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THE INFLUENCE OF RISK FACTORS AND THE PANDEMIC COVID-19 ON THE EVOLUTION OF TUBERCULOSIS WITH MULTIPLE DRUG RESISTANCE AGAINST THE BACKGROUND OF IMMUNOSUPPRESSION AND DIABETES

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Монографія присвячена висвітленню еволюції перебігу множинно-лікарсько стійкого туберкульозу легень у пацієнтів з супутніми захворюваннями, впливу пандемії COVID-19 на епідеміологію туберкульозу у прикордонних регіонах Молдови і Чернівецької області.

Монографія розрахована на широке коло читачів і спрямована на вирішення актуального завдання фтизіатрії – досягнення поставлених цілей Глобальої стратегії «Покласти край ТБ» на період 2016-2035 рр. – звільнення світу від туберкульозу.

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CONTENT

Abbreviations

AIDS	acquired immune deficiency syndrome
ART	antiretroviral therapy
BCG	bacille Calmette-Guerin
BPaLM	bedaquiline, pretomanid, linezolid and moxifloxacin
COVID-19	coronavirus disease 2019
DST	drug susceptibility testing
HBC	high burden country
HIV	human immunodeficiency virus
IGRA	interferon-gamma release assay
LMICs	low- and middle-income countries
MDR-TB	multidrug-resistant TB
NTP	national TB programme
PPPR	pandemic preparedness, prevention and response
RR-TB	rifampicin-resistant TB
SHA	System of Health Accounts
TB	tuberculosis

UHC	universal health coverage
UN	United Nations
VR	vital registration
USAID	United States Agency for International Development
WHO	World Health Organization
WRD WHO	recommended rapid diagnostic test
XDR-TB	extensively drug-resistant TB

Introduction

Despite the achievements in the field of overcoming tuberculosis (TB), this disease still remains a challenge for the medical industry of Ukraine.

Currently, Ukraine is still on the list of 30 countries with a high burden of multidrug-resistant TB and ranks fourth in terms of the incidence of multidrug-resistant tuberculosis in the WHO European Region.

Since the announcement of the TB epidemic in Ukraine, considerable success has been achieved in the fight against this disease, however, the events of recent years in our country have become real challenges that have slowed down the progress of the achieved results.

In particular, in 2020, the COVID-19 pandemic caused significant damage to the work of the phthisiatric service, limiting patients' access to specialized medical care. The year 2021 marked the beginning of a slow phase of recovery of phthisiatric care after the sustained effects of COVID-19 in both national and regional contexts. However, soon after that, Ukraine faced an even bigger problem – military operations in many regions.

The war in Ukraine led not only to internal migration, but also to external migration - to neighboring countries, which causes concern in the health sector of individual countries about the spread of mycobacterial strains from Ukraine to countries with a low incidence of TB. Although there is currently no significant increase in the incidence of TB among the population of host countries, it is expected that the effects of migration may become evident in the coming years.

It is worrying that countries with a low incidence of TB have a limited number of the latest antimycobacterial drugs for resistant strains of the TB pathogen that are common in Ukraine, which, therefore, may worsen the situation with the treatment of patients with resistant forms of TB who arrived from Ukraine. Accordingly, there is a risk of interruption of treatment, expansion of the resistance profile, recovery of the patient's contagiousness. The return of such persons to the territory of Ukraine in the long term may also negatively affect the epidemiological situation.

The combination of the consequences of the COVID-19 pandemic and the movement of migrants and refugees, the

destruction of medical infrastructure, the complication of access to medical care and medicines can significantly complicate the achievement of the goals of the Strategy to overcome tuberculosis, recommended by the WHO.

About 50% of TB patients face catastrophic (more than 20%) costs (direct medical costs and indirect costs such as lost income), while the goal of the "End TB" strategy is to reduce these costs to 0.

About a quarter of the world's population is infected with TB. Approximately 5-10% of them eventually develop active TB.

People living with HIV are 14-18 times more likely to develop active TB. Other risk factors for the development of TB play an important role. In 2022, 2.2 million new TB cases related to malnutrition, 0.89 million to HIV infection, 0.73 million to alcohol abuse, 0.70 million to smoking and 0 .37 million have diabetes.

MDR-TB is a particularly challenging issue, as only 2 out of 5 MDR-TB patients will have access to treatment in 2022. Without proper treatment, about 60% of HIV-negative TB patients and almost all HIV-positive patients with active TB die.

Annually, 13 billion US dollars are spent on the prevention, diagnosis and treatment of TB. Efforts are aimed at achieving the global goal to end TB, approved at the UN TB meeting in 2018. Ending the TB epidemic by 2030 is one of the health objectives of the UN Sustainable Development Goals.

The monograph is devoted to highlighting the evolution of the course of multidrug-resistant pulmonary tuberculosis in

patients with concomitant diseases, the impact of the COVID-19 pandemic on the epidemiology of tuberculosis in the border regions of Moldova and Chernivtsi region.

CHAPTER I

Mycobacteria tuberculosis resistance - stages of drug resistance formation

Tuberculosis continues to be one of the most urgent medical and social problems in the world in the 21st century, including in Ukraine. To this day, there is not a single country in the world where the problem of overcoming tuberculosis (TB) has been solved. The situation with tuberculosis in Ukraine remains difficult and, even, prognostically unfavorable due to the increase in the total number of cases. Thus, the incidence was: 2023 - 48.4 per 100,000 population; 2022 – 45.7%; the increase was +7.3% against the background of an increase in resistant forms of TB both among adults and children [4, 17, 35].

The most sensitive indicator of the state of the epidemiological situation is the incidence rate of TB in children [8]. At the same time, the test of growth of morbidity in children is higher than in adults [4]. The TB rate among children aged 0-14 was 10.4 per 100,000 children (639 cases, which is 3.2% of the total number of registered TB cases in 2023), which is 40.5% more than in 2022 (7.4 per 100,000 children). The increase in the incidence of TB among children aged 0-14 years in 2023 is closely related to the worsening of the epidemic situation among adults. The TB incidence rate among adolescents (15-17 years) increased by 55.3% - from 10.3 to 16.0 per 100,000 people of the corresponding age group (196 cases in 2023 versus 127 in 2022) [12, 46].

The incidence of active TB in combination with the disease caused by the human immunodeficiency virus has increased by 5.1% compared to 2022 and is 8.2 per 100,000 people. (3,350 cases of TB/HIV in 2023 versus 3,191 in 2022) [48].

Ukraine is among the thirty countries with a high burden of multidrug-resistant tuberculosis (MDR-TB). The main threat to the effectiveness of tuberculosis treatment in Ukraine is late detection of the disease and determining the sensitivity of the pathogen, which is the main prerequisite for prescribing adequate treatment. Generalized forms of tuberculosis and cases of MDR-TB are the most difficult in this regard [23, 36].

In the conditions of an epidemic of drug-resistance, only the detection of tuberculosis disease, even with laboratory

confirmation of the diagnosis, is not enough to start treatment. Effective treatment is possible only if the complete resistance profile of the pathogen isolated from the patient is identified [19, 25]. Therefore, the examination for the purpose of prescribing adequate treatment takes time, which depends on the regional features of the organization of anti-tuberculosis care for the population on the one hand, and on the other hand, on how quickly the patient will go through all the stages of diagnosis.

Considering all the above, **the purpose** of review was to assess the situation regarding the evolution of resistance of M. tuberculosis and the stages of the formation of drug resistance based on the materials of available databases.

Materials and methods. The research was carried out in the period from December 2014 to January 2024. A search was performed using key words: pulmonary tuberculosis, resistance, mechanism of formation of multiple drug resistance, immunopathogenesis of tuberculosis, etiotropic treatment. Digital access to the following full-text and abstract databases was used as the main source of research: EBSCO single information base package ; the world's largest single abstract database and scientometric platform *Scopus*; freely available *Google search engine Scholar; MEDLINE with Full text; MEDLINE complete; Dyna Med plus; EBSCO eBooks Clinical Collection ;* abstract scientometric database of scientific publications of the *Web project of Knowledge of the* Thomson company Reuters - Web of Science Core Collection WoS (CC); statistical data of the Ministry

of Health of Ukraine and the Center for Public Health; SCIE (Science Citation Index Expanded); SSCI (Social Science Citation Index); online database of the National Scientific Medical Library of Ukraine; AHCI (Artand Humanities Citation Index).

The next stage, with the use of system topological and metric computer analysis of the obtained data, made it possible to highlight the most informative rubrics for the selected research topic, which are clearly associated with the formation of drug-resistance in pulmonary tuberculosis. As a result, the most complete database of available literary sources was obtained (about 50 out of 502 analyzed arrays).

Results and discussion. In addition to the usual varieties of tuberculosis infection (M. tuberculosis), mutant forms of M. tuberculosis (MTB), which are resistant to the action of many basic antimycobacterial drugs (AMDs), are rapidly spreading in the world: multidrug-resistant tuberculosis (MDR-TB) and tuberculosis with extended resistance (XDR-TB). According to WHO estimates, about 500 thousand inhabitants of the planet are infected with MDR-TB, in which standard therapy is ineffective, and XDR-TB, as experts [15, 39] note, is resistant to almost all drugs known today and has the highest mortality rate among people of working age - 85%. The average life expectancy of ineffectively treated patients is 2.9 years. The probability of successful treatment decreases with the emergence of new resistant strains of MTB with total resistance [42]. According to WHO data, in Ukraine, 16% of patients diagnosed with

tuberculosis for the first time (from 5% in western regions to 16% in eastern regions) have MDR-TB, and 44% of patients with relapse of the disease [18].

State of multidrug-resistant tuberculosis (MDR-TB) in Ukraine during war against the background of the COVID-19 pandemic remains difficult. So, the total number of such cases in 2023 was 1,955 patients, of which 1,326 were new cases. The number of cases of tuberculosis with extended resistance XDR-TB was 228, of which 136 patients were diagnosed for the first time [13]. One of the main problems of tuberculosis control in Ukraine is low treatment effectiveness. The effectiveness of treatment of all cases of tuberculosis in the 2017 cohort was 76% against the world average of 85% [3, 21]. Regularly severe and multidrug-resistant forms of the disease make the biggest contribution to the low national average. Thus, the effectiveness of the treatment of multidrug-resistant tuberculosis (MDR-TB) in Ukraine remains at one of the lowest levels in the world: as of 2019, only in India, Indonesia, Mozambique and Ukraine, the effectiveness of treatment was less than 50% [30].

The set of MTB strains circulating in the population is characterized by significant variability with the presence of high- and low-virulence strains grouped into different families based on genetic features. Modern MTB strains are characterized by the absence of the possibility of horizontal gene transfer, but today there are studies that have shown the presence of rare gene recombinations [32]. Evolution of M. tuberculosis complex is

carried out, in most cases, by deletions and duplications, which causes clonal pattern of evolution of the causative agent and in combination with the absence of recombination can be the cause of the pathogenetic features of the course of individual strains. Genetically different strains of MTB stimulate different immune responses (due to the predominance of concentrations of certain cytokines), which determine not only the difference in pathogenesis, but also in the clinical manifestations of the disease. In general, as recognized by most researchers, the pathogenicity of MTB depends on their ability to survive in macrophages, which captured them and induced a delayed-type hypersensitivity immune response [6, 34].

In Ukraine, the main role in the etiology and epidemiology of TB is played by M. tuberculosis (over 90% of cases), much less often by M. bovis (3-5%). These two species are the main causative agents of diseases.

The results of the first national epidemiological study in Ukraine on the drug resistance of the TB pathogen (DRS) in 2013-2014 revealed the following **genotypes of TB strains :**

- Beijing (50.2%);
- Euro-American Superlineage 16.8%;
- LAM (14.9%);
- Ural (9.6%);
- Haarlem (8.11%);
- S-type (0.3%);
- nd (0.2%).

In most regions of the world, Mycobacterium tuberculosis of the *Beijing* family is currently gaining significant distribution . The main negative characteristic of the *Beijing* family is their ability to form drug-resistance quickly, compared to other families [2]. In a number of studies, it has been shown that MTB of the *W- Beijing family* have copper-resistant strains, and the growth rate in culture is even higher than that of susceptible ones [16]. In general, *Beijing* strains have 41 specific single nucleotide polymorphisms (SNPs), including those in genes involved in the processes of replication, repair, and recombination and having a potential - impact on the evolution and adaptation of representatives of this genetic line [24].

M. africanum causes tuberculous lesions in the inhabitants of Africa . When infected with the bovine type of MBT, *extrapulmonary forms of tuberculosis* develop: lymph nodes, bones and joints, genitourinary system, meninges. MTB of human and bovine types can cause TB not only in humans, but also in cattle, goats, pigs, and less often in horses, dogs, and cats. Almost all vertebrates are affected by TB. Along with the typical pathogenic types of MTB *(M. tuberculosis, M. bovis),* conditionally pathogenic *atypical mycobacteria* were isolated and studied. Under certain conditions, especially when immunity is reduced, diseases similar to TB can develop in humans, which are united by the concept of mycobacteriosis. They differ from the causative agents of TB in the appearance of colonies, growth rate and drug susceptibility to anti-tuberculosis drugs [28].

An important place in epidemiological studies is the study of a person's susceptibility to TB infection. A person has a strong natural resistance to TB. Resistance is not the same throughout life, and the incidence of tuberculosis is affected by gender, age, concomitant diseases, living conditions, etc. It has been shown that there is a genetically determined resistance to TB. The connection of species resistance with immune response genes and the main HLA histocompatibility complex has been proven. A person may be prone to TB if HLA antigens such as DR_2 , B_7 , B_{14} are present on peripheral blood leukocytes [22].

In the study of the evolution of the pathomorphosis of TB of the lungs and, in particular, the formation of drug-resistance, one of the tasks is to study the polymorphism of known candidate genes, as well as the search for new genes which protein products are involved in the pathogenetic mechanisms of the development of the disease [33].

To track the spread of tuberculosis infection in economically developed countries, the method of genotyping the TB pathogen has been introduced. This was facilitated by the discovery of polymorphic repeating regions in the nucleotide chain in M. tuberculosis DNA. This made it possible to study "molecular fingerprints" - the genotype of the TB pathogen [37]. In connection with the above, active research and study, today, requires the question of molecular genetic aspects of the formation of resistance of MTB.

It was shown that the features of the immune reaction in resistant TB are high but rapid expression of TNF-α and inducible isoforms enzyme synthetases nitric oxide (iNOS), which indicates the effective activation of macrophages at the early stage of MTB infection. In turn, interferon-γ (IFN-γ) in macrophages, activated and natural T- killers induce genes which protein products are able to destroy MTB. However, in most cases with MDR-TB, IFN-γ is produced late and weakly, which indicates the benefit of rapid inactivation of macrophages that stimulate the Th1-subtype of lymphocytes. Thus, the activation of Th1 lymphocytes is not effective enough to stop the reproduction of mycobacteria [40].

The expression of almost 527 genes (15% of the total number examined) was detected in different strains of M. tuberculosis. The insertion sequence IS6110, belonging to IS3 transposons, is a sequence widely used as a genetic marker as it is specific for M. tuberculosis strains [7, 14]. Laboratory studies have shown that the emergence of resistance in M. tuberculosis is associated with nucleotide changes (mutations) in genes that encode various enzymes that directly interact with drugs. For example, mutations of the *rpo* gene, which encodes the β-subunit of RNA polymerase in 96% of cases, lead to the formation of M. tuberculosis resistance to rifampicin. Mutations in the *kat* gene lead to the substitution of individual amino acids in the enzymes catalase and peroxidase, which are responsible for the formation of antioxidant protection during the development of inflammatory oxidative stress. Nucleotide changes in the regulatory and

adjacent coding regions of the locus *inh* are associated with resistance of individual MTB strains to isoniazid. Resistance of M. tuberculosis to streptomycin (in 86% of our TB patients) is associated with a mutation in the *rps* gene, which encodes S12 mitochondrial protein, or with nucleotide changes in the *rrs* gene, which encodes 16S RNA [27, 44].

The antigenic (AG) composition of altered forms of MTB is simplified with the loss of at least 33.3-37.5% of AG associated, in most cases, with the cell wall. Some researchers have shown that modified MTB induce the synthesis of antibodies more weak. Probably, these features make it possible to avoid the control of the immune system and create prerequisites for the persistence of MTB in the body. The transformation of MTB into acid-resistant forms is accompanied by a decrease in the concentration of AG in the cell, a simplification of the antigenic composition with the preservation of no more than 62.6-66.7% of AG, including those specific for the M. bovis - M. tuberculosis complex [45].

According to a number of researchers [9, 11, 26], TB, like other infections, is characterized by a cyclic course, which is associated with a certain frequency of reproduction, the degree of virulence and changes in the immunity of the population. In the period of minimum solar activity, morbidity and mortality from TB decreases, which is associated with the influence of solar activity on both humans and MBT (cosmoheliophysical factors, in particular, the 11-year cycle of TB infection activity) [11].

To date, it has been proven that the development of the TB process depends on a number of medico-biological and social factors [43]. The emergence of resistance to antituberculosis drugs is a natural phenomenon, a basic biological law, an expression of the adaptation of species to the environment.

The analysis of literary sources allows us to assert [10, 31, 38] that there is a whole galaxy of theories regarding the formation and essence of drug resistance of MBT.

The theory of adaptation suggests changes in the properties of a microorganism that are adequate to changes in the environment. According to this theory, the development of drug resistance of MTB is considered to be a manifestation of one of the forms of bacterial cell variability under the influence of chemical drugs [9]. That is, the emergence of MTB resistance to anti-TB drugs is caused by the treatment itself, since the population ratio of susceptible and resistant forms of MTB is 90% susceptible and 10% resistant, but in the course of treatment, in the case of choosing the wrong chemotherapy regimen, a significant number of susceptible MTB die, as a result of which the ratio is violated in the microbial population, and the number of resistant MTB exceeds that of sensitive ones.

According to the theory of spontaneous mutations [29], there are resistant mutants in the MTB population. At the same time, anti-TB drugs can play the role of a factor in the further selection of resistant species, or, according to some researchers, mutants. However, the high frequency of spontaneous mutagenesis cannot

always be explained by the speed of the spread of mutations, which contributes to the development of resistance of pathogens to anti-TB drugs.

Numerical studies indicate the possibility of *genetic translocation of mutant genes* from one cell to another and even intergeneric exchange of genetic information. This way of spreading genetic information is described for bacteria, but for *M. tuberculosis* and some substrains of *E. coli,* only indirect signs of intergeneric transmission of genes encoding drug resistance have been identified [41].

Some foreign researchers claim that the reason for the emergence and spread of drug-resistant strains *is the natural biochemical and genetic mechanisms* of bacterial cell life, discuss ways of spreading genetic information that leads to the development of MTB resistance [47]. Of the 3.8-4.2 thousand MTB genes, more than half ensure the synthesis of the cell wall and, under adverse conditions, change its structure and transfer metabolic processes to redundant pathways. This, in most cases, explains the existence of morphologically changed forms of MTB, which are considered as regular stages of the life cycle.

The results of the analysis of a number of studies [8, 13, 38] suggest that *the transformation of the shape and structure of the cell wall* is accompanied by changes in the antigenic composition, which were observed during the immunoluminescent indication of L-forms of MTB. With the help of tuberculin, made from L-forms of *M. bovis*, significantly more animals with latent tuberculosis

infection were found, than in the case of using the drug from the "bacillary" strain. Probably, the persistence of changed (transformed) forms of MTB induces an immune response that differs from the response to the antigen complex of the Koch bacillus, although significance of difference in their antigenic composition is actually unknown.

One of the important types of MTB variability is the formation of L-forms [37]. L-forms are characterized by a reduced level of metabolism, weakened virulence. Remaining viable, they can stay in the body for a long time and induce anti-tuberculosis immunity. L-forms differ in pronounced functional and morphological changes. It was found that the transformation of MTB into L-forms is enhanced with long-term use of antimycobacterial therapy and other factors that disrupt their growth and reproduction, cell membrane formation [9, 24]. It has been found that in the sputum of "non-bacillar" patients with destructive forms of TB, L-forms of MTB can be found, which can reverse (modify) into a rod-shaped variant, thereby causing the reactivation of the tuberculosis process. Therefore, abacillation of the caverns of such patients does not mean their sterilization in terms of MTB.

New discoveries in the genetics of TB are due to the variety of properties of this microorganism, which is determined by its chromosome [3, 31]. Genome *of M. tuberculosis complex* is very conservative. Its representatives have DNA homologies in the range of 85-100%, while the DNA of other representatives of this genus is homologous to *M. tuberculosis* only in the range of 5-

29%. The genome *of M. tuberculosis* is smaller than that of other mycobacteria. In the classical pathogen of human TB, *M. tuberculosis ,* more genes than *M. africanum* and *M. bovis,* which lost part of the genetic material during evolution [25].

In 1998, the nucleotide sequence of the chromosome of the H37Rv *M. tuberculosis* strain was published*,* which is a museum "classic" strain. Chromosomes are toroidal structures - more than 4,000 genes encoding proteins, plus 60 encoding functional components of RNA: a unique ribosomal RNA operon, 10Sα RNA, which participates in the degradation of proteins with atypical matrix RNA, 45 transport RNAs (tRNAs), about 100 lipoproteins [13].

A feature of the genome *of M. tuberculosis complex* is a large number of repeated DNA sequences. *M tuberculosis* H37Rv chromosome has up to 56 copies of IS-elements, which provide DNA polymorphism of MTB (this feature is used in PCR diagnostics). Most of them, with the exception of the IS6110 element, are unchanged. As a rule, 5 to 20 copies of IS6110 are present in the chromosomes of different MTB strains, but there are strains that do not have this element. Differences in the number of copies and localization on the chromosome of these genetic elements are used to differentiate MTB strains in molecular epidemiology.

The most advanced genotyping schemes of mycobacteria are based on the detection of genomic polymorphism caused by the IS6110 element. The divergence of the *M. tuberculosis*

species usually occurs due to recombinations between copies of the IS6110 element, which flank different genes [5]. The use of genotyping in clinical and epidemiological studies is decisive in those cases when it is necessary to distinguish between primary and secondary (acquired) drug resistance.

If the genotype samples of MTB before and during treatment coincide, this indicates the formation of resistance in the course of treatment. The reasons for this can be different:

- biological - insufficient concentration of the drug, individual characteristics of the body (the rate of inactivation of the drug is individual); concomitant diseases that prevent the formation of an adequate concentration of the drug in the blood and in the focus of the tuberculosis lesion;
- behavior and psychological characteristics of the patient (contact with a patient with MDR-TB, irregular medication intake, premature discontinuation of medication, interruptions in treatment, poor drug tolerance);
- disease-related - in the case of a change in the doses of drugs with a large amount of MTB, a change in pH may occur in the areas of the affected tissue, which prevents the active action of the drugs; monotherapy; insufficient dose or duration of treatment; use of drugs with cross-resistance; incorrect prescription of the treatment regimen, inconsistency in drug doses;
- organizational mistakes and inadequate funding of the anti-tuberculosis program and other interested agencies; lack of the

necessary range and quantity of medicines (inferior chemotherapy regime), improper storage of medicines.

If the "molecular fingerprints" are different, then this indicates repeated infection (reinfection) with another strain, which requires correction of antituberculosis treatment.

The use of genotyping can help in clinical and epidemiological studies, when it is necessary to solve the question of the genesis of relapse – either it is the result of the activation of a mycobacterium that was already in the human body, or it is the result of infection with a new strain [9, 32]. Also, this method can detect laboratory cross-contamination.

In fact, from the very beginning of the use of antibiotic therapy, the phenomenon of drug resistance arose. The peculiarity of this phenomenon is that MTB does not have plasmids, and the population resistance of microorganisms to antimicrobial drugs was traditionally described by the presence of R-plasmids in the microbial cell (from the English *resistance*). However, despite this fact, the appearance or disappearance of drug resistance was noted in one strain of MTB. As a result, it turned out that activation or deactivation of genes responsible for resistance are IS-sequences [32].

According to molecular geneticist Ervin Schurr, every third inhabitant of the planet is MTB infected, but only 5-10% of this huge number of carriers can actually get TB. Others somehow manage to keep the disease in a "dormant" state. The scientists focused their attention on the NRAMP1 gene, which is already

known to be related to many diseases. It has been found that variants (alleles) of the NRAMP1 gene control the rate of development of TB, as well as whether the disease develops at all. Erwin Schurr points out that he is the first to encounter the fact that a gene can control the time that passes from the moment of infection to the onset of the disease (the work is published in the latest issue of the Proceedings of the National Academy of Science).

More recently, American scientists from the University of Texas found the exact reason of that MTB infection does not always lead to the development of TB. Previously, it was believed that a hereditary predisposition was to blame for this. Studying the genotype of two groups of patients from Mexico and Korea, American scientists found that the presence of a mutation in the gene located on the 17th chromosome increases the susceptibility to TB by five times. If only a single nucleotide in the gene changes, the production of *MCP-1 protein immediately increases (*it attracts cells of the immune system to areas of inflammation). Mutant human monocytic chemoattractant protein (MCP-1) has a higher affinity for binding to glycosaminoglycans (GAGs) and reduces activity towards transmembrane G protein-coupled receptors (GPCRs) compared to wild-type MCP-1 protein. It is characterized by the fact that the MCP-1 protein is modified, while preserving the structure, by inserting at least one basic and/or electron-donating amino acid residue or replacing at least two amino acid residues with two basic and/or electron-

donating amino acid residues, and the indicated protein includes an amino acid sequence according to the following general formula :

$(M)_nQ(PDAINA(Z1))_mVTCC(X1)NFTN(Z2)(Z3)I(X2)V(X3)RLASYR$
RITSSKCP
KEAVIFKTI(X4) AKEICADPKQ KWVQDSMDHL DKQTQTPKT.

