistance;
E. Resistance to second-line anti-tuberculosis drugs.

41. Advantages of liquid media over solids in the examination of specimens are (MC):
    A. Sensitivity and superior specificity;
    B. Short duration of the period until the final result is obtained;
    C. Inferior sensitivity and specificity;
    D. It is an automated system;
    E. It is the cheapest method of investigation by culture.

42. Advantages of solid to liquid media in examining specimens are (MC):
    A. It is the gold standard in the diagnosis of tuberculosis;
    B. Allows testing for sensitivity to a broader spectrum of anti-tuberculosis drugs;
    C. It is expensive and requires highly qualified staff;
    D. The culture result is obtained in 24 hours;
    E. It is the cheapest method of investigation by culture.

43. Aims of investigating specimens by molecular genetic methods (MC):
    A. Detection of the mutation responsible for resistance to anti-tuberculosis drugs
    B. Microbiological monitoring of antituberculosis treatment
    C. Identification, typing, and genotyping *Mycobacterium tuberculosis*
    D. Detection of the most contagious cases
    E. Evaluation of patients who were in contact with the index case

44. Factors that increase the risk of transmitting tuberculosis infection (MC):
    A. The individual susceptibility of the exposed individual
    B. Contagiousness of the source of infection
    C. Environmental factors
    D. Insufficient ventilation
    E. Poor hygiene conditions

45. The source of *Mycobacterium tuberculosis* infection can be (CS):
    A. Patient with pulmonary tuberculosis;
    B. Patient with extrapulmonary tuberculosis;
    C. Cattle with tuberculosis mastitis;
    D. Mice and rats;
    E. Infected insects;

46. Which risk factors will contribute to the development of pulmonary tuberculosis (CS):
    A. Epidemiological;
    B. Territorial;
    C. Social;
    D. Medical-biological;
    E. Constitutional.

47. What factors will depend on the detection of acid-fast-bacilli in the sputum (MC):
    A. Correct collaboration with the patient;
    B. Informing the patient for the purpose of the investigation;
    C. Specimen contains mucopurulent particles;
    D. Incorrect collection of sputum;
    E. Does not depends how the patient will collect the clinical material.

48. Define positive tuberculin test result with 2 UT (MC):
    A. Presence of induration from 5 mm in nonvaccinated and from 10 mm in vaccinated;
    B. Diameter of the papule is less than 16 mm in children and less than 20 mm in adults;
    C. Positive result cannot differentiate the latent tuberculosis infection from active tuberculosis;
    D. Positive result indicates active tuberculosis;
    E. Presence of lymphangitis and regional lymphadenopathy

49. *In vitro* tests based on interferon-gamma production (IFN-γ) (MC):
    A. Measure the quantity of IFN- γ released by T lymphocytes;
    B. T lymphocytes are stimulated with mycobacterial antigens ESAT6 and CFP 10;
    C. The amount of IFN- γ released is detected by the ELISA method;
    D. Measure the diameter of the skin papule;
    E. It has lower sensitivity than the skin test.

STUDY OF THE CLINICAL CASES

Clinical case № 1

An 18-year-old student was is in continous contact with her father, who is suffering from microscopic positive for AFB tuberculosis. Suddenly, the general state worsened and the fever, chills, headache, night sweats, and dry cough appeared. He was consulted by the family practitioner, who recommended treatment with antibiotics from the group of penicillins and non-steroidal anti-inflammatory drugs. After 5 days of treatment without clinical improvement, the patient was investigated radiologically. The chest X-ray identified multiple microopacities, located bilaterally, over the entire lung area, with the medium intensity and size of 2 mm. The patient was hospitalized with a diagnosis of bronchopneumonia. Clinical examination at admission established: severe general state, pallor, malnutrition, peripheral cyanosis, respiratory rate 24/minute and heart rate 100/minute. On auscultation were distinguished bilateral subcrepitant rales located in the paravertebral area.

Questions:

- Establish the investigation plan of the patient and what investigations can confirm the diagnosis?
- What are the factors that contributed to the development of the disease?
- What detection method was used to identify the patient and which is the recommended?

- What type of the radiological pattern suggestive for tuberculosis can be established in this case?