MCP1 (monocytic chemotaxis protein) is a very important link of the primary immune response when M. tuberculosis enters the body, but if it is produced in excessive amounts, the production of another factor of immunity, interleukin-12 (IL-12), decreases. And it is needed to activate immune cells that have arrived at the infected area to fight bacteria. To date, this is the biggest discovery in the genetics of TB [27]. Scientists hope that it will play its role in the fight against this dangerous disease, the prevalence of which has recently become alarming.

An international group of scientists from the USA and South Africa announced the successful completion of work on deciphering the genome of the TB pathogen [34]. The researchers obtained information about the genetic structure of both drug-susceptible and multi-drug resistant MTB as well as the causative agent of the most dangerous form of the disease – XDR-TB. Scientists from the Broad Institute, Harvard School of Public Health in USA and the Nelson Mandela School of Medicine (South Africa) studied the genome of the XDR-TB strain that led to the loss of more than 50 human lives during a recent outbreak in the South African province of KwaZulu-Natal. When

deciphering four million nucleotide pairs of the MTB genome, a special DNA sequestration technology was used, which allows simultaneous "reading" of hundreds of millions of DNA nucleotides. Scientists found out that drug-resistant and drug-susceptible bacteria differ slightly from the point of view of genetics, they managed to detect only a few dozen small changes in DNA. Some of these differences involved genes which role in the development of drug resistance was known; other changes were detected in new, little-studied genes [34].

MBT by their nature are resistant to many antibiotics. The main reason for resistance is encoded in the structure of the tuberculosis bacillus genome. This property is primarily related to the fact that the highly hydrophobic cell surface serves as a kind of physical barrier for therapeutic agents and antibiotics. It is also reported that cultures of forms of MTB do not always contain specific for *M. tuberculosis complex* insertion element IS6110 and some others, which leads to the absence of synthesis of the corresponding proteins [19].

Laboratory studies have shown that the emergence of resistance in MTB is associated with nucleotide substitutions (mutations) in genes encoding various enzymes that directly interact with drugs.

Moreover, modified MTB are relatively weaker in inducing antibody synthesis. Probably, these features make it possible to avoid the control of the immune system and contribute to their persistence in the body. The transformation of MTB into acid-

resistant forms is accompanied by a decrease in the concentration of AG in the cell, a simplification of the antigenic composition with the preservation of no more than 62.6-66.7% of AG, including those specific for the *M. bovis - M. tuberculosis complex* [8].

The basis for a significant increase in the number of cases of primary drug resistance of MTB may be the widespread use of a few antibiotics that can be used in phthisiology for the treatment of diseases of non-tuberculous etiology. In this regard, recommendations to prescribe one of the antituberculosis drugs - rifampicin as a first-line drug during the treatment of so-called problematic infections caused by gram-positive organisms are of particular concern. The issue of rational use of second-line drugs - respiratory fluoroquinolones [14, 15, 24] remains relevant.

According to a number of researchers [23, 36], due to a variety of factors (demographic, socio-economic, insufficient attention to the problem of combating TB in many countries, the epidemic of HIV infection), the number of patients with abacterial forms of pulmonary TB will increase significantly, and, many of these patients will go undiagnosed and untreated. Even during TB therapy, the irrational choice of drugs and weak control over the use of drugs by patients will lead to an increase in the number of people who excrete resistant MBT.

In our opinion, which coincides with the opinion of a number of researchers [8, 38], the main mechanisms of the development of drug resistance are an inadequate or wrongly chosen treatment

scheme, which leads to the dominance of the drug-resistant strain (selection of significantly resistant strains takes place). Patients who have developed an allergy to one drug are more prone to acquiring resistance to other drugs (drug amplification effect) [13]. According to some authors, the probability of transmission of resistant strains is similar to the degree of transmission of susceptible strains. So, the main cause of the drug-resistance phenomenon is the human factor. All of the above is confirmed by the results of a pilot scientific study provided by the National Institute of Phthisiology and Pulmonology named after F.G. Yanovsky and the University of Illinois (USA), which showed that only 12.8% of tuberculosis patients received treatment according to the standards determined by the orders of the Ministry of Health; 71.1% of patients were prescribed the wrong treatment regimen; 31.6% of patients underwent treatment independently [1, 28].

As part of the implementation of the national TB prevention program, the purchase of equipment for molecular genetic diagnostics is foreseen. The latest molecular platform Xpert MTB/RIF, which has been tested in low- and middle-income countries, is recognized as a first-line test for persons with suspected MDR-TB or HIV-associated TB and as a follow-up test for negative sputum smears from other patients [22]. The implementation of Xpert MTB/RIF on the territory of our country does not require long-term training of medical personnel, modern

laboratories, or the latest methods of biological protection and is extremely promising.

The main disadvantages of traditional direct smear microscopy (low sensitivity and specificity), as well as cultural research (long duration of obtaining the result) have been overcome in a new method [10].

There is promise in separate genetic studies that attempt to use host gene expression in the blood cells of TB patients to identify a disease-specific gene that could later be used to create a diagnostic test and possibly differentiate the stages of the disease. A set of 4 genes has been identified that may help to distinguish between patients with active TB, latent infection and those who have previously received antimycobacterial therapy, as well as a set of three different genes that can be used to distinguish patients with active TB from infected and healthy [8]. An alternative method is the study of gene expression in cells stimulated for the first time by specific MTB antigens. By this method, it is possible to distinguish persons with latent tuberculosis infection from patients with active TB by determining the expression of only 3 genes.

Some researchers found that the relationship between the expression of IL-4 levels and its splicing variant IL-4d2 correlates with the phase of the disease, and changes in the mentioned indicator can be a sign of changes in the microbial load [2, 34].

Since the system of metabolism of xenobiotics is involved both in the protection of the body against the consequences of

the development of inflammatory reactions in TB, and in the metabolism of most anti-TB drugs, it is extremely interesting to study the activity of enzymes that are part of this group. According to the results of many studies, glutathione-S-transferase (GST) polymorphism, in particular, homozygous deletions (null-alleles) of GSTM1 and GSTT1, is one of the causes of increased sensitivity to the damaging effects of environmental factors with damage to the bronchopulmonary system. The role of polymorphic variants of GST genes in the formation of MBT resistance has been shown [3, 8].

Summing up the analysis, it should be noted that scientists are actively researching the molecular and genetic aspects of the formation of resistance in tuberculosis patients in order to prevent its occurrence when applying modern treatment programs. The study and evaluation of the effectiveness of methods for the diagnosis and treatment of susceptible and resistant TB continues .

The issue of studying the genetic aspects of the formation of drug resistance of M. tuberculosis with the determination of the role of polymorphic variants of genes encoding xenobiotic metabolism systems continues to be relevant. with tuberculosis infection to understand the mechanisms of interaction in the process of implementing hereditary information at the organismal level in order to increase the effectiveness of treatment and prevent the formation of resistance.

CHAPTER II

DYNAMICS OF THE MAIN INDICATORS OF THE EPIDEMIOLOGY OF TUBERCULOSIS IN BUKOVINA, THE INFLUENCE OF THE COVID-19 PANDEMIC AND THE CONDITIONS OF THE WAR

According to the WHO, Ukraine is one of the 27 countries in the world where 85% of the incidence of tuberculosis (TB) is concentrated, and it ranks 4th in the world in terms of its prevalence. Almost 100 new cases of TB disease are registered in Ukraine every day. Despite the decrease in morbidity and mortality from TB over the past 5 years, statistical indicators remain above the epidemic threshold. In addition, the epidemic situation with tuberculosis in Ukraine is negatively affected by the

increase in the number of cases of tuberculosis with multidrug resistance (MDR-TB) and extensively drug-resistant tuberculosis (XDR TB) [2,5,11].

A number of scientists and physicians argue that the COVID-19 pandemic has had a potential impact on tuberculosis (TB), including through changes in the organization and provision of medical assistance, as well as a significant impact on people's usual lifestyles and behaviours, as a result of which, according to the WHO, there was a significant global decrease of the number of people diagnosed with newly diagnosed tuberculosis and officially notified in 2020 (compared to 2019). As statistical reports have shown, regression of the incidence rate has occurred in almost all countries of the world [10].

It should be noted, that the pandemic of coronavirus infection (SARS-Cov-2) negatively affected the long-term progress in the fight against TB and reducing the burden of this infection not only in Ukraine, but also in all WHO regions [6,7].

According to WHO annual reports, there has been a significant global reduction in the number of newly diagnosed tuberculosis cases that have been registered, that is, from 2019 to 2020, the number of TB has decreased by 18%, returning to the level of 2012. Limiting access to diagnosis and treatment of TB during the COVID-19 pandemic increased the mortality rate [12].

The armed aggression of the Russian Federation, which has been ongoing in Ukraine for more than a year, has led to a large-

scale humanitarian crisis that has affected the entire population of Ukraine and, probably in the future, along with the impact of the pandemic situation on COVID-19, wich has already caused an increase in the spread of TB. Moreover, on the territory of the conflict zone, as a result of the displacement of the population, the decrease in the standard of living and the deterioration of the humanitarian situation, many people are in danger of acquiring infection from contagious people, especially in conditions of unfitness for living and non-compliance with the requirements of infection control. In addition, migrants and refugees often do not have sufficient access to medical care, which makes timely diagnosis and treatment of tuberculosis difficult [10]. All of the above made it possible to formulate the goal of the fragment of our research.

The aim of the study - is to analyze the regional peculiarities of the epidemiology of tuberculosis in the Chernivtsi region under the influence of unfavorable socio-economic factors.

Materials and methods. The medical records of all newly diagnosed cases of tuberculosis that were registered at the Chernivtsi Regional Clinical Tuberculosis Dispensary from January 2021 to December 2022 (TB MANAGER electronic register of tuberculosis patients) were analyzed.

Descriptive statistics were used to detail the obtained data. Univariate logistic regression analysis was performed for the composite of adverse outcomes (lost to follow-up, treatment failure, death). Cox proportional hazards regression analysis was

performed for the set of adverse outcomes by analysis of survival at the end of treatment. Multivariable logistic regression models for each outcome were developed using a base model that included clinically significant variables (eg, sex, age) and all variables that showed a trend toward association in univariate analysis ($p < 0.1$). The models were then adjusted using backward elimination until a baseline model was reached. After that, the models were tested by gradually adding variables. For logistic regression analysis, the fit of the final models was assessed using the Hosmer-Lemeshow test and the efficiency by the area under the performance curve. For Cox regression analysis, proportional hazards assumptions were tested using Kaplan-Meier curves before model analysis and log-minus-log survival curves after model analysis. Adjusted hazard ratios for Cox regression models and adjusted odds ratios for logistic regression models and 95% confidence intervals were used to assess the association between variables and outcomes. Statistical significance was defined as $p < 0.05$. Subgroup analysis was performed by age, sex, and research center. All analyzes were performed using IBM SPSS Statistics v26.0.

Results and their discussion. The analysis of the incidence of TB among the adult population is shown in Figure 1. Thus, at the end of 2022, 222 patients with newly diagnosed tuberculosis were registered in the Chernivtsi region. The lockdown situation caused by the COVID-19 pandemic and conditions of the war have made their negative adjustments.

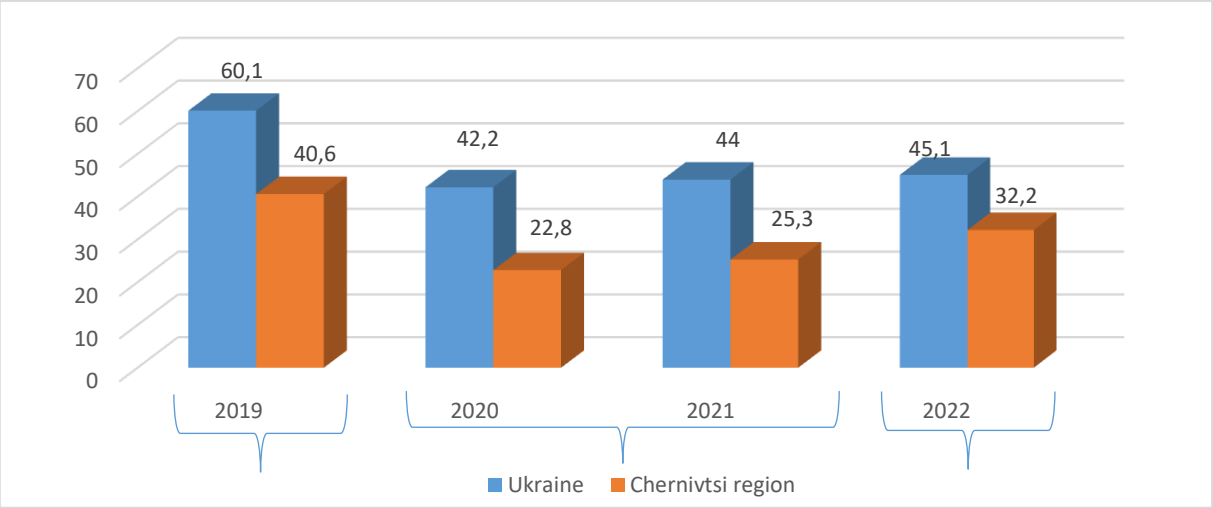

Fig. 1. Dynamics of the incidence of newly diagnosed tuberculosis (2019-2022).

As demonstrated in Figure 1 wich highlights three periods of TB incidence dynamics starting from 2019. The first period is 2019- given as the pre-quarantine period, the second - 2020-2021 - the quarantine period associated with the COVID-19 pandemic, and 2022 - the period of the war in Ukraine [1].

In general, according to the results of the graphic analysis shown in fig. 1, we observe a trend towards a significant decrease in the incidence rate of TB during the Covid-19 period, and the same trend is observed during the martial law both in the state as a whole and in Bukovina [8].

Thus, in 2020, the number of newly diagnosed TB cases in Ukraine decreased by 25% compared to 2019, in Bukovina – by 20.6%.

According to the Center for Public Health, in Ukraine in 2022, the number of first-time registered TB cases, including its

recurrences, was 18,510, or 45.1 per 100,000 population, which is 2.5% more than in 2021 (18 241, or 44.0 per 100,000 population). As for the Chernivtsi region, in 2022 a reliable tendency to increase the incidence rate compared to 2021 by 27.3% was registered [9].

With the onset of the SARS-Cov-2 COVID-19 pandemic in 2020, the number of newly diagnosed TB cases decreased, potentially leading to an accumulation of undiagnosed and latent TB cases that were detected at later stages and in more severe forms and demonstrated a potentially probable increase in the number of active cases in 2022 [3].

The prevalence rate of TB over the last four years has a tendency to decrease across the territory of Ukraine (Fig. 2). Thus, in 2022, the prevalence of tuberculosis infection in Ukraine decreased by 34.5% compared to 2019, and the difference between 2019 (before the quarantine period) and 2021 is 31.5%, between 2020 and 2021 was insignificant, similarly as well as between 2021 and 2022 (the period of the war) [7].

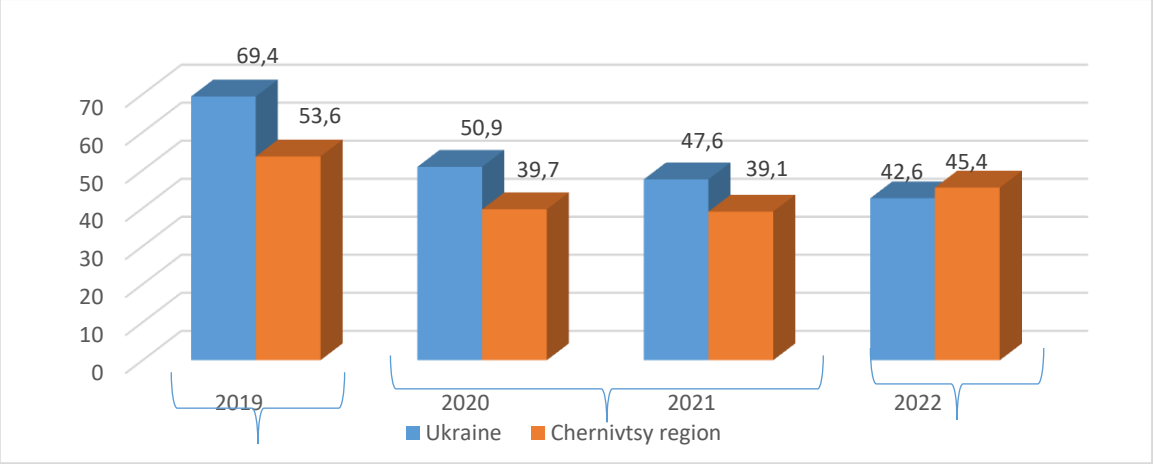

Fig. 2. Prevalence of all forms of active TB among the population of Ukraine in 2019-2021.

The analysis of the prevalence of TB in Bukovina showed a decrease of this indicator for 2020 compared to 2019 by 25.9%, in 2021 the rate of regression decreased to 1.5% compared to 2020, but in the last year - 2022, a probable increase in the indicator can be observed prevalence of TB in the Chernivtsi region - by 16.1% [1].

The analysis of the mortality rate (Fig. 3) due to the tuberculosis among the entire population of Ukraine in the dynamics for 2019-2022 shows its decrease in the state as a whole and, in particular, in Bukovina. There was no increase in the death rate due to TB in the comparative dynamics between 2019-2022 in any region of Ukraine [4].

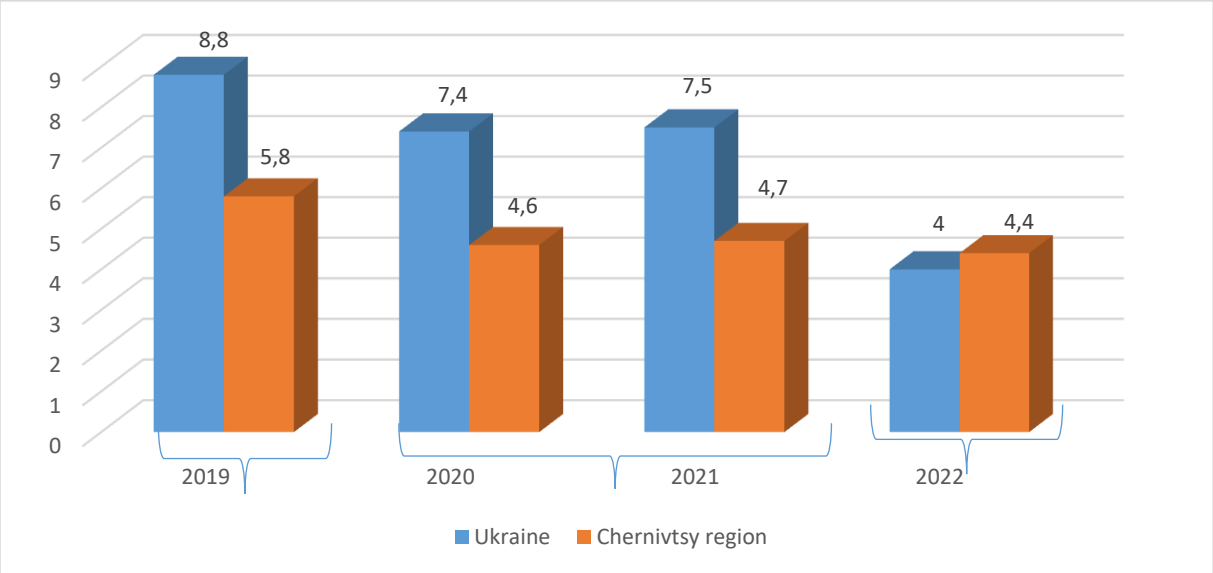

Fig. 3. Dynamics of tuberculosis mortality among the population of Ukraine in 2019-2021.

Attention is drawn to the tendency to increase the death rate from tuberculosis infection in Chernivtsi region (Fig. 3) compared to the similar rate in the state. At the same time, as shown in fig. 3, the difference between the national mortality rate and in the region for 2019-2021 was probably: in 2019 - 34.1%, in 2020 - 37.8%, in 2021 - 37.3%, with a lower value in Bukovina. Conversely, in 2022, we observe a trend towards the equilibrium of this indicator with some potential increase in mortality in Chernivtsi region (by 9.9%) compared to the national level, which, in our opinion, may be due to under-registration of deaths from TB.

The mortality rate in Bukovina under the negative influence of socio-economic factors was 4.7 per 100,000 population in 2021, and 4.4 per 100,000 population in 2022, namely it shows a downward trend.

As demonstrated in Figure 4 the curve of the TB incidence rate estimate per 100,000 population in the period from 2016 to 2022 in the Chernivtsi region. The trend of TB registration is consistent, but with different rates of change. The period of the strict lockdown is characterized by a sharp decrease in the registration of TB cases. Over the past 2 years, a trend towards an increase in the number of cases of active tuberculosis infection has been noted [3].

Fig. 4. Dynamics of TB incidence per 100,000 population in Chernivtsi region in the period from 2016 to 2022.

In fig. 5 are illustrated the incidence of newly diagnosed cases of TB per 100,000 people. Thus, in 2022, 222 patients with active tuberculosis were detected in Bukovina compared to 2021 – 185 patients, the highest number of patients were detected in the Vyzhnytskyi district – 25 patients, which is 36% more than in 2021, where only 16 patients were detected. In particular, it is worth highlighting districts such as Storozhynetsky - where there is a 2-fold increase in the number of patients with newly diagnosed cases of TB: in 2022 - 38 patients, and in 2021 - 19 patients, Hlybotskyi district, where in 2022 - 24 patients were detected, and in 2021 year - 11 patients, Novoselytskyi - the number of patients for 2022 has tripled compared to 2021.

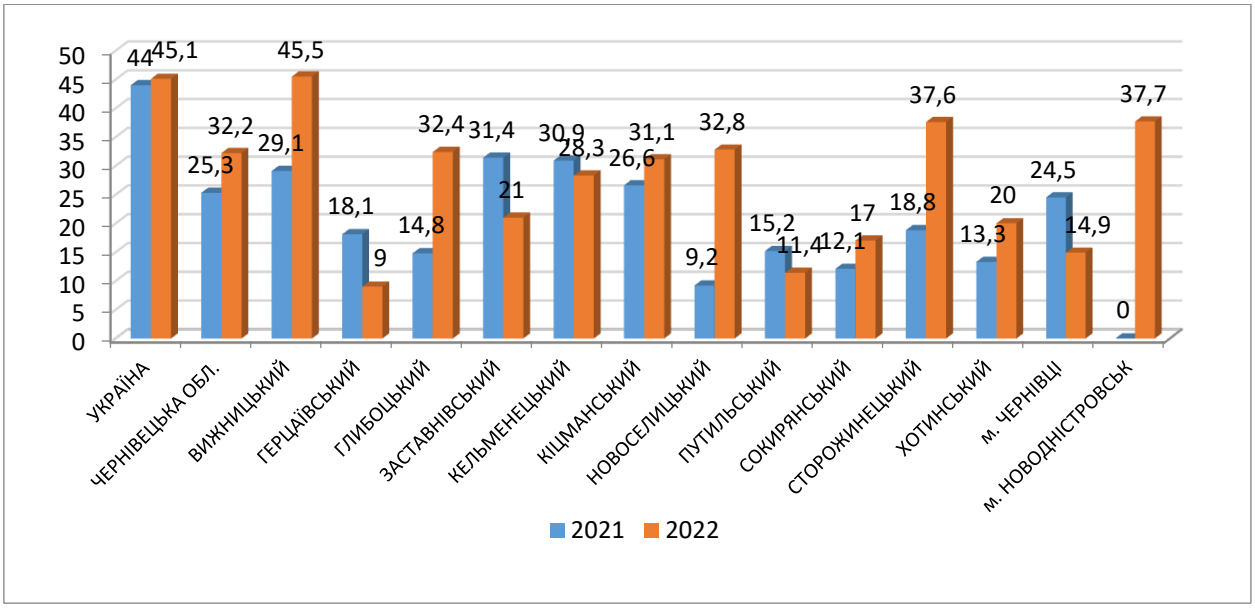

Fig. 5. Incidence of newly diagnosed cases of TB per 100,000 in Chernivtsi region by districts in dynamics for 2021-2022.

However, it is worth noting (see Fig. 5) that in certain regions of Bukovyna, the incidence rate decreased in 2022 compared to 2021. This is, in particular, the city Chernivtsi by 39.1%, Zastavnivskyi district. – 33.1%, Putylsky district. - 25%.

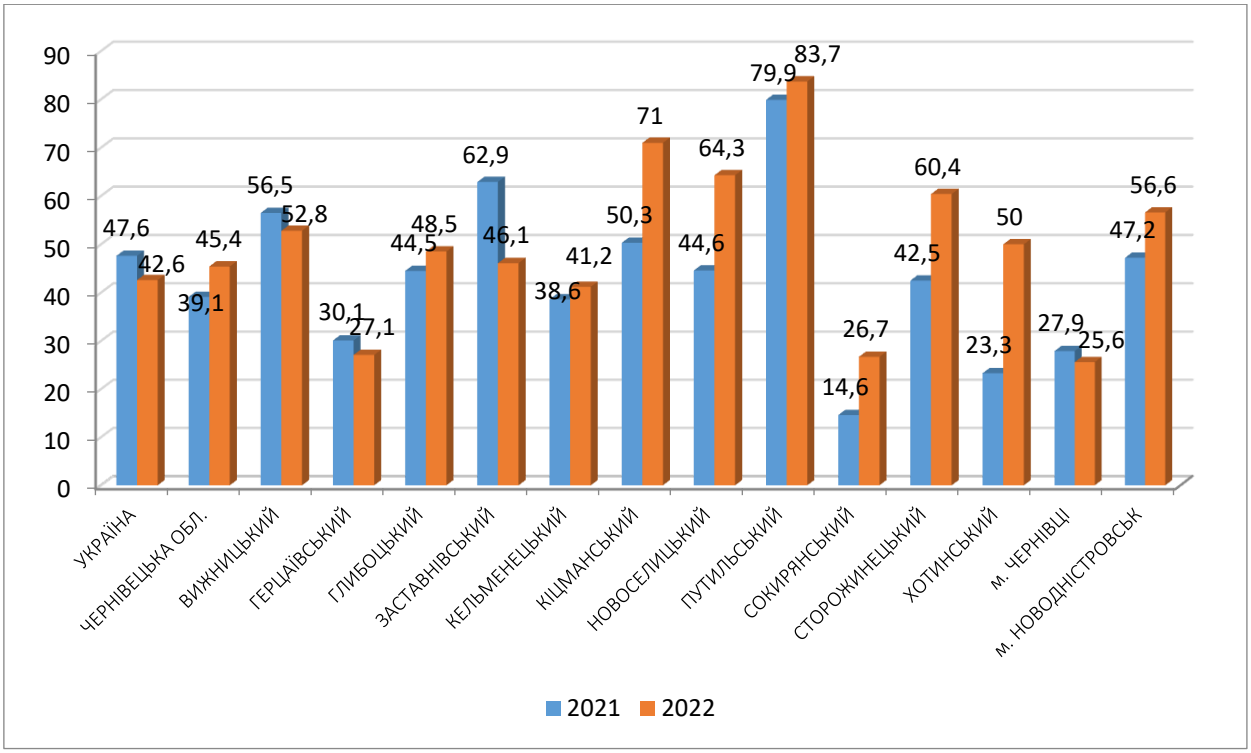

Fig. 6. Prevalence of tuberculosis in the Chernivtsi region by districts in dynamics for 2021-2022.

The data presented in Figure 6 highlight the indicators of the prevalence of TB in the Chernivtsi region compared to the national ones in the dynamics of 2021-2022. Thus, in Bukovyna, the prevalence rate showed an increase in TB in the percentage ratio - by 14%, which is clearly visible in such districts as Storozhinetskyi, Kitsmanskyi, Novoselytskyi, Khotynskyi, with the maximum increase in Putilskyi (mountain district). However, there are regions where a regression of prevalence is observed, in particular, it is the city of Chernivtsi, Zastavnivskyi, Vyzhnytskyi, Hertsaivskyi districts.

Thus, we observe certain probable discrepancies in the indicators of the incidence and prevalence of TB in the Chernivtsi region in the dynamics of 2021-2022, a clear trend of a

synchronous decrease of these indicators is being followed in the city Chernivtsi.

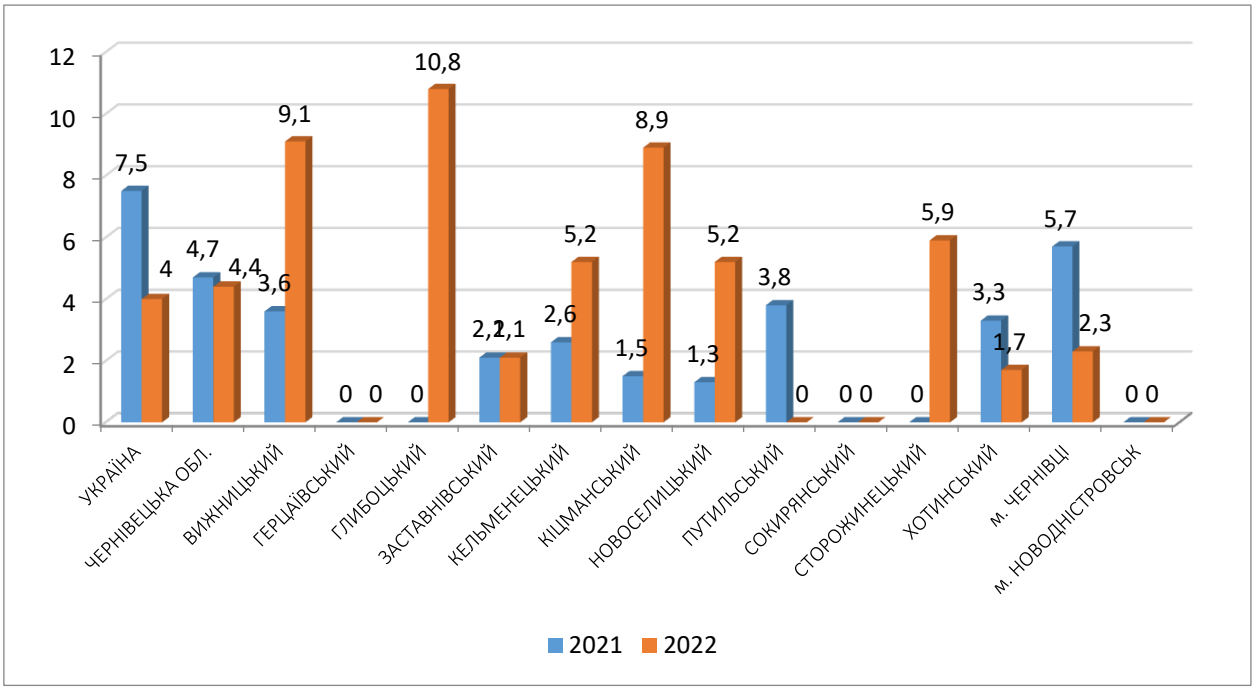

Fig. 7. Dynamics of mortality due to tuberculosis in the Chernivtsi region by districts in dynamics for 2021-2022.

One of the main dimensions characterizing the epidemic situation with TB is the mortality rate. The mortality rate of the region's population from TB was 4.7 per 100,000 population in 2021, and 4.4 per 100,000 population in 2022, which shows a downward trend [4,5].

As you can see, the digital analysis is shown in fig. 7, reflects different results in most districts of the region, where an increase in deaths due to TB infection can be observed compared to last year, in particular, such as: Vyzhnytskyi - the rate has almost tripled, Hlybotskyi - there was no case in 2021, but in 2022 - 10.8

per 100,000 population, Kitsmansky – an increase of almost 6 times compared to 2021. Not all regions of Bukovina have observed an increase in the death rate, in some of them there is a decrease, or no deaths at all, in particular, such as Sokyriansky, Hertsaivskyi districts, and the city of Novodnistrovsk.

Conclusions. In the Chernivtsi region for the period from 2019 to 2022, there is a trend towards a significant decrease in the TB incidence rate, both during the epidemic period and the conditions of war, which correlates with the national trend. Due to the onset of the COVID-19 pandemic caused by SARS-Cov-2, the number of newly diagnosed TB cases decreased in 2020 compared to 2019, potentially leading to an accumulation of undiagnosed and latent TB cases that were detected in more late stages and more severe forms and showed a potentially likely increase in the number of active cases in 2022.

Certain probable discrepancies in the indicators of the incidence and prevalence of TB in the Chernivtsi region in the dynamics of 2021-2022 were revealed, however, a clear trend towards a simultaneous decrease of these indicators in the city Chernivtsi is observed.

The difference between the national and regional mortality rates for 2019-2021 was probably: in 2019 - 34.1%, in 2020 - 37.8%, in 2021 - 37.3%, with a smaller value in Bukovina. Conversely, in 2022, we see a trend towards an equilibrium of this indicator with some potential increase in mortality (9.9%) compared to the national indicator. Thus, the mortality rate in

Bukovina under the negative influence of socio-economic factors was 4.7 per 100,000 population in 2021, and 4.4 per 100,000 population in 2022, i.e. it shows a downward trend.

CHAPTER III

THE ROLE OF CELL-MEDIATED IMMUNITY IN THE PATHOMORPHOSIS OF THE CLINICAL EVOLUTION OF TUBERCULOSIS PATIENTS ASSOCIATED WITH HIV INFECTION

Tuberculosis (TB) represents a burden on the healthcare system of any country [1]. The COVID-19 pandemics caused a reduction in the notification of TB cases from 7.1 million in 2019 to 5.8 million in 2020, respectively by 18% [2]. India, Indonesia and the Philippines were the most affected due to barriers in accessing the medical services. The reduction in the number of reported cases has been associated with an increased number of undiagnosed people with TB co-infected with HIV, untreated patients, increased mortality and the transmission of both infections in the community [2]. The estimated number of deaths among TB patients in 2021 was 1.4 million among HIV-negative people and 187.000 among people living with HIV [3]. The net reduction in TB case-notification in 2021 compared to 2015 constituted only 10%, which was one half of the End TB Strategy milestone. The Strategy put an important accent on the multisectorial approach of the health determinants: poverty, subnutrition, smoking, HIV infection and diabetes [3]. During the high-level meeting of the United Nations in 2023, was showed that the chemopreventive treatment for TB infection was

administrated to 6 million people living with HIV during the period 2018-2022, and a total number of 10.3 million chemopreventive treatments were carried out, respectively 180% more than was predicted [4].

HIV infection represents the most severe state of immunosuppression, considered the major risk factor for TB infection and disease, which is 16-31 times higher compared to people with HIV-negative status [28, 35]. The progression of TB infection into active disease occurs in a proportion of 37% in the first 6 months after the primary infection and in 2-5% in the next 2 years. In people living with HIV (PLH), TB can occur at any stage, regardless the level of CD4+ cells, however, it is most often diagnosed in advanced stages, when immunity is reduced to minimum. According to the Center for Disease Control guidelines, HIV infection is classified in several clinical stages depending on the number of CD4+ cells: stage 0 – asymptomatic, stage I corresponds to the level of CD4+>500/μL; stage II CD4+ level is between 200-500/μL and stage III CD4+≤200/μL [1]. Acquired immunodeficiency syndrome (AIDS) is diagnosed when the CD4+ level is ≤200/μL and/or at least one opportunistic infection is diagnosed. In the absence of antiretroviral treatment (ARV), AIDS usually develops in 8-10 years after primary HIV infection, expressed predominantly through active TB and opportunistic infections [41]. The opportunistic agents that most frequently affects PLH with AIDS are: *Pneumocystis jiroveci*, atypical mycobacteria, human polyomaviruses (JC and BK virus), herpes

viruses, *Cryptococcus neoformans, Cryptosporidium parvum, Candida albicans* and pathogenic germs such as *Mycobacterium tuberculosis, Toxoplasma gondi* and *Salmonella enterica*. Pneumonia with *P. jiroveci* makes the highest challenge in differential diagnosis with pulmonary TB due to the similar clinical-radiological aspects. The microbiological criteria based on the detection of acid-fast-bacilli (AFB) at Ziehl-Neelsen staining of sputum, which is highly specific for pulmonary TB and of intracystic corpuscles in the form of bluish dots in the broncho-alveolar lavage which is highly specific for pneumonia with *P. jiroveci* are considered the gold standards for the etiological diagnosis of these pathologies [6].

In the actual epidemiological context of the Republic of Moldova, the rate of TB/HIV co-infection in new and relapsed TB cases in 2018 was 8.2% (248 TB cases with positive HIV state) [7]. According to the national clinical protocol (NCP)-123, all adults PLH are radiologically examined and children undergo the tuberculin skin test annually. Depending on the results of the clinical and laboratory investigations, patients will receive antituberculosis or chemopreventive treatment. Also, all patients diagnosed with active TB will be counseled and tested for HIV markers - Elisa test for the determination of the p24 antigen and rapid test for the determination of antibodies [49]. The operational studies carried out in the Republic of Moldova demonstrated that the lack of ARV treatment is the main cause of death of TB patients due to rapid progressive evolution with the failure of

respiratory, but the connection with the degree of immunosuppression has not been established [58].

The aim of the study was to assess the role of cell mediated immunity deficiency in the clinical evolution of tuberculosis associated with HIV infection in order to establish recommendations for improvement of the case-management.

Material and methods. A descriptive research designed as a prospective selective and longitudinal study that included 86 patients diagnosed with TB co-infected with HIV was conducted. The research methods were used: historical, epidemiological, direct observational method, documentation analysis, statistical, mathematical and comparative methods.

The including criteria were: a) diagnosis of TB with pulmonary and/or extrapulmonary localization; b) TB was registred during the period 2022-2023 in the public medical institutions of Chisinau, hospitalized in the clinical subdivisions of the Municipal Clinical Hospital for Phthysiopneumology; c) signed informed consent. The general sample that met the including criteria was divided into 2 groups: study group (SG) – consisted of 38 patients diagnosed with TB and AIDS confirmed by the number of CD4+ cells (T helper lymphocytes) lower than 200/μL (83± 34/μL) and control (CG) - 48 patients with CD4+ cell count greater than 200/μL (320±117/μL). The laboratory indicators were compared with a laboratory sample constituited of 50 conventional healthy individuals.

The diagnosis of TB was established according to WHO recommendations based on clinical criteria (presence of clinical signs of general intoxication and bronchopulmonary syndrome), radiological (for pulmonary localization - pattern of infiltrative either nodular opacities, destruction and enlarged hilum) and microbiological (sputum smear microscopy for detection of AFB, GeneXpert MTB/Rif, culture on conventional media). Was operated with the following definitions stipulated in NCP-123: disease localization (pulmonary/extrapulmonary), case type (new case/relapse/failure/lost to follow-up), microbiological status (positive/negative) [29]. The definitions from NCP - 211 "HIV infection in adults and adolescents" were also used regarding the clinical staging of HIV infection from the asymptomatic stage to the AIDS stage according to at least one characteristic condition and the degree of immunodeficiency depending on the number of CD4+ cells/μL [10]. The investigation methods were used such as chest radiological examination in two projections and plane tomography; microbiological examination of sputum (bacterioscopy at Ziehl-Neelson staining for AFB and Giemsa staining for *P. jiroveci*), bacteriological examination (culture on the classic Lowenstein-Jensen and BACTEC media), molecular genetic methods (GeneXpert MTB/Rif and/or GeneXpert MTB /Rif ultra). Other investigations were carried out: general blood analysis, urine analysis, serum biochemical examination (transaminases, urea, creatinine, lactate dehistrogenase), tests

for HIV markers (Elisa for antigen p24, rapid test for antibodies, viral load)

The statistical analysis of the study results was carried out computerized using the variational analysis methods, the quantitative and qualitative verification of the accumulated material, divided into simple and complex groups. Statistical validity was assessed according to Student's t-test. The statistical significance was assessed by the value of $p<0.05$. For risk assessment the multiple linear regression (RML) was performed with the calculation of odds risk (OR) and 95% of confidence interval.

Results. Assessing the indices of the white blood count, it was established that the the absolute number of leukocytes was significantly higher in both groups compared to the laboratory group (LG), which was constituted by the conventionally healthy individuals. The rate of segmented neutrophils was non-significantly lower in both groups compared to LG, but the level was lower in the study group (SG). The rate of band neutrophils was significantly higher in both groups vs. LG. The rate of eosinophils was significantly higher in SG compared to CG and LG. Comparing the groups, was determined that the rate of eosinophils was statistically significantly higher in SG compared to CG, and the rate of lymphocytes was significantly lower in SG vs. CG (Table 1).

Table 1.

Distribution according to laboratory indicators of the white blood count of tuberculosis patients associated with hiv infection (%)

Indicatori	EM (n=59, P%)	ES (n=38, P%)	EC (n=48, P%)
Leucocytes (10^9/L)	6.8±0.25	7.4±1.2 #	6.2±0.9 ##
Segmented neutrophils (%)	62.1±0.33	61.3±3.11	64.5±3.68
Band neutrophils (%)	1.7±0.11	5.7±0.12###	5.6±1.11###
Eosinophyles (%)	2.6±0.12	5.8±1.01#□	2.6±1.24
Lymphocytes (%)	25.8±0.11	17.8±0.22 #□	22.9±0.01
Monocytes (%)	5.6±0.14	6.6±1.8 #	7.3±1.3 ##

Notă: P value at the t-test #≤0.05; ## #≤0.01; ###≤0.001 at the comparision with the laboratory group (LG). □ P value at the t-test #≤0.05 at the comparision of the SG with the CG.

No one patient from the SG was exposed to the ARV treatment for HIV infection prior to the current episode of TB, compared to 38 (79.2%) cases of the SG. Absolutely all patients were treated with ARV during the antituebrculosis treatment, either by continuing or starting after 2 weeks following the onset of the anti-TB treatment. Viral load (number of HIV copies) was

significantly higher in the SG 1.37 e^6/mL vs. 340±100/mL in the CG.

Analyzing the biological characteristics of the patients in the selected groups, was found that in the SG, men constituted 20 (52.6%) and women 18 (47.4%), with a men/women ratio (M/W) = 1.1/1. In the CG, men constituted 31 (64.1%) and women - 17 (35.4%), with a M/W ratio =1.7/1. Distributing patients into age groups according to the WHO classification, was established a significant predominance of the subgroups between 25-34 years and over 65 years in SG, 12 (25%) vs 4 (10.5%) and respectively 0 vs. 4 (8.3%). Re-distributing patients into two categories, younger and older 45 years old, was established a significant predomination of patients younger 45 years in SG 21 (53.7%) vs. 18 (41.5%) in CG, and of older 45 year in CG 30 (62.5%) vs. 17 (44.7%) in SG ($p<0.05$). MLR assessed that age older 45 years was a small risk factor for TB associated with AIDS with OR=1.30 (95% CI: 0.6-2.93). Evaluating the residence of the patients, was found that residents of urban localities significantly predominated in CG (64.6%) vs. 16 (42.1%) in the SG and of the rural localitites in the SG: 18 (47.4%) vs. 12 (25%) in CG, at the same statistical threshold ($p<0.05$). The MLR established that rural residence constituted a moderate risk factor for TB on AIDS, and evaluated with OR=2.2 (95% CI: 1.07-5.74). Homeless were one tenth of patients in both groups (Table 2).

Table 2.

Distribution according to biological characteristics and residence of tuberculosis patients associated with hiv infection (%)

Indicators	SG (n=38, P%)	CG (n=48, P%)	P
Men	20 (52.6)	31 (64.6)	>0,05
Women	18 (47.4)	17 (35.4)	>0,05
18-24 y.o.	1 (2.6)	2 (4.2)	<0,05
25-34 y.o.	4 (10.5)	12 (25.0)	<0,05
35-44 y.o.	12 (31.6)	16 (33.3)	>0,05
45-54 y.o.	16 (42.1)	8 (16.7)	>0,05
55-64 y.o.	5 (13.2)	6 (12.5)	>0,05
65+	0	4 (8.3)	<0,05
Urban	16 (42.1)	30 (62.5)	<0,05
Rural	18 (47.4)	12 (25.0)	<0,05
Homeless	5 (13.1)	6 (12.5)	>0,05

Note: n and P%, absolute number and percentage; P - p-value as the level of significance at the Student's T-test.*

Evaluating the socio-economic status of the investigated patients, was establisehd that the unemployed patients, without any financial source of existence, slightly predominated in ES 21 (55.3%) compared to 20 (41.7%) in EC. Patients with the state-supported financial source such as social allowances for retirement and disability predominated insignificantly in the CG 16

(33.3%) vs 9 (23.7%) in SG. The statistical analysis by MLR established that the vulnerable economic status (unemployed, disabled person, retired) constituted a low risk factor for TB on AIDS, with OR=1.35 (95% CI: 0.83-1.34). Patients with a low level of education predominated statistically significantly in the SG 21 (55.3%) vs. 15 (31.2%), ($p<0.05$) and constituted a moderate risk factor for TB on AIDS, OR=2.71 (95% CI: 1.23-6.54). Patients with mental and behavioral disorders predominated in the SG - 16 (42.1%) vs 9 (18.7%), also those with chronic alcoholism 12 (31.5) vs. 6 (12.5%) at the same statistical threshold ($p<0.05$), active drug users were 2 (5.3%) in the SG.

Both behavioral conditions - mental disorders with OR=2.96 (95%CI: 1.13-7.74) and chronic alcoholism with OR=3.23 (95%CI: 1.42-6.11) were established as high risk factors for TB on AIDS. Social and epidemiological risk factors such as history of detention, recent returning from migration and tuberculosis contact were established in a small and equal proportion in both groups. Active smoking was detected in the majority of patients in both groups, and diabetes mellitus – comorbidity with associated immunosuppressive risk was diagnosed in every tenth patient in both groups (Table 3).

Table 3.

Evaluation of cases according to socio-economic status of tuberculosis patients associated with hiv infection (%)

Indicatori	SG (n=38, P%)	CG (n=48, P%)	P*
Employed	8 (21.1)	12 (25.0)	>0,05
Unemployed	21 (55.3)	20 (41.7)	>0,05
With disabilities	4 (10.5)	6 (12.5)	>0,05
Retired	5 (13.1)	10 (20.8)	>0,05
Low level of education	21 (55.3)	15 (31.2)	>0,05
Mental disorders	16 (42.1)	9 (18.7)	<0,01
Recent history of migration	5 (13.1)	8 (16.7)	>0,05
History of detentions	3 (7.9)	4 (8.3)	>0,05
TB contact	5 (13.1)	8 (16.7)	>0,05
Active tobacco smoking	32 (84.2)	41 (85.5)	>0,05
Chronic alcoholism	12 (31.5)	6 (12.5)	<0,01
Diabetes mellitus	4 (10.5)	6 (12.5)	>0,05

Note: n and P%, absolute number and percentage; P - p-value as the level of significance at the Student's T-test.*

Studying the particularities of the case-detection of the investigated patients, was found that the medical staff of the medical institutions of the primary healthcare assistance (PHC) detected the majority of patients in both groups, with a significant predominance in the CG 41 (72.3%) vs. 14 (52%) in SG.

Phthisiopneumologists or infectious disease specialists diagnosed patients significantly more frequently in the SG - 24 (58.0%) vs 7 (27.7%). Early detection of TB in patients with a progressive evolving symptomatology for up to one month was found only in the CG– 9 (18.7%) cases. All patients from the SG were found to have an evolving symptomatology lasting longer than one month. All patients in both samples had cough with mucopurulent sputum and a limited number of cases had hemoptysis and chest pain. Clinical signs of intoxication syndrome, such as weight loss, asthenia, inappetence/anorexia were established in all investigated patients, and fever/hypofebrile and profuse sweating significantly predominated in ES – 15 (31.2%) versus 3 (7, 9%) cases in EC. Malnutrition, including cachexia (BMI<19 kg/m2) was found in all ES patients, and only in every second in EC (Table 4).

Table 4.

Detection and main clinical aspects of tuberculosis patients associated with hiv infection (%)

Indicator	ES (n=38, P %)	EC (n=48, P %)	P*
Primary healthcare workers	14 (52.0)	41 (72.3)	<0,05
Phthysiopneumologist/other specialists	24 (58.0)	7 (27.7)	<0,05
Early detected (up to 30 days)	0	9 (18.7)	<0,001

Late detected (more than 30 days after the clinical onset)	38 (100)	38 (84.6)	<0,001
Cough and muco-purulent sputum	38 (100)	65 (100)	>0,05
Hemoptysis	5 (13.1)	8 (12.3)	<0,001
Thoracic pain	3 (7.8)	4 (6.1)	>0,05
Undernutrition/cachexia/wasting sdr.	38 (100)	28 (58.3)	<0,001
Feverish	3 (7.9)	15 (31.2)	<0,01
Fever	28 (73,6)	11 (22,9)	<0,001

Note: n and P%, absolute number and percentage; P - p-value as the level of significance at the Student's T-test.*

Pulmonary localization of TB predominated insignificantly in the CG– 38 (79.1%) vs. 25 (66.8%) cases in the SG, and the disseminated TB, including generalized form that includes 2 extrapulmonary and one pulmonary localization predominated in the SG 10 (26.3%) vs. 6 (12.5%) in the CG. The rate of microscopically positive cases for AFB, with an epidemiological risk of spreading the infection in the healthy community, was identified in a similar rate in both groups, and the positive results on culture media was insignificantly higher in the CG 18 (37.5%) vs. 11 (29.4%) in the CG. Rifampicin eithermultidrug resistant tuberculosis was found in a higher number of cases in the CG 8 (16.7%) vs. 3 (7.8%) cases in SG. Evaluating the health-related

conditions associated with HIV and TB, was established the significant predomination of anemia (Hb<11 g/dL in women and <16 g/dL in men) in SG 38 (100%) vs. 29 (60%) in CG. Thrombocytopenia (<150 e^9/L) was found in a small number of cases in both groups. Opportunistic infections such as oropharyngeal candidiasis predominated in the SG 31 (81.6%) vs 19 (39.6%) in CG, *herpes zoster* and toxoplasmosis of the central nervous system were diagnosed only in SG (Table 5).