Clinical case № 2

A 16-year-old boy, a student, lives with his parents, sister, and grandparents in a house in poor conditions. Following an upper respiratory infection, he accused the worsening of the general condition with the appearance of fever, chills, headache, night sweats, dry cough, and continuous chest pain in the right hemithorax. The patient was consulted by the family doctor and treated with antibiotics and anti-inflammatory drugs. After 10 days without clinical improvement, he was radiologically investigated. The pulmonary radiography determined at the level of the right lung, an immense, homogeneous opacity, from the diaphragm to the 4^{th} intercostal space, of medium intensity, with the concave superior border. He was hospitalized, and the clinical examination at hospitalization showed: severe general condition, pallor, malnutrition, peripheral cyanosis, respiratory rate 24/minute, heart rate 80/minute. On auscultation, the decrease of the vesicular murmur in the two thirds of the right lung. Blood count revealed: Hemoglobin 114 mg/L, Leukocytes 9800, Neutrophils 74%, Eosinophils 2%, Lymphocytes 20%, Monocytes 4%, ESR 19 mm/hour.

Questions:

- Establish the investigation plan of the patient and what investigations can confirm the diagnosis?
- What are the factors that contributed to the development of the disease?
- What type of the radiological pattern suggestive for tuberculosis can be established in this case?
- What investigations can confirm the tuberculous etiology of the disease?

Clinical case № 3

The 11-year-old boy was in contact with his uncle, who was suffering from drug-resistant tuberculosis. After an episode of upper respiratory infection, he complained the worsening of his general condition.

Asthenia, fever, chills, night sweats, headache, and dry cough appeared. He was consulted by the family doctor and treated with antibiotics and non-steroidal anti-inflammatory drugs. The clinical examination found a general state which was moderatly modified, pallor, malnutrition, respiratory rate 24/minute, and heart rate 80/minute. At percussion, a dull sound was established in the third upper of the right lung, and at auscultation, subcrepitant at the same level. After 7 days of treatment without clinical improvement, it was radiologically investigated. The pulmonary radiography showed at the S3 an opacity measuring 1.5x3 cm, of medium intensity, heterogeneous with the right hilum enlarged. At the tuberculin skin test (TST) with 2UT was established an induration of 16 mm.

Questions:

- Establish the investigation plan of the patient and what investigations can confirm the diagnosis?
- What are the factors that contributed to the development of the disease?
- Which clinical syndromes can be identified?
- What type of the radiological pattern suggestive for tuberculosis can be established in this case?
- What type of detection has been used and what anti-epidemic measures have to be done in this case?

Clinical case № 4

An 11-year-old child was assessed during the epidemiological investigation of the infectious outbreak. The tuberculin skin test (TST) with 2UT determined an induration of 15 mm. When he was 10 years old the TST resulted with induration of 5 mm. The patient was vaccinated with BCG at birth and revaccinated at 7 years old. The patient complained of asthenia, anorexia, irritability, headache, and night sweats. No pathological changes were established at the clinical examination. The radiological examination was performed. Chest X ray did not identify pathological changes.

Questions:

- Establish the investigation plan of the patient and what radio-imagistic investigation do you recommend to perform additionally?
- Evaluate the results of the tuberculin skin test.
- What are the risk factors for tuberculosis in this case?
- By which way of detection was identified the patient, and what anti-epidemic measures have to be done in this case?

Clinical case № 5

A 10-year-old child, who was in regular contact with a relative suffering from pulmonary tuberculosis treated with multiple interruptions, was hospitalized with the complaints: weight loss, loss of appetite, fatigue, irascibility. The tuberculin skin test with 2UT established a 10 mm papule. Previous tuberculin tests were negative.

The inspection established pale, clean skin, palpable peripheral lymph nodes of hard-elastic consistency. No pathological changes were established during the percussion and auscultation On Chest X-ray was established the enlargement of the pulmonary hilum, predominantly =on the right side.

Questions:

- Establish the investigation plan of the patient.
- What radio-imagistic investigation do you recommend to perform additionally?
- Evaluate the results of the tuberculin skin test.
- What are the factors that contributed to the development of the disease?
- What type of detection was used and what anti-epidemic measures have to be done in this case?