Table 5**.**

Radiological, microbiological and therapeutic peculiarities of tuberculosis patients associated with hiv infection (%)

Indicatori	ES (n=38, P%)	EC (n=48, P%)	P*
Pulmonary	25 (66.8)	38 (79.1)	>0,05
Secpndary extrapulmonary	3 (7.8)	4 (8.3)	>0,05
Diseminated/generalized TB	10 (26.3)	6 (12.5)	>0,05
Microscopic positive for AFB	8 (21)	12 (25.0)	>0,05
RR /TB-MDR	3 (7.8)	8 (16.7)	>0,05
Bacteriologic positive	11 (29.4)	18 (37.5)	>0,05
GeneXpert positive	14 (36.)	21 (43.7)	>0,05
Anemie	38 (100)	29 (60)	<0,001
Thrombocytopenie	7 (18.4)	2 (4.2)	>0,05

Oropharyngeal candidiasis	31 (81.6)	19 (39.6)	<0,001
Herpes zoster	6 (15.8)	0	<0,01
Toxoplasmosis	5 (13.2)	0	<0,05
Pneumonia with *P. jiroveci*	8 (21.1)	0	<0,01

Note: *n and P%, absolute number and percentage; P* - p-value as the level of significance at the Student's T-test.*

Case-type was assessed according to exposure to antituberculosis treatment, which demonstrated that the majority were new cases in both groups, with their slight predomination in the CG 34 (70.8%) vs. 23 (60.5%) in SG. Relapses prevailed in the CG 14 (29.3%) vs. 8 (21.1%) in SG, and patients registered after threatment failure or lost to follow-up predominated in the SG 7 (18.4%) vs. 2 (4.2%) cases. The average timeline until the current diagnosis of TB was 9.1±3.5 years in the SG and 6.5±3.1 years in the CG from the moment of diagnosis of HIV infection. It should be noted that approximately every third patient in both samples was diagnosed with HIV and tuberculosis simultaneously – 11 (28.5%) in SG and 15 (31.2%) in CG.

Treatment with first-line antituberculosis drugs for conventionally susceptible TB was given in a non-significantly higher proportion in the SG 29 (76.4%) vs. 31 (64.6%) in CG, and for MDR-TB in a higher proportion in CG 8 (16.7%) vs. 3 (7.8%) in SG, and the individualized one was administered in a similar

proportion in both groups. Therapeutic outcome defined as success at the antituberculosis treatment completion was established in only every third patient in both groups, being in a similar proportion with those who were still continuing treatment due to clinical and laboratory indications. The death rate was high in SG- 9 (23.4%) vs. 9 (18.7%) in CG, and the rate of patients defined as therapeutic failure or lost to follow-up during anti-TB treatment was found to be very low in both groups– 2 (5.3%) vs . 4 (8.3%). The data are presented in table 6.

Table **6.**

Case-types, treatment types and therapeutic outcomes of tuberculosis patients associated with hiv infection (%)

Indicator	ES (n=38, P%)	EC (n=48, P%)	P*
Nre cases	23 (60.5)	34 (70.8)	>0,05
Relapse	8 (21.1)	14 (29.2)	>0,05
Failure/lost to follow-up case	7 (18.4)	2 (4.2)	>0,05
1st line anti-TB drugs	29 (76.4)	31 (64.6)	>0,05
Treatment for MDR-TB	3 (7.8)	8 (16.7)	>0,05
Individualised regimen	6 (15,8)	9 (18,7)	
Threatment success	15 (39.5)	18 (37.5)	>0,05
Death	9 (23.4)	9 (18.7)	>0,05
Failure/lost to follow-up outcome	2 (5.3)	4 (8.3)	>0,05

Still continuing	12 (31.6)	17 (35.4)	>0,05

Note: *n and P%, absolute number and percentage; P* - p-value as the level of significance at the Student's T-test.*

The research assessed the impact of the severity of the cell mediated immune deficiency associated with HIV infection on the clinical evolution of TB and the final therapeutic outcome. The results of the study demonstrated that the laboratory indicators that characterize TB on AIDS is the general leucocytosis, with lymphocytopenia and eosynophilia, anemia and in rare cases thrombocytopenia. All patients with AIDS were not previously treated with ARV and only one third in early stages of symptomatic HIV-infection.

The biological peculiarities of the tuberculosis endemics in HIV negative patients is characterised by the predilected targeting of the male sex and young age groups, which are not exactly similar compared with gender san age distribution among patients with TB and HIV. Similar results are reported by other local and international studies [1, 7-9]. A biological peculiarity found was young age (under 45 years old) of patients in AIDS stage, and age older 45 years in PLH in earlier stages. The obtained results demonstrated the impact of the complexity of health related conditions associated with sex and young age on the risk of developing TB on AIDS. The predomination of patients from urban environment in the SG demonstrated that residence in the sectors of Chisinau allows optimal accessibility to

screening services for both HIV and TB, and the predominance of patients from the rural localities of the suburbs in the group with AIDS reveals the difficulty for accessing the medical services, confirmed by other researches [7, 10]. The relatively similar proportion of people without a stable place to living confirmed that both infections are socially determined and that screening for TB and HIV needs to be carried out simultaneously for all people living in poverty. The fact that both infections affect socially vulnerable strata was also demonstrated by the predominance of patients with vulnerable economic status, low level of education and active smoking, which were in the same proportion in both groups, similar to the results of national and international research [1, 7 -10]. History of recent migration was established in every sixth patient in both groups and demonstrated the spread of socially determinated infections in hard-to-reach and institutionalized subpopulations. In association with smoking, chronic alcoholism was diagnosed in every third case with AIDS and in a lower proportion in early stages HIV infection. The impact of the TB detection in the symptomatic PLH by primary healthcare worker was more evident in the early stages HIV infection, and phthisiopneumologist or infectious disease specialists more frequently detected AIDS patients. Late-detected forms, such as disseminated and generalized ones, were diagnosed in every fourth AIDS case, and only in every tenth with early detected HIV, similar to other local studies [8-10]. Oropharyngeal candidiasis associated with immune deficiency

was diagnosed in all AIDS patients. *Herpes zoster, toxoplasmosis* and pneumonia with *P. jiroveci* were diagnosed only in AIDS, as a defining factors for this stage [1, 5]. Anemia was established in all patients with AIDS and in a lower proportion in early HIV stages, and thrombocytopenia - only in AIDS stage. Although the clinical components of the bronchopulmonary syndrome, such as cough and expectoration, were constantly etsbalished in the selected patients, the signs of general intoxication - malnutrition/wasting syndrome and fever were more frequently observed in patients with TB on AIDS, similar results obtained by other reseaches. Microbiologically positive results were in a higher proportion in the group with early HIV detected group due to the predomination of pulmonary localization and adequate pulmonary functions for the coughing effort needed for the clinical specimen collection. Despite the differences in the serum level of CD4+ cells, the rate of therapeutic success was low in both groups, and was in the same proportion with those who were still continuing the antituberculosis treatment, constituting every third case respectively. The death rate was insignificantly higher in the group of patients with TB on AIDS - 9 (23.4%) vs. 9 (18.7%) cases in early HIV stages, caused by comorbidities associated with HIV infection.

Conclusions. The study identified that the risk factors for TB in patients diagnosed with AIDS stage of the symptomatic HIV infection were: residence in rural areas, low level of education,

mental and behavioral disorders, associated with multiple causes, such as consumption of alcohol, drug use and toxoplasmosis of the central nervous system.

Primary health care workers contributed to a higher proportion in the detection of TB in patients in the early stages of HIV infection, and specialists – in the AIDS stage. Late detected forms, including pulmonary disseminated and generalized, were diagnosed in a higher proportion in patients with AIDS. Oportunistic infections in patients with AIDS was confirmed by the presence of oropharyngeal candidiasis, infections with *herpes zoster, toxoplasmosis, P. jirovecii* pneumonia.

The reduced rate of therapeutic success was associated with a similar rate of those who were still continuing treatment due to therapeutic indications, and the death rate was high regardless of the level of immunosuppression. Which was determined by the underlying conditions associated with HIV infection

As practical recommendations with application value we have established:

The complex clinical, radiological and serological screening for TB and HIV infection is recommended to be done by all primary healthcare workers with a vulnerable economic status, with a low level of education, residents of poor or rural areas, with mental illness or behavioral disorders.

The standardized management of TB patients with HIV infection causes a high mortality regardless the degree of immunosuppression, and this fact requires the implementation of

a complexity preventiona; measures for both infections, as well as an individualized therapeutic approach.

CHAPTER IV
THE ROLE OF MICROBIOLOGICAL AND RADIOLOGICAL TESTS IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS IN THE CONDITIONS OF THE PANDEMIC COVID-19

Tuberculosis (TB) is a social-determinate disease with a high prevalence in the Eastern European Region (EER), including in the Republic of Moldova [1, 2, 3, 4]. The main barriers to achieving TB control in EER are social, economic, educational and psychological issues [1, 18]. According to WHO, EER is a high-risk zone for communicable diseases, with an inadequate concern regarding social determinants of health [4, 5, 14-16]. Almost all countries listed in the WHO list with the highest burden of multi-drug resistant tuberculosis (MDR-TB) are located in the EER [3, 12, 13, 16]. The countries with the highest rates of tuberculosis (TB) are the poorest and have many unequal social inequalities including very limited access to healthcare [1, 14, 16]. The studies showed that affected by TB people usually live in absolute poverty, which provides the ideal condition for the spreading of Mycobacteria tuberculosis [1, 4, 5, 8, 9]. Often in those households, poverty is associated with malnutrition and HIV infection, which both conditions diminish the immune resistance of the organism making them vulnerable to tuberculosis [5, 9, 10].

In the Republic of Moldova (RM) tuberculosis is one of the priority public health problems, and its prevention and control are

strategic objectives of the Strategy ,,European Moldova" which integrates the principles of the National Public Health Programme [11]. Statistical data from recent years demonstrated an improvement in the epidemiological indicators, as a result of the provision of patient-centred TB healthcare services, based on prevention, detection and treatment [2, 6, 7]. The constantly positive trend from the pre-pandemic period (until 2019) experienced an alarming decline in the context of the COVID-19 pandemic [6, 7, 8, 11, 12, 13]. The monitoring indicators assessing the response to TB, which included data from 2019, known as the pre-pandemic year established a 38.7% reduction in the number of new, relapse cases detected in 2020 compared to 2019 and a slight increase of 2.7% in 2022 compared with 2021. Starting with 2020, followed by 2021 and 2022 the TB epidemiological indicators were reported to the Moldovan population of 3.079.908 people (including right bank– 2.053.238, left bank – 378.329) and children – 648.341 (right bank – 561.961, left bank – 86.380). The incidence of new cases and relapses in 2022 was 68.8/100.000 population (2.121 cases) and in 2021 - 67.1/100.000 population (2.068 cases) with the rate of TB-HIV co-infection of 13%. In 2022, 1.666 new cases of TB were register compared with 1.614 new cases in 2021 the incidence was calculated to 54.1/100.000 population and was compared to 52.4/100.000 population in 2021. The incidence of TB relapses in 2022 was 14.8/ 100.000 population (457 cases), compared to 2021- 14.7/100.000 population (454 cases), constituting 22% of

the global incidence. The rate of pulmonary forms with lung destruction in new pulmonary TB cases in 2022 was 37% (559 cases) and in 2021 - 41% (591 cases), which is double compared with pre-pandemic period [6, 7]. The mortality was 5.7/100.000 population and death registered until one year after detection constituted 43% [6, 7]. Exposed data demonstrated delayed diagnosis of late detected forms of pulmonary TB, but no clinical and radiological aspects were assess in correlation with the methods of diagnosis – microbiological and radiological. The aim of the study was to establish the role of microbiological and radiological tests in diagnosis of pulmonary tuberculosis in the actual epidemiological context of the COVID-19 pandemics.

Material and methods. The research was conduct as retrospective, selective, and descriptive and included a cohort of 172 patients diagnosed with pulmonary tuberculosis during the period 1.1.2020-31.12.2022 in Chisinau, RM. The inclusion criteria were: age older than 18 years, tuberculosis diagnosed by the pulmonologist, detected as the new case and signed informed consent. The patients were distributed in two groups, 1-st group included 116 patients diagnosed with pulmonary TB through radiological investigations, without microbiological positive tests and were compared with the 2-nd group of 56 patients with positive for tuberculosis assays (Ziehl-Neelson staining, PCR assay Gene Xpert MTB/Rif). The diagnosis of pulmonary TB was established according to the criteria provided by the national policy Nr. 123. Every patient was investigated through the

sputum examination at Ziehl-Neelson staining, PCR assay Gene Xpert MTB/Rif and culture on the Lowenstein-Jensen and liquid BACTEC media. In the 1st group, the radiological methods were applied - chest X-ray examination in 2 incidences -116 (100%), tomosynthesis-23 (20%) and high-resolution computed tomography -14 (12%). In 35 (30%) cases, 3D bronchial fibroscopy with the aspiration of tracheo-alveolar liquid was performed. The study's schedule included data about a) biological and social peculiarities, demographic characteristics (living in urban/rural areas); b) economic peculiarities: economic state (employed, unemployed, retired, disabled,) and health-insurance coverage (presence/lack of health insurance); c) characteristics with high risk: homelessness, migration, history of detention, infectious contact; d) case-management: health care seeking behaviour and addressability to healthcare settings, methods used for TB detection, the medical staff involved in the detection, comorbidities, complications and HIV status; e) TB-related characteristics: localization (pulmonary/secondary extrapulmonary), results of microbiological assays, anti-tuberculosis treatment and the outcome. Other investigations performed were: hemoleucogramme, urine analysis, serum biochemical tests (transaminases, urea, creatinine, lactate dehydrogenase), and tests for HIV markers. The statistical assays have been used for the analysis of variation (ANOVA test) and Student's t-test. Statistical significance was determined by the

value of $p<0.05$. Multiple linear regression (RML) was performed to calculate the Odds Ratio for the evaluation of the risks.

Results and discussion. Distributing patients by sex, was determined that in the 1st group 62% were men and 38% women, the male/female ratio=1.6/1, while in the 2nd group 78% were men and 22% women with the rate of 3.7/1. The comparative analysis showed that men were more often diagnosed through microbiolgoical methods, which is associated with high contagiousness, with an OR=3.1 (95%CI: 1.25-4.19), χ^2 =0.03. By distributing the patients into age groups according to the WHO classification was estabished in the 1-st the highest rate were integrated in the group between 35-44 years old (y.o.) - 27%, followed by 45-54 y.o. 24% and 25-34 y.o. and 55+ y.o. with a similar rate 17%, then of the youngest 18-24 y.o. - 14% of patients. In the 2-nd group predominated patients between 35-44 y.o. 28%, followed by 45-54 y.o. 25%, 25-34 y.o. - 22% and in the lowest proportion 55+ y.o. 14% and 18-24 y.o. 11%. Distributing patients in 2 age groups, younger 35 and older 35, there were no statistical differences between them. The biological peculiarities of the patients confirmed that both young age people and older have a similar chance of developing TB and are to be detected through microbiological or radiological assays. So, for patients of any age we will apply the prevention measures to reduce the risk of developing TB and in cases with clinical suspicion to be investigated according to the national policy, performing microbiological and radiological tests. Evaluating the place of

residence of patients when TB was diagnosed, the urban location was established in majority of patients of all groups - 76% in the 1st group and 65% in the 2-nd group. Patients from the 2-nd group were living more frequently in rural localities 36% vs. 24% cases in the 1st group. So, radiological methods for diagnosis were more accessible to patients living in urban sectors compared with rural areas, without statistic evidence. The homeless were identified in a small proporion in both groups: 3% vs 9%. The data are displayed in Table 7.

Table 7.

Distribution according to the biological peculiarities and demographics (%)

Indicators	**1st group (n=116, P%)**	**2nd group (n=56, P%)**	**P**
Men	72 (62)	44 (78)	**<0,05**
Women	44 (38)	12 (22)	**<0,05**
18-24 y.o.	16 (14)	6 (11)	>0,05
25-34 y.o.	20 (17)	12 (22)	>0,05
35-44 y.o.	32 (27)	16 (28)	>0,05
45-54 y.o.	28 (24)	14 (25)	>0,05
55+	20 (17)	8 (14)	>0,05
Urban	88 (75)	38 (65)	>0,05
Rural	20 (17)	20 (36)	>0,05
Homeless	4 (3)	5 (9)	**<0,01**

Note: n is P%, absolute number and percentage of media; P* - significance at Anova test.

The patients were evaluate according to the last level of academic education and divided into several groups: primary, incomplete secondary, completed secondary (general school, specialised secondary) and superior studies. In the 1st group predominated the patients with secondary level 48%, followed by incomplete secondary 27%, then specialised level 14% followed in a minor proportion primary 7% and superior studies-2%. In the 2-nd group every second had secondary graduated education 50%, followed by incomplete secondary studies 25%, then primary education 14% and specialised degree in 11% cases. Low level of academic education slightly predominated in the 2-nd group – 50 (89%) vs. 98 (84%) cases in the 1st group. This finding demonstrated that the low level of academic education predisposes the development of TB, but has no impact on access to certain detection methods. Tuberculosis affects socially vulnerable groups and while evaluating the economic status was found that in the 1st group predominated employed individuals 42%, then unemployed 34% and in a lower proportion students 10 % and retired 7 %. In the 2nd group the patients with a stable economic state as being employed were only 25% and the majority were socio-economical vulnerable – 42 %, retired 14%, disabled 11% and students 7%. So, this finding demonstrated the fact that the low socio-economical level predisposed to detection by microbiological methods as being symptomatic, because they have a limited access to radiological methods, with an OR=2.9

(95%CI: 1.19-3.96), χ^2 =0.02. The obtained data confirmed that socio-economical support and awareness should be provide to all socially vulnerable groups, giving them accessibility to radiological investigations. The distribution of patients according to marital status classified them in groups: married, single, divorced, widowed. In the 1-st group predominated married individuals - 60%, followed by single state – 24 % and in a similar proportion divorced/widowed 16 % of cases. In the 2-nd group predominated married 57 %, followed by singles 33 % and divorced/widowed 10 %. Single either married state was established in a similar proportion in patients from both groups. Exposed data are reflected in the Table 8.

Table 8.

Distribution according to the social peculiarities (%)

Indicators	**1st group (n=116, P%)**	**2nd group (n=56, P%)**	**P**
Primary	8 (7)	8 (14)	>0,05
Incompleted secondary	32 (27)	14 (25)	>0,05
Secondary general	56 (48)	28 (50)	>0,05
Specialised training	16 (14)	6 (11)	>0,05
Superior studies	2 (2)	0	>0,05
Employed	48 (42)	14 (25)	**<0,05**
Unemployed	40 (34)	24 (42)	>0,05
People with disabilities	8 (7)	6 (11)	>0,05

Student	12 (10)	4 (7)	>0,05
Retired	8 (7)	8 (14)	>0,05
Married	70 (60)	32 (57)	>0,05
Single	28 (24)	18 (33)	>0,05
Divorced/widowed	16 (16)	6 (10)	>0,05

Note: n is P%, absolute number and percentage of media; P* - significance at Anova test.

Considering the totality of the particularities with an impact on the case-detection was established that men and patients residing in rural localities, with the socially vulnerable state were more predisposed to the detection through the microbiological methods, which involves an important epidemiological risk and women. Patients residing in urban localities and economically stable were more detected through the radiological investigations without a positive microbiological state.

Analyzing the scientific review, which demonstrated that the harmful habits with high risk for TB are: smoking, alcohol consumption and the illicit drug use was identified that in the 1-st group active smoking was identified in 62 %, chronic or abusive alcohol consumption in 2 % and no drug use. While in the 2-nd group smoking was established in 77 %, alcohol consumption 16 % and 4 % of patients were drug users. Epidemiological contact was established at 21 % of the 1-st group which involved the systematic screening and in 11 % of the 2-nd group and represented a contributing and facilitating factor for radiological investigation in scope of diagnosis

OR=4.1 (95%CI: 2.1-5.8), χ^2 =0.01. Returned from abroad during the last 12 months 7 % in the and 4 former detainees (3 %). All these characteristics related to the socio-economic peculiarities exposed an important impact on the diagnosing methods of tuberculosis in the epidemiological conditions of COVID-19 epidemics.

National policy recommends the detection of the new cases of the pulmonary tuberculosis through the microbiological examination of the symptomatic cases. Studying the case-detection characteristics of the patients, it was found that in the 1-st group 27 % of the cases were people from high-risk groups and were detected through the systematic screening compared with 15 % from the 2-nd group, represents an indicator of accessibility to the preventive healthcare services. By the other side an increased use of the systematic screening in the 1-st group was conditioned by a higher rate o patient with high risks: HIV-infection 10 % vs. 4 %, comorbidities such as diabetes mellitus 4 % vs. 1 %, mental disorders in 6 % vs 1 % in the 1-st group vs 2-nd group, respectively, immunosuppressive conditions and chronic immunosuppressive treatment for autoimmune pathologies in 2 % in the 1-st group. Passive detection of symptomatic cases predominated in the 2-nd group 73% vs. 48% and constitute an indicator of the late detection of contagious forms. The direct addressing to the specialised services in physiology was used in a slightly higher proportion by the patients of the 2-nd group 32% vs. 25% in the 1-st group

which is an indicator of reduced accessibilities either of lack of confidence in the primary healthcare services.

Clinical aspects when diagnosis of tuberculosis was established were characterized by subacute onset (7-30 days) in 67% patients from the 1st group vs. 9% in the 2nd group and a lost lasting evolution (>90 days) in the 2nd group 60% vs. 7% in the 1st group. A similar proportion of the patients in both groups complained of tuberculosis with suspicious symptomatology between 30 and 90 days till diagnosis 25% vs. 31%, respectively. Obtained data demonstrated the presence of a significant number of patients detected in more than 1 month after the onset of the symptoms in the 2-nd group as being characteristic for accessing the microbiological methods of investigations. Clinical signs of the intoxication syndrome – asthenia, lost of weight, night sweats, fever/feverish and the bronchopulmonary syndrome – cough, mucopurulent expectorations were identified in all patients of the 2-nd group and only in 76% of the 1-st group. Data demonstrated an important number of symptomatic individuals in the group detected by microbiological assays as being characteristic for passive detection. Distributing patients into the groups according to the clinical diagnosis was established infiltrative form in all patients from the 1-st group vs. 86% in the 2-nd group. While disseminated form and cavernous were diagnosed only in the 2-nd group, with 7% for each form. Both lungs were involved in the infectious process in every second patient from

2-nd group and in every fourth in the 1-st group and extensive forms to more than 3 lung segments were detected in all patients from the 2-nd group vs one fourth in the 1-st group (Table 9). So, infiltrative form of pulmonary tuberculosis ware diagnosed in the majority of patients in all investigated groups, in similar rates reported by the epidemiological indicators for the Republic of Moldova. While extensive forms on more than three segments was confirmed in the majority of patients from the 2-nd group as being straightly and positively correlated with the presence of the bronchopulmonary symptomatology (r=0.89) and low correlated with the general intoxication syndrome (r=0.21). In the mean, the bilateral location of the infectious process was tightly positively correlated with the bronchopulmonary symptomatology (r=0.82) and tightly positively correlated with the general intoxication syndrome (r=0.11).

Table 9

Distribution according to the radiological peculiarities (%)

Indicator	**Lot 1 n=116 P(%)**	**Lot 2 n=56 P(%)**	**P**
Infiltrative	116 (100)	48 (86)	>0,05
Diseminated	0	4 (7)	>0,05
Fibro-cavernous	0	4 (7)	>0,05
Both lungs affected	30 (26)	32 (57)	**<0,001**
One lung affected	86 (74)	24 (43)	**<0,001**
Extensive TB on 3+	31 (27)	56 (100)	**<0,001**

lung segments			
Limited TB	85 (73)	0	**<0,001**

Note: n is P, absolute number and percentage of media; P* - significance at Anova test.

The most important indicators of the interruption of the infection transmission and epidemiological danger are those representing the treatment effectiveness (Table 10). So, in the 1st group the therapeutic success was registered at 82 % vs 62 % in the 2-nd group, which included all patients who completed the treatment. In the 2-nd group the therapeutic success was established at 62 % of which all the cases were cured. Therapeutic failure was identified in a similar low proportion of cases – 2 % vs 4 %, respectively, and the patients lost to follow-up were as well a few 6 % in both groups. Deaths were estbalished only in a few cases of the 2-nd group 4 (8 %). Therapeutically interruptions and incompliance were more often detected in the 1-st group 23 % vs 7 %, argued by a higher rate of comorbid patients which interrupted due to clinical intolerance, but a few cases interrupted as the clinical state improved and they left the country. Adverse drug reactions were registered in every seventh patient in both groups.

Table 10.

The indicators of the treatment's effectiveness in the groups of patients (%)

Indicator	**Lot 1 n=116 P(%)**	**Lot 2 n=56 P(%)**	**P**

Treatment success	95 (82)	36 (62)	**<0,05**
Therapeutical failure	4 (2)	2 (4)	>0,05
Interrupted/lost to follow-up	3 (6)	3 (6)	>0,05
Died	0	4 (8)	**<0,05**
Fully compliant	89 (77)	52 (93)	>0,05
Adverse drug reactions	16 (14)	9 (16)	**<0,05**
Limited TB	95 (82)	36 (62)	**<0,05**

Note: P-absolut number and percentage of media; P* - significance at Anova test.

The research evaluated the clinical-radiological peculiarities of the patients diagnosed with pulmonary tuberculosis through the radiological and microbiological methods in the actual epidemiological context associated with COVID-19 pandemics. The results of the study demonstrated that the biological peculiarities of the patients was male gender, with no differences while distributing them in age groups and the predomination of cases residing in urban sectors having detected TB through the radiological methods and in rural areas having diagnosed TB through the microbiological assays. Similar results are often reported in local and international studies [1, 3-10]. Social peculiarities of the patients regardless the methods of diagnosis were economical vulnerable state, primary and secondary incomplete academic level and harmful habits, such as tobacco smoking and alcohol abuse. The epidemiological risk factor which was more involved in the development of TB among patients

detected through the radiological investigations was TB contact, similar to other research [8-10]. The medical factors, such as comorbidities with high risk for TB (HIV infection, diabetes mellitus, mental disorders and immune suppressive treatment/conditions) were more involved in the same group [8-10]. Both TB contact and associated diseases are eligibility criteria for active screening for TB, which are fully recognised by local studies [8-10]. Synthetically analyzing the groups, was concluded that patients diagnosed by microbiological methods showed severe, late detected and long lasting evolution compared with those diagnosed through the radiological methods [11]. The correlation was very tight between the radiological extensiveness and clinical expressiveness of the bronchopulmonary and general intoxication syndromes in patients diagnosed by microbiological methods. The indicators of the treatment effectiveness was highly superior in the groups diagnosed through the radiological methods, in which were not detected died patients. Therapeutic failure was identified in a similar proportion of cases, and the loss to follow-up were found in a limited number of cases in both groups.

C**onclusions.**

The case-control study identified that the main peculiarities of patients diagnosed with TB regardless of methods of investigation were: male gender, low social and economic state and high prevalence of harmful habits such as smoking and alcohol consumption.

Patients diagnosed through the microbiological methods were residing more frequently in rural areas, with low accessibility to healthcare services, concluded through a long-lasting symptomatology, late detected forms of TB, high expressiveness of the clinical symptomatology and severe evolution, followed by a high rate of poor treatment outcomes, including death.

Patients diagnosed through the radiological methods were more frequently from urban sectors, from TB clusters, with comorbidities with high risk for TB sickness, such as HIV infection, diabetes and mental disorders which constituted included in active screening programs. The lack of positive microbiological assay results was the consequence of the unilateral and limited forms of pulmonary and determined a higher rate of treatment success rate.

As practical recommendations with applicative value were established:

Screening of the subpopulations with high risk should be supported through various ways including clinical, radiological and microbiological investigations for TB.

The standardized clinical-case management of TB patients should be oriented to actions aiming to address the main challenges for early detection and improve the treatment effectiveness, such as: social economical vulnerability, harmful habits and associated diseases.

CHAPTER V

FEATURES OF CLINICAL STATUS AND ASSOCIATED PATHOLOGY IN PATIENTS MLS-TB / HIV DEPENDING ON THE LEVEL OF IMMUNOSUPPRESSION

The SARS-CoV-2 pandemic has highlighted numerous issues in medical triage and the timely provision of care due to the massive influx of patients into healthcare facilities. Although the risk group for COVID-19 is well-defined [30], and it is well-known that age, sex, and comorbidities, including cancer, cardiovascular diseases, and especially diabetes mellitus, are major risk factors for COVID-19 patients, not all patients in the risk group experience severe disease-most recover without oxygen therapy and intensive care.

To address the strain on hospital beds and expand the capacity for outpatient care, it is crucial to study the pathogenetic mechanisms and identify effective prognostic markers for COVID-19.

Established criteria for COVID-19 severity include low albumin levels, high levels of D-dimer, ferritin, procalcitonin, IL-6, C-reactive protein, lactic acid, LDH, anisocytosis, and the count of polymorphonuclear leukocytes [26, 14, 5, 22].

In our previous studies, we have thoroughly analyzed the diagnostic significance of markers such as extracellular neutrophil traps (NETs), extracellular plasma DNA, hyaluronic acid levels, and the long non-coding RNA HAS2-AS in the leukocytes of

COVID-19 patients [2-3]. In this study, we examined the relationship between the expression level of the long non-coding RNA HIF1A-AS1 and the severity of COVID-19 in high-risk patients with diabetes, obesity, and hypertension.

ARDS, a severe form of acute lung injury, is one of the life-threatening complications of viral respiratory infections, including SARS-CoV-2 [12]. Fluid accumulation in the alveoli during ARDS leads to severe hypoxia [16, 25]. The transcription factor HIF—hypoxia-inducible factor—is the main regulator of the body's response to hypoxia [17-10]. HIF-1α transcriptional activity increases the survival of phagocyte cells and stimulates the expression of critical factors, including vascular endothelial growth factor (VEGF), as well as pro-inflammatory cytokines (TNF, IL-1, and IL-12) at the infection site [18]. It has been shown in numerous studies that viral infection can induce HIF-1α expression, which, upon activation, may favor the pathogen rather than the host [24, 11, 20, 21]. For instance, excessive production of pro-inflammatory cytokines, and ultimately, the cytokine storm, as a key factor in severe pneumonia in patients with H1N1 infection, is mediated by HIF-1α, which can induce the production of pro-inflammatory molecules at the site of inflammation [8].

The role of HIF-1α in the context of COVID-19 has also been demonstrated. Mingfu Tian et al., in their study, present findings showing that the mRNA level of HIF-1α is significantly higher in peripheral blood monocytes (PBMC) of COVID-19 patients compared to healthy individuals [30].

According to the authors, SARS-CoV-2, through the transmembrane protein ORF3a, induces HIF-1α, which in turn enhances viral replication and inflammatory responses [30]. In another study, Jahani also emphasizes that in severe cases of COVID-19, the activation of HIF-1α is responsible for the progression of the cytokine storm [10].

Recently, considerable attention has been given to the investigation of long non-coding RNAs (lncRNAs), which are involved in numerous physiological and pathological processes through epigenetic regulation and complex signaling pathways. The expression level of HIF-1α is regulated by various factors, including, we believe, potentially through the signaling pathways of the long non-coding RNA HIF1A-AS1.

Long non-coding RNAs are generally defined as endogenous cellular RNAs longer than 200 nucleotides, which are ubiquitously found in eukaryotic genomes but lack protein-coding potential. lncRNAs play an important role in maintaining cellular homeostasis [34].

The functions of HIFA-AS1 are still insufficiently understood. Only a few studies have documented the biological significance of HIF1α-AS. Increased expression of HIF1A-AS1 has been reported in thoracoabdominal aortic aneurysm [31]. HIF1A-AS1 has also been proposed as a biomarker for colorectal carcinoma [7]. Functionally, HIF1A-AS1 is pro-apoptotic and anti-proliferative in vascular smooth muscle cells, Kupffer cells, and umbilical vein endothelial cells [34, 37].

However, the expression of HIF1A-AS1 in COVID-19 has not been studied. In our work, we publish the results of our own research, which show that the expression levels of HIF1α and HIF1A-AS1 increase in severe COVID-19 patients from the risk group at the pre-hospital stage, prior to the onset of clinical complications and severe hypoxia. We hypothesize that the expression level of HIF1A-AS1 can be considered a prognostic marker for severe COVID-19 at the pre-hospital stage.

To date, tuberculosis (TB) remains one of the biggest global burdens on the health care system [10]. According to the World Conservation Organization of Health (WHO), in 2021, 10.6 million people fell ill with TB and 1.6 million died from it [17, 18]. To the emergence of the coronavirus infection caused by the SARS-CoV-2 (COVID-19) virus, TB was the main one the cause of death from one infectious agent [2, 5, 10, 11, 17, 18]. Ukraine is one of 27 countries of the world, where 85 % of the incidence of TB is concentrated, and it ranks 4th in the world in terms of prevalence. In almost 100 new cases of TB disease are registered in Ukraine every day [10, 11, 12, 16].

One of the main reasons for the epidemically tense situation with TB in the world and in Ukraine is spread of TB with multiple drug resistance (MDR-TB) [14, 16]. Total number such cases in 2023 amounted to 1,955, of which 1,326 were new cases [10, 12, 13, 15]. Number cases of tuberculosis with extended resistance - 228, of which 136 were diagnosed for the first time [11, 16].

At the same time, TB continues to be the main opportunistic disease against the background of HIV-infections [1, 4, 8, 9]. In patients with deep immunodeficiency, not only lung diseases are often registered, but and extrapulmonary localization of TB, especially against the background of a significant violation of immunity, which occurs after the number of CD4+ lymphocytes below 200 cells/μl [2, 3, 6, 13, 14, 15]. In addition to severe immunosuppression and generalized TB, in such a category of patients there is a variety of accompanying pathology, which worsens the clinical condition of patients and becomes the basis for the development of etiotropic side reactions therapy and formation of multiple organ failure [7, 12, 15, 16]. Therefore, clinical research the status of patients with MDR-TB / HIV and their accompanying pathology, is an actual direction for further study and development of new methods of pathogenetic treatment aimed at reduction of adverse reactions of antimycobacterial therapy (AMBT) and antiretroviral therapy (ART), improving the course of MDR-TB / HIV co-infection and treatment efficiency indicators.

The purpose of the work is to study the features of the clinical status and accompanying pathology in patients with MDR-TB / HIV co-infection depending on the level of immunosuppression.

Materials and methods. 104 patients aged 20 to 55 participated in the study, the average age was 37.2±7.8 years. All min ori were HIV-positive with laboratory confirmation MDR-TB

with mycobacterium resistance to first- and second-line drugs. Patients with MDR-TB/HIV were homogeneous in terms of prior treatment history, adherence level, and profile resistance Depending on the level of immunosuppression, they were distributed as follows:

- 1 group (LH-1) – 52 patients with MDR-TB/HIV with a level of CD4+ lymphocytes < 50 cl/μl;

- 2nd group (LH-2) – 52 patients with MDR-TB/HIV with the level of CD4+ lymphocytes from 200 to 50 kl/μl.

Research design: simple, open, randomized.

Inclusion criteria:

- the patient's consent to participate in the study;

- newly diagnosed MDR-TB against the background of HIV infection;

- age of patients from 20 to 55 years;

- patients with MDR-TB/HIV who have not previously taken second-line AMBPs and ART;

Exclusion criteria:

- refusal of the patient;

- repeated cases of MDR-TB against the background of HIV infection;

- patients who were previously treated with second-line drugs;

- patients who interrupted treatment and were transferred to palliative treatment in the previous period case;

- patients who were in the terminal phase of MDR-TB and HIV infection;

- presence of acute kidney or liver failure;

- mental disorders.

The results of the research were processed using variational methods statistics, calculated averages of absolute and relative values, their errors. Used parametric criteria for differences: M - arithmetic mean; m is the error of the mean arithmetic, expresses the reliability of the obtained average value of the investigated characteristic; t - confidence coefficient (Student-Fisher difference reliability criterion); p - level indicator significance.

To compare organized groups, we used the determination of frequency of occurrence studied criteria. Quality indicators are presented in the form of Q ± mq (Q is the frequency of appearance of the feature, mq - standard deviation). Statistical significance was reliable at p < 0.05.

Statistical data processing was carried out using software Microsoft Excel 2016 (license no. 00201-10554-16848-AA351), all calculations were performed by means of Statsoft Statistica 8.0 (license number STA862D175437Q).

Results and discussion. According to the data of the conducted research, the clinical condition of the patients on MDR-TB/HIV with pronounced immunodeficiency upon admission to the inpatient department was assessed as "extremely severe" in 11 patients with LH-1 (21.2%) and in 7 patients with LH-2 (13.5); "heavy" - in 78.8 % of cases LH-1 (41 patients) and 67.3 % - LH-2

(35 patients). Condition of medium severity in LH-1 patients were not registered, however, among LH-2 patients such a state assessment took place in 10 cases (19.2 %). Patients of both treatment groups also did not have a satisfactory condition registered.

Before the start of taking AMBT and ARVT, patients of both groups presented a large number of complaints, which was associated, on the one hand, with a severe immunodeficiency condition caused by infections: HIV, generalized TB with extrapulmonary localizations, opportunistic and fungal processes, and, on the other hand, the presence of various concomitant somatic pathologies, addictions (alcohol, narcotics), and the presence of unsuccessful previous episodes of treatment.

Complaints of an intoxication nature (fever, weight loss, weakness, sweating) dominated over broncho-pulmonary symptoms (cough, shortness of breath, chest pain) (Table 11).

Fever with a body temperature above 38 0 C was registered in 94.2 % of patients on the 1st group (49 cases), and in 78.8 % (41 patients) of the 2nd group ($p < 0.05$). Such a high rate of patients with fever confirmed that most patients had active inflammatory processes, which were accompanied by pronounced intoxication, progression of MDR-TB, HIV, and others opportunistic diseases against the background of deep immunosuppression.

Table 11

Characteristics of clinical signs in patients with MDR-TB/HIV depending on the level of immunosuppression (LH-1 n=49; LH-2 n=51; %)

Clinical signs / Complaints		Patients with CD4+ level < 50 cells/μL (LH-1)		Patients with CD4+ level from 200 to 50 cells/μL (LH-2)	
		Together (n=49)		Together (n=51)	
		абс	Q ± m_q%	абс	Q ± m_q%
Complaints of intoxication genesis	fever	49	94,2 ± 3,2	41	78,8 ± 5,7 *
	weight loss	40	76,9 ± 5,8	29	55,8 ± 6,9 *
	cachexia	17	32,7 ± 6,5	9	17,3 ± 5,2 **
	inquisitiveness	42	80,8 ± 5,5	33	63,5 ± 6,7 *
	weakness	52	100,0 ± 0,0	45	86,5 ± 4,7 *
Respiratory symptoms	dyspnea	29	55,8 ± 6,9	17	32,7 ± 6,5 *
	cough	29	55,8 ± 6,9	22	42,3 ± 6,9 *
	chest pain	7	13,5 ± 4,7	5	9,6 ± 4,1 *
General somatic symptoms	loss of appetite	45	86,5 ± 4,7	37	71,2 ± 6,3 *
	pain in the epigastrium	32	61,5 ± 6,7	30	57,7 ± 6,9
	zatrud.kovtan	20	38,5 ± 6,7	14	26,9 ± 6,1 *
	diarrhea	33	63,5 ± 6,7	19	36,5 ± 6,7 *
	bloating	13	25,0 ± 6,0	9	17,3 ± 5,2 *
	jaundice	7	13,5 ± 4,7	4	7,7 ± 3,7 *
	pain in the lower back	35	67,3 ± 6,5	24	46,2 ± 6,9 *
	headache	15	28,8 ± 6,3	9	17,3 ± 5,2 *
	irritability	25	48,1 ± 6,9	15	28,8 ± 6,3 *
	euphoria	37	71,2 ± 6,3	22	42,3 ± 6,9 *
	insomnia	19	36,5 ± 6,7	11	21,2 ± 5,7 *

	convulsions	3	5,8 ± 3,2	0	0,0 ± 0,0
	lower vision	23	44,2 ± 6,9	17	32,7 ± 6,5 *
	important n/ends.	30	57,7 ± 6,9	15	28,8 ± 6,3 **
	dysuria	27	51,9 ± 6,9	13	25,0 ± 6,0 *
	enlarged lymph nodes	46	88,5 ± 4,4	38	73,1 ± 6,1 *
	skin rash	21	40,4 ± 6,8	11	21,2 ± 5,7 **
	back pain	15	28,8 ± 6,3	9	17,3 ± 5,2 *
	joint pain	35	67,3 ± 6,5	21	40,4 ± 6,8 *

Notes:

1. *- the difference is significant between LH-1 and LH-2 groups ($p < 0.05$);
2. **- the difference is significant between LH-1 and LH-2 groups ($p < 0.01$);
3. #- the difference is significant between LH-1.1 and LH 1.2/LH-2.1 and LH-2.2 groups ($p < 0.05$).

Weight loss was observed on average by 11.0 ± 3.2 kg in 40 patients (76.9 %) of LH-1 and in 29 patients (55.8 %) LH-2 ($p < 0.05$). Some patients developed cachexia: LH-1 patients a cachectic state occurred in 32.7 % (17 cases), against 17.3 % (9 patients) of LH-2 ($p < 0.01$). A significant decrease in body weight was accompanied by asthenia in a very pronounced form. Significant weakness, as one of the earliest complaints, was characteristic of all LH-1 patients (100 % of cases), and 86.5 % of LH-2 patients. Sweating, especially at night, was also noted in more patients - 80.8 % of LH-1 and 63. 5% of LH-2 ($p < 0.05$).

Shortness of breath prevailed among respiratory symptoms, which was registered in patients with LH-1 in 29 cases (55.8 %) and in 17 (32.7 %) persons LH-2 ($p < 0.05$).

The cough was mostly dry and unproductive, with difficult discharge sputum, and bothered 29 patients with LH-1 (55.8 %) and 22 (42.3 %) with LH-2 ($p < 0.05$) (see Table 1).

Pain in the chest associated with the act of breathing was explained by damage to the leaves pleura, it was noted in 7 (13.5%) and 5 (9.6%) cases of LH-1 and LH-2 ($p < 0.05$).

Patients of both groups presented a large number of complaints on the side of the gastrointestinal organs tract: loss of appetite was the most common concern – 86.5% of LH-1 patients (45 cases) and 71.2% of LH-2 patients (37 people) ($p < 0.05$); difficulty swallowing and discomfort when food passes through the esophagus – 38.5% of patients with LH-1 and 26.9% of LH-2, which was the most complex manifestation of oropharyngeal.

Candidiasis (OFK), due to total fungal damage to the oral mucosa cavity, tongue and esophagus. In addition, many were diagnosed with a fungal infection of the intestinal mucosa, which was clinically manifested by diarrhea in patients of both groups (63.5 % LH-1 and 36.5 % LH-2), and abdominal distension – 25 % LH-1 and 17.3 % LH-2 ($p < 0.05$) (see Table 11).

In patients on MDR-TB/HIV in a state of pronounced immunodeficiency, when examined the presence of polymorbid pathology with damage to numerous organs and systems was confirmed (Table 12).

On the part of the respiratory system, respiratory insufficiency (PN) 1 was most often diagnosed degree, which was determined in 19 patients with LH-1 (36.5 %) and 11 (21.2 %)

with LH-2 ($p < 0.05$). More 2nd degree DN, pronounced by symptoms, occurred in 10 LH-1 (19.2 %) and 6 LH-2 (11.5 %) patients ($p < 0.05$).

Chronic bronchitis in the phase of remission was determined in 16 patients (30.8%) with LH-1 and in 15 patients with LH-2 (28.8 %), in a state of exacerbation - in 13 (25 %) and 7 (13.5 %) people, respectively ($p < 0.05$). Chronic obstructive pulmonary disease (COPD) of the 1st degree occurred in 17 patients (32.7 %) of LH-1 and 12 (23.1 %) LH-2 ($p< 0.05$), in comparison with more complicated COPD of the 2nd degree – 11 cases (21.2%) LH-1 and 5 (9.6%) LH-2 ($p < 0.01$) (see Table 12).

Pronounced intoxication had a negative effect on the work of the cardiovascular system as well subsequently led to the development of carditis with heart failure (HF). CH of various degrees nu (1,2A and 2B) was diagnosed in 38 patients with LH-1 (73.1 %) and in 23 (44.2 %) with LH-2 ($p < 0.05$), and was determined 1.7 times more often than in the group of patients with more pronounced immunodeficiency.

The hepatobiliary system had certain disorders of the functional state of the liver and biliary tract in patients of both groups, which was caused by the presence of viral infections in the anamnesis hepatitis: hepatitis B (38.5 % and 26.9 % of patients, $p < 0.05$), and hepatitis C (63.5 % and 36.5% of cases, p < 0.05) in LH-1 and LH-2, respectively. Viral activity of hepatitis was not determined under the conditions anti-tuberculosis hospital. In 50 % of patients LH-1 and in 32.7 % of LH-2 ($p <$

0.05), there was chronic toxic hepatitis, which was caused by the influence of narcotic substances, alcohol abuse, as well as taking ineffective antimycobacterial drugs (AMBP) in previous episodes of treatment before obtaining the results of drug sensitivity (see table 12).

Table 12

Characteristics of concomitant pathology in patients with MDR-TB/HIV depending on immunosuppression (LH-1 n=49; LH-2 n=51; %)

Organ systems / Clinical diagnoses		Patients with CD4+ level < 50 cells/μL (LH-1)	Patients with CD4+ level from 200 to 50 cells/μL (LH-2)
		Together (n=49)	Together (n=51)
Respiratory system	Chronic bronchitis, exacerbations/remissions	25,0 ± 6,0/ 30,8 ± 6,4	13,5 ± 4,7 */ 28,8 ± 6,3 *
	Respiratory insufficiency 1 st./2 st.	36,5 ± 6,7/ 19,2 ± 5,5	21,2 ± 5,7 */ 11,5 ± 4,4*
	COPD 1 st./2 st.	32,7 ± 6,5/ 21,2 ± 5,7	23,1 ± 5,8 */ 9,6 ± 4,1 **
	Pneumocyst. pneumonia	3,8 ± 2,7	0,0 ± 0,0
Cardiovascular system	Pericarditis	17,3 ± 5,2	7,7 ± 3,7 **
	SN 1 Art	51,9 ± 6,9	36,5 ± 6,7 *
	CH 2 A/	13,5 ± 4,7/ 7,7 ± 3,7	7,7 ± 3,7 */ 0,0 ± 0,0
	SN 2B	13,5 ± 4,7	9,6 ± 4,1 *
Gastrointestinal tract	Hypertensive disease	86,5 ± 4,7	71,2 ± 6,3 *
	Oropharynx. candidiasis	61,5 ± 6,7	57,7 ± 6,9 *
	Chronic gastritis	28,8 ± 6,3	21,2 ± 5,7 *
Hepato-biliary system	Expression. min. DPK, st. rem.	38,5 ± 6,7	26,9 ± 6,1 *
	Viral hepatitis B	63,5 ± 6,7	36,5 ± 6,7 *
	believe hep C, not	50,0 ± 6,9	32,7 ± 6,5 *

	specified act		
	Chronic hepatitis	25,0 ± 6,0	13,5 ± 4,7 **
	Cirrhosis	17,3 ± 5,2	7,7 ± 3,7 **
	Ascites	75,0 ± 6,0	55,8 ± 6,9 *
Urinary system	Chronic pyelonephritis	25,0 ± 6,0/	17,3 ± 5,2 */
	Chronic renal failure of the 1st-2nd century	63,5 ± 6,7/	38,5 ± 6,7 */
Gender system	Prostatitis	48,1 ± 6,9	26,9 ± 6,1 *
	Epididymorchitis	25,0 ± 6,0	13,5 ± 4,7 **
	Dysplasia cervix	80,8 ± 5,5	53,8 ± 6,9 *
Nervous system	Meningitis	7,7 ± 5,3 #	11,5 ± 6,4
	Toxoplasma. head of the brain	3,8 ± 2,7	0,0 ± 0,0
	Encephalopathy	100,0 ± 0,0	71,2 ± 6,3 *
	Polyneuropathy	38,5 ± 6,7	25,0 ± 6,0 *
The organ of vision	Enter the virus-bacterium.	34,6 ± 6,6	17,3 ± 5,2 **
Endocrine system	Idiopathic hypothyroidism.	63,5 ± 6,7	32,7 ± 6,5 **
	Diabetes, type 2	9,6 ± 4,1	3,8 ± 2,7 **
Skin diseases	Psoriasis	9,6 ± 4,1	3,8 ± 2,7 **
	Seborrhea	25,0 ± 6,0	17,3 ± 5,2 *
	Allergic dermatitis	21,2 ± 5,7	13,5 ± 4,7 *
	Herpes generaliz. 1/2 type	17,3 ± 5,2	7,7 ± 3,7**
Locomotor system	Arthritis	36,5 ± 6,7	25,0 ± 6,0 *

Notes:
1. *- significant difference between LH-1 and LH-2 groups ($p < 0.05$);
2. **- significant difference between LH-1 and LH-2 groups ($p < 0.01$);

3. #- the difference is significant between groups LH-1.1 and LH 1.2/LH-2.1 and LH-2.2 ($p < 0.05$).

In 13 patients with LH-1 (25%) and 7 patients with LH-2 (13.5%), the above-mentioned hepatitis led to the formation of cirrhosis of the liver, as well as ascites in 17.3% of cases of LH-1 and 7.7% of LH-2, ($p < 0.01$) (see table 12).

Chronic renal failure (CKD) developed in patients of both groups in the background generalized combined infection, accompanied by hypercreatininemia, hyperuricemia and slowing down the rate of glomerular filtration. Grade 1 CKD was registered in 63.5 % patients with LH-1 and 38.5 % of LH-2 (p < 0.05), while only 17.3 % of LH-1 and 13.5 % had CKD of the 2nd degree LH-2 ($p < 0.05$), which was accompanied by a greater increase in urea and creatinine (see table 12).