Clinical case № 6

A 42-year-old man, unemployed, smoker, alcohol abuser, complains during 3 months of the worsening of the general condition, with the appearance of the remittent evening fever, night chills and sweats, cough with

mucopurulent sputum, rarely with streaks of fresh blood. The clinical examination was revealed a bad general state, pallor, malnutrition, peripheral cyanosis, respiratory rate 21/minute, heart rate 90/minute. At percussion, a dull sound was established in the superior areas of both lungs, tympanic sound in the lower areas of both lungs; and on auscultation, diffuse subcrepitant rales. On chest Xray were revealed multiple, micronodular and macronodular opacities, of different sizes, medium intensity, with hypertransparencies localized in S2 on both lungs, described as cavities with thin walls.

Questions:

- Establish the investigation plan of the patient and enumerate the methods, which can confirm the diagnosis of pulmonary tuberculosis?
- What radiological lesions are suggestive for tuberculosis in this case?
- What are the high-risk factors for tuberculosis identified at the patient?
- What type of detection was used and what anti-epidemic measures have to be done?

Clinical case № 7

A 36-year-old man, a driver of a public bus, was investigated during the prophylactic medical examination. Digital radiography determined a group of opacities of size 0.5 to 1 cm, with medium intensity, heterogeneous located in the S1, S2 segments of the left lung. The patient is an active smoker and has a cough with mucous sputum, asthenia, and night sweats. The clinical examination found a patient in a satisfactory general condition without pathological changes.

Questions:

- What type of screening was used?
- Establish the investigation plan and enumerate the methods, which can confirm the diagnosis of pulmonary tuberculosis?
- What radiological lesions are suggestive for tuberculosis in this case?

Clinical case № 8

A 30-year-old nurse was in continuous contact with her father, who died from tuberculosis. Gradually, the general condition worsened. Appeared a low-grade fever, headache, night sweats, and dry cough. She was treated with antibiotics and non-steroidal anti-inflammatory drugs. After 5 days of treatment without clinical improvement, was radiologically investigated. At the chest X-ray was determined an infiltrative opacity with an average size of 5 cm, of medium intensity, heterogeneous, with unclear borders localized the segment S6 of the left lung. At the clinical examination, it was established a moderately modified general state, pallor, respiratory rate 20/minute, heart rate 68/minute, absence of pathological changes.

Questions:

- What type of screening was used and which is recommended in this case?
- Establish the investigation plan and what methods confirm the diagnosis of pulmonary tuberculosis?
- What radiological lesions are suggestive of tuberculosis in this case?

Clinical case № 9

A 39-year-old patient, the unqualified worker after exposure to cold, felt ill complaining of remitting fever and chills, headache, night sweats, cough with mucopurulent sputum, continuous chest pain in the left hemithorax. The patient was treated with antibiotics from the penicillin group and non-steroidal anti-inflammatory drugs, without clinical improvement. Gradually the general condition worsened. The patient lost 4 kg in weight. After an episode of coughing, hemoptysis appeared and was transported by ambulance to the emergency hospital. At the clinical examination, the patient had medium modified general state, pallor, fever, chills, profuse sweating, mucopurulent cough, chest pain, respiratory rate 24/minute, and heart rate 80/minute. On percussion, a dull sound was

established in the superior third of the left lung, on the auscultation subcrepitant rales at the same level. Chest radiography determined in the left upper lobe, an opacity in the entire lobe, of medium intensity, heterogeneous, with destruction, with multiple nodules of dissemination in the adjacent region.

Questions:

- What type of screening was used to detect the patient?
- What clinical syndromes were found at the objective examination?
- Establish the investigation plan and what methods would confirm the diagnosis of pulmonary tuberculosis?
- What radiological lesions are suggestive for tuberculosis in this case?

Clinical case № 10

A 32-year-old nurse lives in an apartment with her 3-year-old son and his parents. After the divorce, 1 year ago, she felt mentally depressed and lost 6 kg. During the investigations carried out to complete his child's admission to kindergarten, the radiological examination of the lungs determined an opacity of medium intensity, of 3x3.5 cm, with unclear borders, located in the upper segments of the right lung with hypertransparency sector and adjacent multiple opacities of medium intensity.

Questions:

- What type of detection was used in this case and the epidemiological focus group?
- Establish the investigation plan and what methods confirm the diagnosis of pulmonary tuberculosis?
- What radiological lesions are suggestive of tuberculosis in this case?

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