From the side of the nervous system, concomitant diseases were determined, which were mainly HIV-associated and developed as a result of the activity of opportunistic infections. 5 patients have LH-1 (9.6 %) and in 2 LH-2 patients (3.8 %) meningitis, cryptococcal, cytomegalovirus, of herpetic origin ($p < 0.01$). Cerebral toxoplasmosis occurred only in LH-1 patients 2 cases (3.8 %). HIV-associated encephalopathy was diagnosed in 100 % of LH-1 patients, and only 71.2 % of LH-2 ($p < 0.05$), which confirms the direct connection between the state of the nervous and immune systems, the latter was in a suppressive state and unable to restrain the activity of viruses- opportunists, tropic to nervous tissue.

As a result of the action of viruses and other infectious agents, not only the nervous system suffers, but also organ of vision, which was registered in 18 patients of LH-1 (34.6%) and 9 (17.3%) of LH-2 ($p < 0.01$), the lesion was accompanied by congestion of the optic nerve disc, corneal edema and the formation of precipitates on it, the formation of inflammatory nodes on the iris, clouding of the vitreous body, the development of chorioretinal foci on the fundus, which eventually led to decrease in acuity, or even loss of vision (see Table 12).

On the part of the endocrine system, the development of hypothyroidism was noted as a result of exhaustion body (pronounced intoxication syndrome, weight loss, cachexia), idiopathic

hypothyroidism was 1.9 times more common in patients with more severe manifestations of immunosuppression, and occurred in 33 patients with LH-1 (63.5 %) and in 17 (32.7 %) with LH-2 ($p < 0.01$) (see Table 12).

Conclusions. As a result of the conducted research, the following conclusions can be drawn: Symptoms of intoxication in the studied patients occurred more often than signs respiratory origin. Thus, fever was registered in 94.2 % of patients of the 1st group (49 cases), and in 78.8 % (41 patients) of the 2nd group, weight loss in 40 patients (76.9 %) of LH-1 and 29 patients (55.8 %) LH-2. Pronounced weakness was present in 100% of cases in the 1st treatment group, and in the 2nd group - only in 86.5% of patients. Shortness of breath, which was registered, prevailed

among the respiratory symptoms more often in LH-1 patients (29 people – 56.1 %) than in LH-2 patients (17 people – 31.3%). Cough was registered in 29 LH-1 (54.3 %) and 22 (42.3 %) LH-2 patients. Chest pain bothered 7 (13.5 %) and 5 (9.6%) patients with LH-1 and LH-2.

Numerous complaints from other organ systems were presented as follows: loss appetite 86.5% LH-1 (45 cases) and 71.2% LH-2 (37 patients), difficulty swallowing and discomfort when passing food through the esophagus in 38.5% of patients with LH-1 and 26.9% with LH-2, diarrhea in 63.5% of LH-1 and 36.5% of LH-2, bloating – 25% of LH-1 and 17.3% of LH-2; pain in the right hypochondrium in 67.3%
LH-1 and 46.2% LH-2; edema 57.7% LH-1 and 28.8% LH-2; insomnia - 36.5% and 21.2% on the 1st and 2nd groups respectively.

Patients with MDR-TB / HIV are diagnosed with many forms of concomitant pathology in the form of: respiratory and heart failure, oropharyngeal candidiasis, chronic gastritis, duodenal ulcer, viral hepatitis C, B and intoxication hepatitis genesis, phenomena of cirrhosis, hypothyroidism, chronic renal failure against the background of renal pathology, diseases of the genital organs (prostatitis, cervical dysplasia), encephalopathy with symptoms polyneuropathy, viral-bacterial uveitis, lesions of the skin and musculoskeletal system, etc.

All this indicates the presence of multiorgan pathology, which is more pronounced in patients in a deep state

immunosuppression, with the content of CD4+ lymphocytes from 50 µl/ml and below.

CHAPTER VI
THE IMPACT OF THE RISK FACTORS ON GENERALISATION OF TUBERCULOSIS INFECTION DURING COVID-19 PANDEMIC

COVID-19 infection was assessed by WHO as pandemic on 11th March 2020 and was established as a public health

emergency of international concern [1, 2, 13-16]. The COVID-19 pandemic reduced the number of notified TB cases by 18% from 7.1 million in 2019 to 5.8 million in 2020 with a slight increase to 6.4 million in 2021, 7.5 million in 2022 and stabilization to 10 million in 2023-2024 [13-15]. The dynamics of TB epidemiological indicators reflected the changes in accessibility of TB pecialized services and restructuring of the healthcare system, temporary closure or transformation of healthcare facilities into COVID-19 dedicated centers, reallocation of the healthcare staff to COVID-19 centers, financial barriers, isolation and difficulties in accessing transportation to medical facilities and lack of information [1, 2]. These barriers disproportionately affected certain high risk groups, including people with chronic health conditions, older adults, and socioeconomically disadvantaged populations, marginalized subpopulations, which finally led to an increased rate of late detected generalized TB. The COVID-19 pandemic shad a significant impact on specialized in TB healthcare system [16]. The main changes were: financial (loss of revenue), operational (shortage of personal, equipment, supplies, delayed care), modification in healthcare delivery (compulsory hospitalisation was replaced with ambulatory anti-TB treatment) which led to long-term changes (weakned the preparedness to a larger heath crisis), exacerbated existing healthcare disparities, making vulnerable risk groups to miss the screening measures (chest X-ray/ tuberculin skin test), and in recovery period was identified the requirement to reinforce the BCG vaccination [1,2].

Despite the decreasing of the epidemiological indicators of TB during the COVID-19 pandemic (by 39%) from 2019 till 2020 there was identified an increased rate of late detected and generalised forms [3-9]. Generalised TB (GTB) occurs when uncontrolled *Mycobacterium tuberculosis* infection disseminate through the blood stream, spread in the lungs and other extrapulmonary organs [1-3]. According to the specialized publications, the most affected extrapulmonary sites in GTB were: peripheral lymph nodes, bone and joints, meninges and brain, urogenital and gastrointestinal tracts [5]. Findings from multiple studies revealed that GTB follows after the primary tuberculous infection in severely immunocompromised patients (children under 5 years old, elders (65+), in people living with HIV and patients taking the immunosuppressive treatment or anti-TNF drugs) [3-11]. Less frequently GTB develop after the reactivation of latent tuberculous infection localized in the dormant foci on the background of diabetes mellitus, malnutrition, smoking and associated chronic respiratory diseases, silicosis or heavy alcohol consumption [5,9]. The social-epidemiological conditions which were frequently encontered in patients with GTB were close contact with a source of TB infection, lack of BCG vaccination and probably genetic susceptibility. Poverty and overcrowding were identified in almost all patients with TB [3-11]. Academic publications showed that beside the complexity of the risk factors, the non-specific symptomatology contributed to late detection and dissemination of the pulmonary TB infection in extrapulmonary

sites [6-9]. General and systemic symptoms which were encountered in patients with GTB were weakness, loss of appetite, weight loss, fever/feverish, night sweats and chills. Constant pulmonary signs were persistent cough lasting more than 3 weeks, progressive dyspnea, hemoptysis and thoracic pain [6-9]. The extrapulmonary symptoms revealed the affected site or organs. In meningeal TB the patients complains were headaches, nausea and vomiting, which progress in stupor and coma. In genitourinary TB there were lumbar pain, pyuria, hematuria and can progress with the enlargement of the scrotal mass and sterility. The patients with abdominal and peritoneal TB often complained abdominal pain, from mild till acute abdomen and melena. In pericardial TB mostly evident are the chest pain, dyspnea and tachypnea, pericardial tamponade with hypotension, tachycardia, peripheral edema, neck vein distention, paradoxal pulse or week/absent peripheral pulses. For tuberculous lymphadenitis there was an enlargement of the peripheral lymph nodes, more often of the posterior cervical and supraclavicular chains with possible fistulation on the skin and scrofuloderma [6-9].

Scholarly publications concluded that ehe diagnosis of GTB is a challenge due to a large spectrum of clinical signs and low sensibility of laboratory tests in extrapulmonary TB [3-12]. A limited number of researches targeted the clinical and laboratory aspects of GTB, and no one identified the evolving risk factors,

difficulties in diagnosis and treatment during the COVID-19 pandemic, which constituted the premise of our study.

The aim of the exposed research was to establish the impact of the risk factors on progression and generalisation of TB infection in epidemiological context of COVID-19 pandemic (2020-2023) for identification of targeted interventions to mitigate the pandemic's impact on healthcare control efforts, addressing both clinical and social determinants of TB.

Material and methods. The research was a prospective case-control study in which were included 290 patients diagnosed with TB, distributed in two groups: study group (SG) - 84 patients diagnosed with generalized TB (one pulmonary and more than two TB extrapulmonary sites) and control group (CG) - 186 patients with pulmonary TB. All patients were registered during the period 1.1.2020-31.12.2023 and they signed the informed consent. Selected period was defined by the declared by the national authorities as COVID-19 pandemic [10].

The including criteria in the SG were: age older 18 years, TB diagnosed through the conventional methods (clinical, radio-imagistic, microbiological, histological tests), primary location - pulmonary TB and at least 2 extrapulmonary TB locations. In the CG the including criteria were: age older 18 years, TB diagnosed through the conventional methods (clinical, radio-imagistic, microbiological) and pulmonary location. The patients were investigated by collecting the sputum and other relevant clinical samples from which were performed the Ziehl-Neelson staining

for the detection of the acid-fast-bacilli, molecular genetic test GeneXpert MTB/Rif and were performed the conventional cultures (solid Lowenstein-Jensen and liquid BACTEC).

The study's schedule included data about: a) biological and social peculiarities, demographic characteristics (living in urban/rural area); b) economical peculiarities: economical state (employed, unemployed, retired, disabled,) and health-insurance coverage (presence/lack of health insurance); c) characteristics with high risk: homelessness, migration, infectious contact; d) case-management: methods used for TB detection, medical staff involved in detection, comorbidities, complications and HIV status; e) TB-related characteristics: location (pulmonary/extrapulmonary), results of radiological, microbiological and histological assays, the anti-tuberculosis drugs and the final treatment outcome. Associated investigations - hemoleucogramme, urine analysis, serum biochemical tests (transaminases, urea, creatinine, lactate dehidtrogenase) and the tests for HIV markers were performed in all patient.

The statistical assays used were: the analysis of variance (Anova test) and Pearson chi-square test. Statistical significance was determined by the value of $p<0.05$. Multiple linear regression (RML) was performed for calculating the Odds Ratio (OR) for evaluation of the risks, which were classified in low (1.1-1.59), medium (1.6-2.59) and high (>2.61)

Results and discussions. Assessing the extrapulmonary sites, which associated affected lungs was established that

abdominal organs (small intestine, liver, peritoneum and mesenteric lymph nodes) were affected in 26 (25%) cases, followed by the lymph nodes in 19 (18%), pleura in 19 (18%), kidneys in 16 (15%), spine in 13 (12%), upper respiratory ways in 12 (11%), skin-subcutaneous tissues in 4 (3%) and central nervous system-meninges in 3 (3%) patients. The most common associations were between pleura, abdominal organs and kidney, which was diagnosed in 19 (22%) cases, followed by the association between pleura and upper respiratory ways in 12 (14%). All patients with scrofuloderma were diagnosed with the TB of latero-cervical lymphnodes. Diagnosis of pulmonary TB was established by microbiological methods in 55 (65%) and by clinical-radiological methods in 24 (35%) patients. Extrapulmonary TB was confirmed using the clinical-radiological methods in 28 (33%), microbiologically in 17 (20%) and histologically in 9 (11%) patients. Other investigations established etiological diagnosis in 30 (36%) cases with extrapulmonary involvement

Assessing patients general data, was determined that in the SG predominated men vs. women, with the male/female rate 6/1 compared with 1.4/1 in the CG. Men were 72 (86%) in SG vs. 126 (67%) cases in the CG and male gender represented a high risk factor for progression and generalisation of TB (OR=2.9 (95%CI: 1.45-5.63), p=0.04). While distributing patients in the age groups according to the WHO classification was identified that the youngest groups (between 18 and 44 y.o.) predominated in the

SG compared with CG, 61 (73%) vs. 38 (20%) patients and the age less than 45 was a high risk factor for progression of GTB (OR=9.1 (95%CI: 4.5-16.5), p=0). The rate of patients residing in urban localities was statistically higher in the CG 156 (84%) *vs.* 60 (71%), while the patients from rural localitites were more often in the SG 24 (29%) vs. 28 (16%). Living in rural area, which is associated with lack of specialised healthcare services was a high risk factor for progession of TB infection in GTB (OR=3.8 (95%CI: 1.9-7.4), p=0). The demonstrably higher proportion of patients without a stable place of living was found in the SG and was identified as a high risk factor for GTB (OR=3.1 (95%CI: 1.4-6.9), p=0.02). So, patients with such risk factors: male gender, young age, residing in rural localitites and in disadvantaged social economical state should be the targeted by the systematic screening procedures aimed to promote early detection and reduce the risk for generalisation of TB infection. The data were displayed in Table 12.

Table 13.

Distribution of patients with tuberculosis by biological and demographic characteristics (%)

Indicators	**Study group (n=84, P%)**	**Control group (n=186, P%)**	**P ***
Men	72 (86)	126 (67)	<0.001
Women	12 (14)	60 (33)	<0.001

18-24 y.o.	2 (2)	0	>0.05
25-34 y.o.	18 (21)	8 (4)	<0.001
35-44 y.o.	41 (49)	30 (16)	<0.001
45-54 y.o.	14 (17)	42 (23)	>0.05
55-64 y.o.	11 (13)	57 (30)	<0.001
+65 y.o.	2 (3)	49 (26)	<0.001
Urban	60 (71)	156 (84)	<0.05
Rural	24 (29)	18 (19)	<0.05
Homeless	15 (18)	12 (6)	<0.05

Note: n și P%, absolut number and percentage of media; p* - p value in Anova test

The social peculiarities, which were assessed were: the last level of the academic education, economical state, harmful habits (tobacco smoking, alcohol abuse) and migrational history (returned from abroad, refugee, displaced). The academic state was divided in two cathogories: the low level (primary and incomplete secondary), which statistically predominated in the SG 68 (81%) vs. 88 (47%) and optimal level (secondary and high education), which was more often identified in the CG 118 (63%) vs. 16 (19%). The low academic education was identified as a high risk factor for GTB (OR=4.6 (95%CI: 2.6-8.1), p=0). Disadvantaged economical state (unemployment, people with disabilities, retired and students) was established in a statistically larger share in the SG 76 (90%) vs. 125 (67%) cases, and was

identified as a high risk factor for GTB (OR=3.1 (95%CI: 1.5-6.1), p=0.04).

Patients without a medical insurance statistically were substabtially more identified in the SG – 54 (64%) vs. 87 (47%). Single state patients, were more frequently in the SG 54 (64%) vs. 76 (41%), being established as a medium risk factor for GTB (OR=2.4 (95%CI: 1.5-4.9), p=0. The harmful habits, such as active tobacco smoking predominated in the SG 74 (89%) vs. 71 (40%) cases in the CG, and the chronic alcoholism/heavy drinking in 32 (38%) vs. 29 (21%), respectively. So, both unhealthy habits were identified as high risk factors, with a more elevated value attributed to tobacco smoking (OR=11.5 (95%CI: 5.4-23.6), p=0.01), then to chronic alcoholism/heavy drinking (OR=3.5 (95%CI: 1.9-6.2), p=0). Every tenth patient from both groups, was a migrant returned from abroad during the last 12 months, after a staying for more than 3 months. The TB contact in the frame of the family cluster was established in a higher proportion in the SG, 21 (25%) vs. 19 (10%) cases in CG, constituting a high risk factor for GTB (OR=2.9 (95%CI: 1.4-5.8), p=0).

Concluding exposed data, was established that the social unfavourbale characteristics and harmful habits were the risk factors TB with multiple sites and it would be recommended to be annualy screened to prevent the late detection of TB. Exposed data were reflected in the table 13.

Table 13

Distribution of tuberculosis patients by social characteristics and bad habits (%)

Indicators	Study group (n=84, P%)	Control group (n=186, P%)	P*
Low level of acadedmic education	68 (81)	68 (36)	<0.001
Optimal level of acadedmic education	16 (19)	118 (64)	<0.001
Employed	12 (14)	61 (33)	<0.01
Unemployed, students, people with disabilities	76 (90)	125 (67)	<0.001
Single, divorced, widowed	54 (64)	76 (41)	<0.001
Active smokers	74 (89)	71 (40)	<0.001
Active/past drug users	8 (8)	12 (6)	>0.05
Heavy alcohol drinkers	32 (38)	29 (15)	<0.001
Returned from abroad	10 (11)	21 (11)	>0.05
TB contact	21 (25)	19 (10)	<0.01

Note: n și P %, absolut number and percentage of media; p* - p value in Anova test

National clinical policy based on international recommendations requires the examination of the symptomatic patients through microbiological assays of the symptomatic cases and the radiological investigation of the high risk groups in the frame of the systematic screening [6]. Studying the case-detection of the patients, it was found that the rate of the new cases was higher in the CG 165 (89 %) vs 60 (71 %) in the SG

and those who were already exposed to anti-tuberculous drugs predominated in the CG 21 (11 %) vs 24 (19 %) cases.

By the general practitioners were detected more often the patients from the CG 171 (92 %) vs 51 (61%) and by the different specialised physicians in the SG 33 (39%) vs 15 (8 %) in the CG. Through the symptomatic screening were detected majority of the cases from the SG 168 (91 %) vs 61 (73%) in the CG and through the systematic screening predominantly in the CG 23 (27 %) vs 18 (10 %) cases. While collecting the anamnesis, the acute onset (0-30 days) of the diseases was perceived in 25 (13 %) cases and subacute (30-90 days) in 82 (44 %) cases. All patients from the SG and 79 (42 %) of the CG complained for more tha 90 days before diagnosis was established. Such signs of the intoxication sydrom – asthenia, lost of weight, night sweats, fever/feverish and the bronchopulmonary syndrome – cough, muco-purulent expectorations were identified in all patients of the SG and in 112 (60 %) cases of the CG. Assessing the clinical diagnosis, infiltrative form statistically predominated in the CG, 186 (100%) vs. 61 (72%) in the SG. Disseminated and cavernous forms were diagnosed more often in the SG, 21 (25%) vs. 2 (3%) in CG, respectively. Both lungs were affected in 65 (77%) cases and extenssive TB in more than 3 lung segments was detected in all patients from the SG compared with 23 (13%) cases of CG.

Lung parenchimal destruction or cavitation was radiologically identified in a statistical higher proportion in the SG 61 (73%) vs. 41 (22%) cases of CG. Microbiological tests were positive in a

higher proportion in the SG: AFB assays in 46 (56%) vs 38 (20%) cases of CG, GeneXpert MTB/Rif 51 (61%) vs 61 (33%) and conventional cultures in 55 (65%) vs 40 (21%) cases, respectively. Despite important differences between the rate of positive microbiological tests between the groups, the rate of MDR-TB was similar 7 (5%) in SG vs. 11 (6%) in CG. To assess the correlation between the indicators was used Pearson correlation test. It was established a strong correlation between the positive sputum smear results for AFB assays and parenchimal lung destruction (Pearsons R=0.7; (p=0)), positive GeneXpert MTB/Rif results (Pearsons R=0.81; (p=0) and conventional conventional cultures (Pearsons R=0.84; (p=0). The microbiologically positive results in feces were obtained in 18 (21%), in pleural liquide in 12 (14%) and in urine in 9 (11%) cases of the SG. It is important to emphasize a higher rate of comorbid patients in the SG – 80 (95%) vs. CG 96 (51%). HIV-infected was every fouth in the SG and every tenth in CG. Other comorbidities such as diabetes mellitus were diagnosed in 6 (7%) vs 10 (11%), mental disorders in 12 (15%) vs 31 (32%), respectively (Table 14).

Table 14.

Distribution of tuberculosis patients by case of detection and types of cases (%)

Indicators	Study group (n=84, P%)	Control group (n=186, P%)	P*

New cases	60 (71)	165 (89)	<0.001
Previously treated	24 (19)	21 (11)	<0.001
Detected by the primary healthcare staff	51 (61)	171 (92)	<0.001
Detected by the specialist	33 (39)	15 (8)	<0.001
Detected through the symptomatic screening	61 (73)	168 (91)	<0.001
Detected by active case-finding	23 (27)	18 (10)	<0.001
HIV-infected	21 (25)	20 (11)	<0.01
Presence of co-morbidities	80 (95)	96 (51)	<0.001
Lung destruction	61 (73)	41 (22)	<0.001
AFB positive (sputum)	47 (56)	38 (20)	<0.001
GeneXpert MTB/Rif positive	51 (61)	61 (33)	<0.001
LJ/BACTEC culture positive	55 (65)	40 (21)	<0.001
MDR-TB	7 (5)	11 (6)	>0.05

Note: n și P%, absolut number and percentage of media; p* - p value in Anova test

The standardised anti-TB treatment for drug susceptible TB followed 77 (92 %) patients of SG and 175 (94 %) of CG. The treatment for MDR-TB was administrated in 7 (5 %) patients from the SG and 11 (6 %) in the CG. Adverse drug events were detected in 9 (11 %) patients fn the SG vs. 15 (8 %) in the CG. The treatment outcome was unfavourable in 55 (64 %) cases of the SG and mong them 37 (41 %) died. Successfuly treated were 31 (36 %) patients. Pearsons correlation test was used to assess

the correlation between the poor treatment outcome (failure, lost to follow-up and death) with the high risk factors. In the SG it was tightly correlated with low level of acadedmic education (Pearsons R=0.56; (p=0), unfavourable economical state (Pearsons R=0.9; (p=0), tobacco smoking (Pearsons R=0.5; (p=0), and parenchimal lung destruction (Pearsons R=0.7; (p=0).

The actual research evaluated the impact of the conditions associated with COVID-19 pandemic on the share of the risk factors and case-detection of TB, taking into as a study group the patients diagnosed with severe, generalised TB with multiple locations. The results demonstrated that the biological peculiarities, which contributed to late detection and generalisation of TB were complex, intricated and were related to disadvantaged social economical state and other assigiated risk factors-harmful habits (active tobacco smoking, alcoolism) and TB contact. Similar results were reported in several local and international research papers [3-12]. Other associated risk factors with the greatest impact on the generalization of TB were commorbidities – symptomatic HIV infection, diabetes mellitus, mental disorders similar data obtained in reseach papers [3-12].

Assessing the exposure to the anti-TB treatment was established that those patients were in a larger share diagnosed with GTB and were at the evidence of pneumophtysiological service. Such data were not found in international reseaches, because the dispensarisation of TB patients is characteristic for former soviet republics. Because the pulmonary TB location was

the including criteria in the research and the lung parenchmal destruction was radiologically detected in a higher share in patients with GTB, the rate of microbiologically positive patients was higher in that group. While evaluating the extrapulmonary location, was established a higher rate of patients which had affected the organs of the gastro-intestinal system, pleura and kidneys, which were confirmed by the microbiological investigations. Synthetically analyzing the clinical research results, was concluded that patients with GTB had a long lasting evolution, clinically expressed and microbiologicaly positive which contributed to the spread of the infection into the body. The treatment outcome was unfavourable and was tightly correlated with disadvantaged social economical level, tobacco smoking and parenchimal lung destruction.

C**onclusions.** The case-control study which included patients diagnosed with generalised tuberculous infection diagnosed during the COVID-19 infection identified that the major risk factors for progression and generalization of TB were: male gender, patient’s disadvantaged social economical state, and harmful habits (tobacco smoking and alcohol abuse).

The late detection was caused by the barriers in accessing the healthcare services specific for COVID-19 pandemics period, lack of health insurance and low rate of microbiologically confirmed cases.

A strong correlation was obtained between unfavourable treatment outcome and patient's disadvantaged social economical state harmful habits, and lung destruction.

As practical recommendation with applicative value: screening of the subpopulations with multiple high risks should be

supported and continously ongoing despite the barriers caused by associated pandemic infection aiming early detection, prevent generalisation of TB and ensuring high treatment effectiveness.

CHAPTER VII

The Impact of the Glucose Metabolism Disorders on the Risk of Acquiring Tuberculosis and Predicting the Outcome according to the drug resistance spectrum of Mycobacterium tuberculosis. A case-control study in the Republic of Moldova.

Subsection 7.1. Epidemiological data on diabetes and tuberculosis at the global and regional levels in real epidemiological conditions

7.1.1. Diabetes mellitus.

Diabetes mellitus (DM) is an important public health, one of four priority non-communicable diseases targeted by the United Nations Development goals []. According to the World Health Organization (WHO) and the International Diabetes Federation (IDF), diabetes represents one of the most urgent burden on the public healthcare system globally. Statistical data demonstrated the continuous increase of the number of patients with DM [].

So, according to the IDF the total number of people with DM in 2021 was 537 million adults, and by 2030 it will reach 643 million, followed by 2045 – 632 million. Almost 318 million adults were registered with glucose metabolism disorders (GMD), with a high risk of developing diabetes in the near future. Worse is the epidemiological situation in low- and middle-income countries were live three thirds of individuals living with DM [].

The social impact of GMD lies in the fact that the disease leads to early disability and mortality of the working age population, through the development of late and chronic complications: microangiopathy (retinopathy, nephropathy and neuropathy), macroangiopathy (acute myoc ardial infarct, cerebral ictus, aneurism) [8].

GMD is an economic burden for the healthcare system, the patient and his family due to the expensive treatments associated with chronic complications [24]. Such costs were related to the health services - diagnosis, treatment, monitoring and follow-up. To those costs are added the loss of economical productivity and following disabilities caused by chronic complications and premature death [27]. According to reports from different countries, the expenses for a diabetic patient are approximately 2-3 times higher than for a non-diabetic patient. More than 80 % of countries report that they spend between 5 % and 20 % of their health expenditure on people with DM at the global level [].

According to WHO Global Report on Diabetes at the global level the hyperglycemia ranks on the 3rd place in the list of risk factors for premature mortality, after hypertension and smoking. The data presented by the IDF shows that over 90% of people with DM have diagnosed type 2 diabetes and only one fifth with type 1 [31, 37].

According to the Moldovan National Agency for Public Health every tenth adults lives with diabetes and almost 48 % are not diagnosed. Most of the Moldovan patients are affected by the type 2 DM. In the Republic of Moldova, the rate of diabetes among all endocrine, nutritional and metabolic diseases is almost 48 %. Between 2020 and 2023 there was an increase in 1.5 time in incidence from 280.8 cases/100.000 population in 2020, to 409.9 cases/100.000 population in 2022. The mortality rate in 2022 was 16.5/100.000 population.

According to the STEPS study, Prevalence of the Risk Factors for Non-communicable Diseases in R. Moldova in 2021 every fifth person had never measured their glycemia. Every tenth Moldovan person was found with an abnormal high level of fasting plasma glucose test and 6.3% had either diabetes or prediabetes. From the total number of comorbid patients, every third person with blindness and kidney failure suffers from diabetes and almost 70% of lower limb amputations are caused by diabetes. In the Republic of Moldova, the DM causes, on average, a 10-year reduction in life expectancy. The study confirmed that in most of the Moldovan patients were involved the nutritional risk factors, such as excessive consumption of saturated fats and trans fats, sugars and salt, low consumption of fruits and vegetables, as well as physical inactivity, which were established as the main causes for the high burden of type 2 diabetes [21, 42].

According to IDF in 2021 there were registered worldwide approximately 5 million deaths due to DM in people between 20 and 79 years old, which is the equivalent of one death every 6 seconds due to diabetes. Diabetes represents 14.5 % of all global deaths in adults and almost 46.6 % occur during the working age [5, 34].

Other study confirmed that in the Republic of Moldova, 56 % of population were overweight, and 22.9 % of people were obese in 2019 [11]. The proportion of obese women was 1.6 times higher than that of men in 2019 []. One of the main predisposing

condition that that about a third of the Moldovan population consumes processed food products with increased salt content, and 66.6 % – less than 5 servings of fruits and/or vegetables [8]. Also, 12.3 % of Moldovan population have a high basal glycemia, and 29.4 % have high total cholesterol blood level (Table 15). Four out of ten people had high blood pressure and only 23.8 % of them were on medication for high blood pressure in 2019 [22]. The statistical yearbook presented by the Moldovan Ministry of Health, Labor and Social Protection shows a significant increase in the number of people diagnosed with DM, which practically doubled in the last 20 years [23].

Table 15.

Epidemiological Indicator of Diabetes Mellitus in the Republic of Moldova (%)

	Disease type	Indicators	2022	2015	2010	2004
PREVALENCE						
	DM total -	Number	100.408	90.392	60.936	38.819
		/ 10.000 population	131.6	254.3	171.1	107.7
	Aduts	Number	99.745	90.023	60.586	38.464
		/ 10.000 population	419.2	314.1	215.9	141.2
	Children	Number	663	369	350	355
		/ 10.000 population	6.4	5.4	4.6	4.7

INCIDENCE						
	DM total	Number		10.387	7.726	6.026
		/ 10.000 population		29.2	21.7	16.7
	Aduts	Number		10.318	7.678	5.933
		/ 10.000 population		36	27.4	21.8
	Children	Number		69	74	93
		/ 10.000 population		1,0	0,6	1,1

Note: Epidemiological indicators calculated were

Incidence – established how commonly is the new diagnosed diabetes in Moldovan population reported at 100.000 population in a specific period of time of 12 months.

Prevalence - established how commonly are patients with diabetes in Moldovan population reported at 100.000 population in a specific period of time of 12 months.

7. Tuberculosis.

Tuberculosis is a social-determinated disease with a high burden in the Eastern European Region, including the Republic of Moldova. The main barriers in achieving a high disease control in the Eastern Europa are social, economic, educational and

psychological issues [9]. Eastern Europe is a high risk zone with an inadequate concern regarding social determinants of the health [5]. Almost all countries listed in the WHO list with the highest burden with multi-drug resistant tuberculosis are located in the Eastern Europe [22]. The countries with the highest rates of tuberculosis are the poorest and have a lot of unequal social inequalities including very a limited access to health care settings. The people affected by tuberculosis usually live in asbolute poverty because they live and work in overcrowding which provide the ideal condition for spreading of *Mycobacteria tuberculosis*. Often the poverty is associated with malnutrition and HIV infection, which both conditions diminishe the immune resistance of the organism making them vulnerable to tuberculosis [17].

Tuberculosis is one of the priority public health problems, and its prevention and control are strategic objectives for the Public Health Programme of the Republic of Moldova. Statistical data from recent years demonstrated an improvement of the epidemiological indicators, as a result of the provision of person-centered tuberculosis care services (prevention, detection and treatment). The constantly positive trend from the pre-pandemic period experienced an alarming decline in the context of the COVID-19 pandemic. For the first time in the last two decades, a regression has been reported, driven by a significant reduction in tuberculosis detection, with a particular impact on tuberculosis response programmes. In monitoring indicators of the response to

tuberculosis, the data for the year 2019 (pre-pandemic) remain the reference [6].

In the Republic of Moldova in 2020, there was established a 38.7% reduction in the number of new and relapsed TB cases detected compared to 2019 and a slight increase in the epidemiological indicators for the year 2022 compared to the year 2021, with a 2.7% increase in the global incidence. Starting with 2020, following with 2021 and 2022 the TB epidemiological indicators were reported to the Moldovan population of 3.079.908 for the last 3 years. According to the new estimations TB incidence in 2021 was 84/100.000 population and the TB mortality - 5.7/100.000 population, respectively. The total average annual population in the Republic of Moldova was estimated with 3.079.908 people (including right bank– 2.053.238, left bank – 378.329) and children – 648.341 (right bank – 561.961, left bank – 86.380). The incidence of new cases and relapses regarding tuberculosis in 2022 was 68.8/100.000 population (2.121 cases); in 2021 it constituted 67.1/100.000 population (2.068 cases). There was an increase of the incidence with 2.5% during the year 2022. In 2022, 1.666 new cases of tuberculosis were registered compared to 1.614 new cases in 2021, the incidence was calculated to 54.1/100.000 population and was compared to 52.4/100.000 population in 2021. So, an increase with 3.2% was recorded. The incidence of TB relapses in 2022 was 14.8/ 100.000 population (457 cases), compared to 2021- 14.7/100.000 population (454 cases). The incidence of TB relapses increased

by 0.7% in 2022 compared with 2021. The rate of destructive forms in new pulmonary TB cases in 2022 was 37% (559 cases) and in 2021 - 41% (591 cases). Tuberculosis affects men more than women, with a ratio of 61.4% men to 38.6% women in new cases and registered relapses in 2022. The highest rates of tuberculosis were reported among adults aged 35-44. The average age at diagnosis was 43 years. The incidence of new and relapsed TB cases in children in 2022 was 16.3/100.000 population (106 cases), in 2021 - 17.3/100.000 population (112 cases) and a decrease with 5.8% was calculated. In 2022, 103 new cases of tuberculosis in children were registered compared to 106 new cases in 2021 with the incidence of 15.9/100.000 population (compared to 16.3/100.000 population in 2021) and a decrease with 2.5% was estimated. Relapse of TB in children in 2022 was 0.5/100.000 per 100 thousand population (3 cases) and in 2021 – 0.9/100.000 (6 cases). In 2022, 14 new cases in children with destructive pulmonary TB were detected which were 27% of total new pulmonary cases and in 2021 – 10 cases with destruction, which represented 28% of new pulmonary cases in children [5, 14].

The most important immune suppressive condition detected at the global level which determines the highest risk for acquiring tuberculosis is the infection with HIV [12].People living with HIV have a 15-22 times greater risk of developing tuberculosis than people without HIV. Tuberculosis is the most common disease

among people living with HIV, including those on antiretroviral therapy, and is the leading cause of death [14].

According to the national clinical protocol Tuberculosis in adults NCP-123, all HIV-positive adults are radiologically examined annually, and children will be tested through the tuberculin skin test (TST). Depending on the results of clinical and laboratory investigations, to the patients with positive or hiperergic TST results will be given the anti-tuberculosis or chemopreventive treatment. Also, patients diagnosed with active TB will be counseled and tested for HIV markers - the Elisa test. Will be assessed the seric concentration of the p24 antigen and will be performed the rapid test for the determination of antibodies []. The operational studies carried out in the Republic of Moldova have demonstrated that untreated HIV infection is the main cause of death in TB patients due to the rapidly progressive evolution with the deterioration of all vital functions, but the connection of these phenomena with the degree of immunosuppression has not been established [28].

The rate of TB/HIV co-infection among new and relapses of TB cases in 2022 was 11.1% (235 cases) and in 2021 it was 10.3% (214 cases) in the Republic of Modlova. TB mortality in 2022 was 6.7/100.000 population (207 cases) and in 2021 it was 6.5/100.000 population (199 cases) with an increased by 3.1%. In 2022, 31 cases detected post-mortem were registered which were 15% of all TB deaths and in 2021, 24 (12.1%) cases detected post-mortem. The proportion of TB deaths up to one

year after TB detection for the 2022year was 51.2% (106 cases) and in 2021 it was 47.2% (94 cases) [39].

Tuberculosis treatment is free for insured and uninsured patients. The success rate among new and relapsed cases of drug-susceptible tuberculosis for patients who initiated treatment in 2021 was 80%. With the introduction of new antituberculosis drugs such as Bedaquiline and Delamanid into the multidrug-resistant tuberculosis treatment regimens, the treatment success rate among new cases with multidrug-resistant tuberculosis increased. The treatment success rate for the 2021 cohort of sensitive TB, new pulmonary, bacteriologically confirmed cases was 79.3% (576 from 726 cases) []. For the 2020 cohort, the success rate was 81.4% (507 from 623 cases). The success rate of MDR TB treatment in new cases for the 2020 cohort is 75.4% (178 from 236 cases), for the 2019 cohort it was 79.5% (322 from 405 cases) [].

Addressing the social determinants of health is a shared responsibility between local public authorities and stakeholders within and outside the health sector. The associations between diabetes, smoking, alcoholism, chronic lung disease, cancer, immunosuppressive treatment and tuberculosis are well recognized. It is necessary to focus on the interactions, synergies and challenges of integrating tuberculosis care with non-communicable and communicable disease management strategies. The need for sustained and growing funding for such initiatives is greater than ever and requires increased

commitment. Progress in tuberculosis prevention and care has been deeply affected by the COVID-19 pandemic. The detection of tuberculosis cases was most dramatically affected, being linked both to the availability of resources in the health system and to the population's access to services [51].

Subsection 7.2. Influence SARS-COV-2 on the dynamics of cytokine status during the inflammatory process in the lungs on the background disorder of carbohydrate metabolism.

The pandemic of coronavirus infection, which began in December 2019 in China, on at the beginning of 2020, the WHO declared an emergency of international significance in the field of health care. The overall mortality rate from COVID-19 is quite low and is 1.4–2.3 % [26, 40], however, several risk groups have been identified that experience more high risk of developing a severe form of the disease and, therefore, have a higher mortality. In particular, cardiovascular diseases, arterial hypertension, chronic respiratory diseases, metabolic syndrome, and diabetes mellitus (DM), apparently play an important role in the development of a severe form of the disease with a number of complications [8, 24, 42, 43]. Clear evidence has been provided that DM is one of the leading risk factors for COVID-19 [8, 42, 43]. It should be noted that, despite the epidemic of COVID-19, wewe are facing an epidemic of type 2 diabetes. According to the WHO Global Report on diabetes [29] 422 million adults worldwide were

living with DM in 2014 compared to 108 millions in 1980. According to IDF (International Diabetes Federation – International Diabetes Federation), in 2019 an estimated 463 million adults (aged 20-79) were living with diabetes, and by 2045 this number will increase to about 700 million [12]. In addition to clinical evidence that DM is an important risk factor for COVID-19 is still missing scientific data that would give us a better understanding of the physiological processes involved in relationship between DM and COVID-19.

Today, we have deepened our knowledge in the peculiarities of the pathogenesis of the disease and immune mechanisms of protection, which made it possible to discover new directions of pathogenetics treatment of this disease. However, the mechanisms of action remain unclear pro-inflammatory cytokines in pneumonia caused by COVID-19 against the background of metabolic ones disorders.

The purpose of the study. Analyze and summarize literary sources related to the study modern concepts of understanding pro-inflammatory cytokines in pneumonia caused by COVID-19 against the background of metabolic disorders.

Research materials and methods. The research used analytical and bibliosemantic methods. A literature search was conducted during the scientific search studies using the Medline, Google Scholar and PubMed databases. For keywords were used in the search: “pro-inflammatory cytokines”, “COVID-19”,

“inflammation”, “tuberculosis”, “insulin resistance”, “diabetes type 2”, “metabolic disorders”.

Research results and their discussion. The connection of viral infections with metabolic disorders acquired a new vision after the appearance of a new coronavirus 2019 disease (COVID-19), caused by severe acute coronavirus respiratory syndrome 2 (SARS-CoV-2) [11, 33]. Spectrum of clinical manifestations of COVID-19 varies from asymptomatic cases to severe multiple organ dysfunctions. Among the various risk factors predicting adverse outcomes after SARS-CoV-2 infection, an important role is played by insulin resistance (IR) and type 2 diabetes [11, 38]. Type 2 diabetes is neuroendocrine disease characterized by chronic hyperglycemia and accompanied by vascular complications due to endothelial dysfunction. Except type 2 diabetes has an increased susceptibility to respiratory infections, especially to pneumonia It is known that the infection of COVID-19 can cause direct damage pancreas, which in combination with insulin resistance can worsen hyperglycaemia in people with diabetes or lead to new hyperglycaemia and diabetes in people, who do not suffer from this disease [2, 21].

Several hypotheses have been proposed to understand the pathophysiological mechanism that underlies the occurrence of hyperglycemia and new-onset diabetes in patients with COVID-19. SARS-CoV-2 uses ACE2 (angiotensin converting enzyme 2 converting enzyme 2) as a receptor for penetration into human cells. Virus SARS-CoV-2 has viral spike-like glycoproteins present

on its surface, which bind through the receptor-binding domain to human ACE2 receptors on the cell-host [10, 22, 34]. Violation of glycemic homeostasis with β-cell dysfunction of the pancreas causes a decrease in the level of ACE2. Decreased levels of ACE2 will increase the level of Ang-II (Angiotensin-II) in the serum, which will lead to increasing the number of Ang-II receptors in β-cells and strengthening the renin-of the angiotensin system, thereby increasing oxidative stress in β-cells [7]. ACE2 through signaling of the ACE2 receptor type 1A (AT1R) gives a powerful respiratory respond. Upregulation of AT1R through ACE2 causes inflammation as immune cells, as well as cells present in tissues. This interaction creates an increased vascular permeability, which is associated with the production of reactive oxygen species, cytokines and chemokines, including prostaglandins, VEGF (Vascular endothelial growth factor - growth factor vascular endothelium), NFκB (NF-κB Nuclear factor kappa B), TNFα (TNF-α Tumor necrosis factor α), IL-1β, IL-6 and IFNγ (interf eron γ - interferon γ). In fact, initial plasma concentrations of IL1B, IL1RA, IL7, IL8, IL9, IL10, IFNγ, TNFα, interferon-gamma-induced protein 10 (IP10), monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein 1α (MIP1A), MIP1B and VEGF were higher in patients with COVID-19 than in healthy individuals [13, 42].

In addition, recruitment of immune cells [25] and overexpression of TLR4 (TLR Toll-like receptor - Toll-like receptor), TLR2, CD40 and matrix metalloproteinase 9 (MMP9) provoke a

hyperinflammatory state [15], which leads to a cytokine storm. At the COVID-19 cytokine storm begins as a result of the penetration of the virus into pneumocytes and the endothelium of the pulmonary vessels, as a result of which cells of the immune system are stimulated, which leads to the synthesis of a large number of various pro-inflammatory cytokines, which mainly mediate and increase inflammation in lung tissue.

So, COVID-19 can be classified as an inflammatory disease by its nature. In turn metabolic syndrome and type 2 diabetes are also associated with inflammatory dysregulation and are are chronic inflammatory conditions. It is believed that most of this inflammation is caused accumulation, hypertrophy and rupture of adipocytes and inflammation of visceral fat fabrics It should be taken into account that the final product of almost all of the above-mentioned ways is hyperactivity of the inflammatory state [3, 23, 27, 36, 39].

Inflammation causes an increase oxidative stress, which can damage proteins, lipids and DNA both systemically and locally, both in the liver and in the muscles, that is, in the organs that regulate production and metabolism glucose, increasing insulin resistance. Inflammation can cause insulin resistance and β-cell dysfunction through various mechanisms that mediated by inflammatory mediators such as IL-1, IL-6, TNFα, INF-ɣ and MCP1 [3, 19].

Proinflammatory cytokines block insulin signaling receptors in β-cells [25]. IL-1β, as the main pro-inflammatory mediator,

binds to IRS-1 (Insulin receptor protein – Receptor insulin protein) with the help of ERK-dependent (extracellular regulated kinases – extracellular regulated kinases) and ERK-independent mechanisms at post-transcriptional levels, which leads to insulin resistance [14].

The mechanisms involved in the pathogenesis of IR mediated by IL-6 are universal and consist of an overdose of non-oxidative glucose metabolism [18], an increase lipoprotein lipase, which increases the level of triglycerides [17], which activates SOCS (The suppressor of cytokine signaling - Suppressor of a cytokine signal) [9], and it counteracts the action of insulin [35].

Chronic hyperglycemia suppresses ACE2, which normally has an anti-inflammatory effect, promotes excess secretion of pro-inflammatory cytokines, such as IL-6 and TNF-α, and stimulates renin-angiotensin system (AS), which leads to IR. IR is characterized by increased release of glucose by the liver, reduction of glucose utilization by muscles and strengthening lipolysis In this condition, the response to insulin decreases [6, 30, 32].

Acute inflammation is characterized by a relative deficiency of insulin, an increase lipolysis and an increase in the level of free fatty acids [5], which cause stress hyperglycemia due to cytokine storm.In fact, the cytokine storm is characterized higher levels of inflammatory markers, such as CRP (C-reactive protein), more a high rate of erythrocyte sedimentation and an increase in the number of leukocytes [20].

At in type 2 diabetes there is an increase in the level of certain cytokines, such as TNFα, IL-1β and IL-8, which contribute to the emergence of infection. Moreover, compared to patients with COVID-19 without Diabetes, who were hospitalized in the intensive care unit (ICU) in patients with COVID-19 and DM had significantly higher levels of CRP, procalcitonin, ferritin, and IL-6 [31]. In patients, infected with SARS-CoV2, it was noted that inflammatory cytokines such as IFN-γ and MCP-1, activate Th1 cells, which trigger specific immunity. However, on the other hand, these patients also express IL-4 and IL-10, which are secreted by Th2 cells and responsible for suppressing inflammatory reactions. In addition, Th17 cells will be activated through various cytokines such as IL1-β and TNF-α are present in the cytokine storm [41]. There is a noticeable difference in the cytokine profile between patients infected with COVID-19 in ICU compared with confirmed cases of COVID-19 outside VIT, which convincingly indicates a connection between cytokine storm and disease severity.

Also, this cytokine storm will inevitably lead to multiple organ dysfunction due to hyperinflammation which in fact observed in most critical cases of COVID-19 worldwide [4]. Raised IL-17 levels have also been found in patients with type 2 diabetes to be associated with inflammation in adipose tissue. IL-17 regulates the expression of TNF-α and NF-kB, which can lead to increased expression of pro-inflammatory cytokines, causing chronic inflammatory reactions to system level. It plays an important role

in the development of insulin resistance patients with type 2 diabetes. A direct molecular connection was confirmed #39; the connection that was before proved the role of inflammatory cytokines in inducing insulin resistance in peripheral tissues [1].

TNF-α phosphorylates serine 307 in IRS-1 and induces insulin resistance through activation NF-κB and Jun NH2-terminal kinase (JNK) [28]. MCP-1 products that promote growth proliferation of macrophages in adipose tissue [16], in combination with increased production CCR5 [19] can cause insulin resistance. Oxidative stress caused by inflammation, overactivates stress signaling pathways such as JNK and NF-kB [28], and promotes secretion of TNF-α and IL-6 - a condition that causes an increase in glucose in the peripheral tissues.

Thus, it can be concluded that inflammation may be the cause dysregulation of glucose metabolism and hyperglycemia occurring during COVID-19. Imbalance of other powerful cytokines that modulate mild chronic inflammation in adipose tissue, may contribute to SARS-CoV-2-induced insulin resistance.

Conclusion. We examined the molecular mechanisms by which pro-inflammatory cytokines cause insulin resistance and have a detrimental effect on the depletion of β-pancreatic cells in patients with T2DM who are in critical condition with COVID-19. We have also discussed the mechanisms by which hyperglycemia contributes by the "cytokine storm" characteristic of severe SARS-CoV-2 infection stimulation by monocytes and macrophages of the production of pro-inflammatory cytokines in the epithelium

respiratory tract. We believe that the viral infection of COVID-19 causes multiorgan distress disease, creating an imbalance between cellular and cytokine immune system, leading to a hyper-inflammatory cytokine storm that affects the systemic homeostasis. Viral infection of COVID-19 in patients with diabetes, who already have there is a violation of the imbalance of immunity, lead to further deterioration of their general state, which makes it more difficult. In addition, β-cells are classically susceptible to oxidative stress, experience increased inflammation and depletion of ACE-2 receptors during viral infections of COVID-19, which may adversely affect the function and survival of β-cells, thereby increasing the severity of the disease.

Subsection 7.3. Risk factors for development of diabetes in the population of the Republic of Moldova.

Moldovan scientific papers identified that there are several risk factors which contribute to the development of diabetes mellitus in the population of the Republic of Moldova, including []:

1. Family history of a close family member with diabetes, which increases the risk for type 1 diabetes. It was established the risk to develop DM was two times higher if the disease was diagnosed on maternal line.

2. Obesity - overweight or obesity, especially with excess abdominal fat, increases the risk of type 2 diabetes. The risk of development of DM was three times higher in obese women

compared with obese men. The medium IMB of Moldovan diabetic patients was 30 kg/m^2.

3. Hypercholesterolemia - high seric levels of VLDL and LDL cholesterols and triglycerides increase the risk of type 2 diabetes. The association between overweight or obesity, hypercholesterolemia and hypertension constiute the most potent complex risk factor for type 2 DM and was identified in every third Moldovan patients.

4. Unhealthy diet which contains processed foods, refined carbohydrates, sugary drinks, and low in fruits, vegetables, and whole grains increases the risk of type 2 diabetes. Usually poor diet is associated with low or lask of physical activity which have ab increased risk for type 2 DM.

5. Age more than 55 years increases the risk of type 2 diabetes, and early childhood - of type 1 diabetes.

6. Ethnicity such as african americans, hispanic americans, native americans, and asian americans, have a higher risk on the American continent and roma population in the Republic of Moldova.

7. Gestational diabetes or being diagnosed with diabetes during the pregnancy or after the new born delivery increased the risk of developing type 2 diabetes in the course of the life.

8. Polycystic ovary syndrome in women determines a three times higher risk of developing insulin resistance, leading to type 2 diabetes than women without sexual endocrinological disturbances.

9. Arterial hypertension - high blood pressure (primary or secondary hypertension) is linked to an increased risk of developing type 2 diabetes. Hypertensive women had two times higher risk for DM than hypertensive men.

11. Active smoking increases the risk of type 2 diabetes and cardiovascular diseases, including arterial hypertension, which both are potent risk factors.

12. Obstructive sleep apnea are associated with insulin resistance and an increased risk of type 2 diabetes

13. Exposure to certain chemicals, including alcohol addiction increase the risk of diabetes.

14. Chronic stress can contribute to insulin resistance and increase the risk of type 2 diabetes.

Moldovan epidemiological studies carried out in the last decades by the Diabetes Centers demonstrate that the number of patients with diabetes doubles every 10 years [36]. A cohort study which included 2.676 patients registered in the Republic of Moldova established that the main risk factors for the development of type 2 diabetes were different depending on the gender and age groups. Highlighting the risk factors for the occurrence of type II DM was established that the majority of patients with diabetes were women, older 45 years, with excess body weight, sedentary lifestyle, family history of DM and pre-existing arterial hypertension. The risk was lower in overweight men, but was higher in men from urban areas with sedentary lifestyle, arteria arterial hypertension, hypercholesterolemia,

active smoking and alcohol consumption in the antecedents [42]. This fact confirms the relationship between several risk factors such as arterial hypertension, metabolic disorders of the glucose and lipid metabolism, obesity and insulin resistance is tightly straight. The study concluded that managing the risk factors through lifestyle changes, such as maintaining a healthy weight, being physically active, and adopting a balanced diet, avoiding excessive stress, active tobacco smoking and exposure to chemical agents, such as alcohol and toxin can prevent the onset of type 2 diabetes [47].

7.3.1. Risk factors for development of tuberculosis in the population of the Republic of Moldova

The main risk factors for tuberculosis includs the determinants that influence the risk of progression of latent micobacterial infection into active disease. Often they are classified in three cathegories: social, epidemiological and biological. In 90% of cases at least three different factors are associated, the most prevalent being social factors. The major social factors are overcrowidng, poor indoor ventilation, close contact with an infectious source, medical conditions that diminish the immune host defences against micobacterial infection, such as malnutrition, tobacco smoke, indoor air polution, alcoholism, and comorbid conditions-HIV infection, diabetes, gastro-intestinal diseases, chronic respiratory diseases, silicosis, malignancies and immunosuppresive treatment.

Due to severe socio-economic vulnerability the airborne infection and spreading of *Mycobacteria tuberculosis* by infected environment is commonly identified in the poorest settings. The WHO elaborated a strategy for addressing the poverty with the aim to improve the TB control at the local level, and was based on several active steps:

1. identifying poor and vulnerable groups: ethnic minorities, subpopulation disadvantaged by gender-related factors, other special situation) in the regions with high TB burden;

2. determination the barriers that reduces the accessibility of vulnerable groups to TB diagnosis methods and specialized treatment services;

3. assessment of actions that could improve the accessibility of TB patients to specialized health care;

4. systematic review of the pro-poor addressed characteristics on the population groups requiring special consideration concerning TB control actions;

5. systematic review of the impact of measures on TB epidemic indices.

The most important condition which diminishes the immune resistance and increases the risk for tuberculosis is poor personal hygiene that in most of the cases is associated with socioeconomic vulnerability. A good personal hygiene is the background of a good health. The personal hygiene, which reduces the risk to be infected with *Mycobacteria tuberculosis* consists in such personal hygiene habits as washing hands after

coming from the community facilities or other settings with a high risk for contamination. The poorest people or those with mental disorders have usually a low personal hygiene, showed through the dirty clothes, body odor, breath odor, missing teeth. Such aspects are a frequent cause for discrimination in the healthy community [22]. For reducing the risk for infection, the community, as well the patients should be advised to maintain their person hygiene, wash the hands regularly, to wash the body and the hair often. An important aspect for diminishing the risk for infection is to keep fingers and toenails trimmed and in correct shape, which will prevent the infection through the cutaneous way, which is often in patients with GMD. It prevent the keeping the infection with *Mycobacteria tuberculosis* under the nails and the infection under the nail beds. The brushing of the teeth regularly, at least two times per day minimize the accumulation of the *Mycobacteria tuberculosis* in the mouth and spreading the infection in the upper respiratory tract. The unhealthy gums frequently established in patients with DM cause the loose of teeth, which makes difficulties to the patients to eat food and cause the undernourishment and malnutrition. It is a very important the concern of the washing of hand after coughing or sneezing. The coughing is spreading in average 3500 *Mycobacteria tuberculosis* per ml of sputum. The sneezing can spread in average 1 million of *Mycobacteria tuberculosis* per one sneezing reflex. The use of hygienic products like alcohol-based sanitizing gel, handy soap

and clear water should be used every time after a contact with an well known patient with diagnosed tuberculosis [11].

Malnutrition and food insecurity increases the risk for contracting tuberculous infection. As well, tuberculosis is common in populations with tobacco use, alcohol abuse and drug use. Cultural and financial barriers of socio-vulnerable populations can contribute as major obstacles in seeking of the health care. It results in delayed detection and severe illness with frequently fatal outcomes.

In the Republic of Moldova, every 10^{th} citizen has a glucose metabolism disorder [12]. Patients with both types of diabetes mellitus are an important risk groups for tuberculosis development and require annual radiological examinations [24]. The association of DM and pulmonary tuberculosis is established most frequently in patients whose tuberculosis was diagnosed on the background of diabetes [41]. If tuberculosis and diabetes are detected simultaneously, diabetes worsens the evolution of tuberculosis [50]. The factors that contribute to the reactivation of the latent infection to progressive tuberculosis are the hyperglycemia and metabolic disorders. It is well known that high level of basal glucose determines a high degree of inflammation, deficiency of innate resistance, cellular immunity through the dysfunction of alveolar macrophages [27]. The late detection of tuberculosis in diabetic patients, error in the treatment of tuberculosis and diabetes, contributes to the unfavorable evolution of the tuberculosis process with a high risk for

complications and death [27]. Almost every fifth diabetic patient with tuberculosis developed complications in 2021 in Republic of Moldova. The late detection of tuberculosis in patients with diabetes mellitus is determined by the low specificity of clinical signs associated with the atypical radiological aspects. Although the relevant localization for tuberculosis is the upper and posterior segments of the lungs in patients with diabetes mellitus, tuberculosis is established in segments III, IV and V and is associated with pulmonary destruction [9]. The highest number of patients with lung destruction was determined among diabetic cases in 2022 [11]. The most important therapeutical issue is the high rate of adverse drug events, which were recorded in 28% of diabetic patient in 2022. Antidiabetic therapy in patients with tuberculosis must be individualized due to the high frequency of glycemic balance disturbances and adverse reactions to anti-tuberculosis preparations [13]. The rate of drug resistance in patients with pulmonary tuberculosis is increasing and reaches 20-30% in different scientific resources and in different regions [].

The risk factors for drug resistance in patients with diabetes are: history of anti-tuberculosis treatment, patient's young age, HIV infection, smoking, alcohol consumption and use of psychotropic substances [26]. Factors with a lower risk for drug-resistance are: age over 45, obesity and female sex [16]. In the Republic of Moldova almost 5% of cases with pulmonary tuberculosis were diagnosed with DM annually. In the Republic of Moldova the patients diagnosed with DM are annually screened for

tuberculosis and the rate of patients detected by the active way achieved 65%. Late detection and late onset of the therapy, dietary errors and inadequate treatment represent one of the cause of the worsening of tuberculosis and premature death associated to the synergy of both diseases [9]. Due to difficulties in the glycemic control the antidiabetic therapy in patients with tuberculosis should be individualized. According to the national protocol patients with DM should be hospitalized for the initiation of the anti-tuberculosis treatment. Before initiating the anti-tuberculosis treatment, it is necessary to compensate the metabolic disorders with an anti-diabetic diet and appropriate treatment. The anti-tuberculosis treatment should be administrated with caution due to a high rate of adverse drug reactions and the treatment effectiveness should be strictly monitored [15]. Managing these risk factors through lifestyle changes, such as maintaining a healthy weight, being physically active, and adopting a balanced diet, can help prevent or delay the onset of type 2 diabetes.

According to WHO reports the main risk factors of tuberculosis include:

- Close contact with a sick person because *Mycobacterium tuberculosis* spread through the air when an infected person coughs, sneezes, or talks. Being in close contact with a person with active TB increases the risk of transmission of tuberculous infection.

- Weakened immune system due to HIV/AIDS, chemotherapy, recipients of organ transplants, and individuals taking certain immunosuppressive medications.
- Malnutrition: poor nutrition weakens the immune system, making individuals more vulnerable to *Mycobacterium tuberculosis* infection.
- Age: tuberculosis can affect individuals of any age, but the risk is higher in young children and older adults, particularly those aged 65 years and older.
- Living conditions: overcrowded and poorly ventilated living spaces increase the risk for tuberculous infection transmission. This is especially relevant in settings such as prisons, shelters for homeless and refugee camps.
- Healthcare settings: medical workers and individuals spending time in healthcare facilities can be exposed to *Mycobacterium tuberculosis* infection and getting active disease
- Substance abuse: intravenous drug use, alcohol consumption can weaken the Immune system and increase the risk of *Mycobacterium tuberculosis* infection.
- Travel to high-prevalence areas, especially in countries with limited access to healthcare resources, can increase the risk of exposure to *Mycobacterium tuberculosis* infection
- Active smoking, which damages the respiratory system, reduce the muco-cilliar clearance and weakens the immune

system, making smokers more susceptible to *Mycobacterium tuberculosis* infection.

- Chronic medical conditions: diabetes, gastro-intestinal diseases, chronic respiratory diseases, chronic kidney disease, and cancers can increase the risk of *Mycobacterium tuberculosis* infection.

It's important to note that one third of the global population is infected with *Mycobacterium tuberculosis* but not everyone will becomes sick. Many individuals have latent infection, where the bacteria are present in a dormant state and not causing symptoms. However, when the immune system weakens, latent infection can progress into active tuberculosis. Assessing the results of the Moldovan systematic cohort studies was established that the main risk factors were social ones– unemployment, low financial income, living in poverty, followed by medical risk factors which were associated with suppressed immune response: HIV-infection, treatment with immune modulators and corticosteroids, anti-neoplastic chemotherapy, followed by diabetes mellitus and psychiatric diseases.

Men were affected more frequently in both groups, however more expressed were in Chernavtsy group. The employed patients prevailed in the group from UA and disease disabled in the group from MD. Lack of health insurance was identified in one half of Moldovan group that demonstrated the high rate of health vulnerability. Bad living conditions prevailed in the Chernavtsy group, but the extreme poverty in Chisinau group. Migrants and

people with the history of detention were identified only in the Moldovan group.

Low rate of patients from TB outbreaks in both groups demonstrates the low degree of activities performed in the infected outbreaks.

Associated diseases prevailed in the Chernavtsy group, but the most influent immune suppressive condition HIV infection prevailed in Moldovan group. The chronic alcohol abuse was indentified in a similar proportion in both groups.

In the epidemiological context the infection with *Mycobacterium tuberculosis* is concentrated in areas with high density of the population, poor enviromental and sanitation conditions: poverty, food insecurity and low living conditions. In the Republic of Moldova the most affected groups by the infection with *Mycobacterium tuberculosis*, are defined as hard-to-reach groups are: homelesses, migrants, individuals living with HIV, drug injection users and alcohol abusers. Accumulating evidence suggested that not only the defficiences in performing an effective antituberculosis treatment is a problem for the public health care system, but also the lack of intervention to resolve social and economic problems of those groups are a challenge. All factors that diminish the success rate of control measures are unproprely addressed.

According to International Diabetes Federation the main causes of DM are: socio-economic vulnerability, glucose metabolism disorders, environmental poverty and food insecurity,

and genetic factors. The key contributors to the increased level of diabetes mellitus in Moldovan population include: high level of the urbanisation, ageing population, decreasing levels of physical activity, increasing overweight and obesity prevalence. Systematic reviews established the main risk factors with the highest impact on the development of diabetes mellitus and tuberculosis are listed below :

1. Weakened immune system, for both diabetes and TB, the risk factors which can compromise the immune system. DM particularly when is not correctly managed, weakens the body's ability to maintain the infections in latent form, including tuberculosis.
2. Chronic inflammation, which is often in diabetes mellitus is associated with chronic hiperglycemia, which can create an environment favorable for the progression of the latent infection in active tuberculosis.
3. Malnutrition or imbalanced food intake, poor in vitamins, proteins is a risk factor for both DM and tuberculosis. Unhealthy nutrition can contribute to the development and severe evolution of both conditions – diabetes mellitus and tuberculosis.
4. Age older 65 years is a risk factor for both diabetes and tuberculosis, due to lowered immune state.
5. Poor living conditions and overcrowded environment, unventilated living facilities can increase the risk for

mycobacterial infection. Socioeconomic factors may play a role in the prevalence of these conditions.

6. Geographical location: tuberculosis and diabetes are more prevalent in certain regions of the world, with low and middle income countries.
7. Access to healthcare: Limited access to healthcare services can contribute to the prevalence and severity of both tuberculosis and diabetes. Lack of early detection and treatment may lead to complications.
8. Smoking: is a risk factor for both tuberculosis and diabetes. It can contribute to the development and progression of these conditions and severe evolution.
9. Substance abuse: including alcohol abuse and illicit drug use, is associated with an increased risk of both tuberculosis and diabetes.
10. Healthcare settings: healthcare workers exercising in healthcare settings, have an increased risk of TB exposure and nosocomial transmission.

7.3.*2. Case-management of patients with diabetes mellitus and tuberculosis. Screening, diagnosis and treatment.*

Given the synergy between diabetes and tuberculosis risk factor, it's important for healthcare providers to be aware of the shared risk factors and consider comprehensive care for individuals with both conditions. Monitoring and managing diabetes effectively can contribute to reducing the risk of

tuberculosis in the population. Additionally, individuals with diabetes should be vigilant about tuberculosis symptoms and seek medical advice promptly if they suspect exposure or develop symptoms.

The actual costs determined by patient with DM and tuberculosis for hospitalizations, days of incapacity for work, as well as long-term treatment of complications are extremely high. DM are the main cause of disability in Moldova, reduce quality of life and contribute to premature mortality. Tuberculosis, is the main cause of public health threats due to communicable infection, reduce quality of life and increase the economical disability.

In recent years in the Republic of Moldova, statistical data on the structure of diabetes complications have been collected, but the real number of patients with chronic complications (retinopathy, nephropathy, neuropathy) and acute complications (hypoglycemia, ketoacidosis) is underestimated. On tuberculosis side, estimative 10% of patients develop tuberculosis and the acute evolving tuberculosis constitute a medical associated condition with a high risk for death.

Actually the access to primary healthcare is granted to all people with DM and tuberculosis, regardless they are insured or not. The burden of DM treatment is only on endocrinologists, and family doctors are limited in prescribing hypoglycemic treatment, due to insufficient qualification in the diabetology. The burden of tuberculosis is only on pneumophtysiologist as only they can prescribe and follow-up the treatment. The family doctors are

limited in screening and monitoring the continuation phase of the anti-TB, due to insufficient qualification. For an optimal management in the field of diabetes treatment, was concluded the importance of the delimitation of the the functions between the family doctor, the nurse and the endocrinologist, so that the family doctor is responsible for the screening and diagnosis of diabetes and that of type 2, as well as the initiation of treatment with oral antidiabetics and the screening of complications, and the endocrinologist should be responsible for the coordination of treatment, especially of patients with type 1 diabetes and those with type 2 diabetes, transferred to insulin therapy. Insufficient information of people with risk factors for the development of diabetes and patients with diabetes requires the involvement of nurses in informing and educating patients.

Adverse drug reactions (ADRs) in diabetes are common. In a recently published study the most of potential adverse reactions which were detected during the treatment of tuberculosis associated with the treatment for diabetes were toxic hepatitis, neurotoxical events, gastrointestinal disorders and decrease of the visual accuity. The insulin can determine hypoglycemia which is the most frequent side effects during the injectable anti-diabetic treatment. This clinical signs are shakiness, confusion, sweating, and, if severe, can lead to unconsciousness. The oral hypoglycemic agents, such as Sulfonylureas, Biguanides, Thiazolidinediones can determine the gastrointestinal issues: nausea, vomiting, or diarrhea. The Glucagon-like peptide 1-based

therapies for the treatment of the type 2 of DM can determine nausea, and pancreatitis. The DM&TBLT-2 Inhibitors, called as gliflozins, are a class of drugs that lower the glycemia by preventing the kidneys from reabsorbing sugar produced by the body. The most frequent ADRs are genital and urinary tract infections, genital and urinary tract infections, dehydration and low blood pressure, increased urination, potentially causing dehydration. The Dypeptidyl Peptidase IV inhibitors (Sitagliptin, Saxagliptin, Linagliptin, and Alogliptin, Vildagliptin) can increase the risk for upper respiratory tract infections and the patients can experience cold-like symptoms, such cough, expectoration and fever. It's important to note that individual responses to medications can vary, and not everyone can develop the same side effects. Additionally, healthcare providers carefully consider a patient's overall health, other medical conditions, and potential drug interactions when prescribing medications for diabetes. Regular monitoring and communication with healthcare professionals are crucial to managing diabetes effectively and safely [9].

A study on medical human resourse of the Republic of Moldova established that there is a lack of specialized medical personnel and limited access of specialists in the field tuberculosis and endocrinology [11]. There are not enough specialist doctors who are trained in the management of chronic complications of diabetes - cardiologists, nephrologists, neurologists, surgeons, orthopedists. Worse is the situation in

phtisiological services, because the lowest number of specialists were recorded in pneumophtysiology [15]. There is also a lack of nutritionist educators and health life style trainnees, compulsory needed during the course of the treatment. Currently, the endocrinological service in the Republic of Moldova is maintained by about 40% of specialists who are of retirement age, which creates an impediment in the provision of medical assistance according to international requirements and recommendations [21]. The training of staff in endocrinology was carried out through clinical residence. For economic reasons, but also because of the lack of bases equipped with laboratories, young specialists refuse employment in the remote districts of the Republic of Moldova [13]. In the pneumophtisiogy, the situation with the human resources is even worse. The majority of the medical staff is in retirement age and the peripheric localities are without any referent pneumologist [33].

International clinical guidelines emphasized that diabetes mellitus screening methods require a periodical review of methodology for screening and risk stratification. Screening for diabetes involves identifying individuals who may be at risk of the condition or who already have diabetes but are asymptomatic [53]. Screening and early detection is crucial for timely intervention and management. Screening guidelines may vary based on factors such as age, risk factors, and the type of diabetes [45]. Also, was stated that the screening recommendations may vary based on individual factors and

guidelines from national health organizations. At the global level the common risk factors for diabetes which should be evaluated at the level of every primary healthcare unit are: age 45 years or older, family history of diabetes, obesity or overweight, physical inactivity, history of gestational diabetes, arterial hypertension and cholesterol levels [17]. Taking into account the consequences of gestational diabetes on the health of the mother, fetus and the newborn, international recommendations asked in a mandatory way to do screening in all pregnant women and rigorous monitoring of all women with a confirmed diagnosis. It was confirmed that it is important for individuals to discuss their risk factors with healthcare providers and follow recommended screening guidelines for early detection and appropriate management of diabetes.

7.3.4. The role of endocrinologist - diabetologist in the clinical case-management of diabetes mellitus patients from the Republic of Moldova

In Moldovan medical system the specialist in endocrinology, known as a *diabetologist* have the main role in the diagnosis and treatment of diabetic patients. The responsibilities of the diabetologist include:

- Interpretation of the Fasting Blood Glucose Test , which is a test measures blood sugar levels after an overnight fast. Typically, a blood sample is taken in the morning before eating or drinking anything other than water.. Fasting blood

glucose levels of 126 milligrams per deciliter (mg/dL) or higher on two separate occasions may indicate diabetes. The results should be interpreted by the diabetologist and follow-up actions recommended.

- Interpretation of the Oral Glucose Tolerance Test (OGTT) involves the fasting overnight and then drinking a glucose solution. Blood samples are taken at intervals to measure how the body processes glucose. A two-hour blood glucose level of 200 mg/dL or higher during the OGTT may suggest diabetes.
- Hemoglobin A1c test measures the average blood glucose levels over the past two to three months. It does not require fasting and if A1c level is above 6.5% or higher may indicate diabetes.
- Risk assessment through the questionnaires and clinical assessment by the healthcare providers may use questionnaires and clinical assessments to evaluate risk factors such as family history, age, obesity, physical inactivity, and ethnicity. Individuals identified as high-risk through assessments may be recommended for further diagnostic testing.
- Random blood glucose test measures blood glucose levels at any time of the day, regardless of when the individual last ate. A random blood glucose level of 200 mg/dL or higher, along with symptoms of diabetes, may suggest the need for further testing.

- Point-of-care testing are testing devices, such as fingerstick glucose meters, that can provide immediate results. Elevated blood glucose levels on point-of-care testing may establish further laboratory evaluation.

A specialist in tuberculosis, known as a *pulmonologist, pneumophtysiologist* or an infectious disease specialist, have the main role in the prevention, diagnosis, and treatment of tuberculosis patients. The responsibilities of a phtysiopeumologist include:

1. Diagnosis: clinical evaluation integrated in the conduct a thorough medical history and physical examination to assess symptoms and risk factors associated with tuberculosis.
1. Diagnostic testing: order and interpret diagnostic tests such as tuberculin skin tests (TST), interferon-gamma release assays (IGRAs), chest X-rays, and sputum tests for acid-fast-bacilli, Gene X-pert MTB/RIF to confirm or exclude tuberculosis infection or disease.
2. Treatment planning and prescribtion of medications as appropriate treatment plans, which often involve a combination of anti-tuberculosis drugs for the specified duration to treat active forms. Drug resistance testing to identify any drug-resistant strains of *Mycobacterium tuberculosis* and adjust treatment accordingly.
3. Patient management: monitoring and follow-up the response to treatment, adjust medication regimens as needed, and ensure compliance with the prescribed medications, manage

side effects or complications arising from tuberculosis medications.

4. Prevention and control through the contact investigation to identify and screen individuals who may have been exposed to tuberculosis, providing preventive treatment when necessary. Infection control and promote measures to prevent the spread of tuberculosis in healthcare settings and the community.
5. Education and counseling of patients about tuberculosis, its transmission, treatment, and the importance of adherence to medications. Prevention strategies in term of providing guidance on preventive measures for those at risk of tuberculosis, including individuals with weakened immune systems.
6. Collaboration and consultation with other specialist, with other healthcare professionals, such as infectious disease specialists, primary care physicians, respiratory therapists, and public health professionals, to ensure comprehensive care. Public Health collaboration with public health authorities and organizations to contribute to tuberculosis prevention and control efforts at the community and population levels.
7. Research and advocacy through the support of the research efforts aimed to improving tuberculosis diagnostics, treatment modalities, and prevention strategies. Advocacy

for policies and practices that promote tuberculosis awareness, prevention, and research funding.

8. Follow-up post-treatment monitoring which provides a long-term follow-up care for individuals who have completed anti-tuberculosis treatment to monitor the risk for relapse or potential complications. Specialists in tuberculosis are the main professionals in the global efforts to control and eliminate tuberculosis. Their work extends beyond the individual patient to community-level interventions and public health initiatives.

The general practitioners, known as family doctor, play an important role in the screening, diagnosis, and management of tuberculosis at the primary care level. While definitive diagnosis and treatment often involve specialists, general practitioners are often the first point of contact for individuals seeking healthcare. The tasks and aspects of screening for tuberculosis and management by the general practitioners may include the:

1. Clinical assessment as the general practitioners conduct a medical history and physical examination to assess for signs and symptoms associated with tuberculosis. Common symptoms include persistent cough, weight loss, night sweats, and fatigue, hemoptysis, thoracic pain, fever, feverish.
2. Tuberculin Skin Test (TST) or Interferon-Gamma Release Assay (IGRA) as the general practitioners may administer and interpret TST or IGRA to assess exposure to

Mycobacterium tuberculosis, the bacterium causing TB. These tests help identify individuals with latent tuberculosis infection.

3. Chest X-ray because the general practitioners may order a chest X-ray or computed tomography to check for abnormalities in the lungs, such as the characteristic pulmonary infiltrates, cavities, nodules, calcinates, pleurisy suggestive for pulmonarytuberculosis.
4. Sputum testing: general practitioners may order the collect of 2-3 sputum samples for laboratory testing to confirm the presence of *Mycobacterium tuberculosis* in individuals with suspected active TB as acid-fast-bacilli stainig, GeneXpert and cultures of the conventional media.
5. Contact investigation: the general practitioners may initiate contact investigations to identify and screen individuals who have been exposed to a person with infectious tuberculosis.
6. Referral to specialists in pneumophtysiology if tuberculosis is suspected or diagnosed. General practitioners may refer patients to infectious disease specialists or pulmonologists for further evaluation and management.
7. Treatment initiation - general practitioners may initiate treatment for latent tuberculosis infection, knew as chemopreventive treatment or refer patients with active tuberculosis to specialists for appropriate treatment.

At the level of the primary health care the anti- tuberculosis chemopreventive treatment schemes are administrated as following:

1. Rifapentin administered daily for one month;

2. Rifapentin and Isoniazid administered weekly for 3 months;

3. Isoniazid and Rifampicin administered daily for a period of 3 months;

4. Isoniazid administered daily for 6 months.

Chemopreventive treatment is recommended with a high degree of certainty of therapeutic effectiveness in people with HIV-positive status and in people exposed to tuberculosis, with intradomestic and close contact. It is administered on an empty stomach under the supervision of specially trained medical personnel.

Before inititation of any antibiotic treatment all respiratory patients should be investigated through the screening procedures which include the steps:

- Access to accessible medical services for tuberculosis screening.
- Referral to a phtisiopneumologist for clinical examination with the identification of disease risk factors and the presence of clinical signs suggestive of tuberculosis.
- Examination of the patient to identify tuberculosis infection: radiological examination of the chest and/or tuberculin test (children 0-14 years).

- Microbiological examination of the patient's sputum with symptoms suggestive of tuberculosis. The general purpose of tuberculosis screening is to identify suspects in the targeted subpopulation for the early diagnosis of evolving tuberculosis or to establish the alternative diagnosis of tuberculosis infection. As a result, to the patient diagnosed with tuberculosis will be recommended antituberculosis treatment, and the one with an alternative diagnosis of tuberculosis infection will be prescribed chemopreventive treatment.
- Education and counseling as the general practitioners play an important role in educating patients about tuberculosis, its transmission, and the importance of adherence to treatment. Counseling may include discussions about medication side effects, the importance of completing the prescribed course of treatment, and infection control measures.
- Monitoring and follow-up performed by the general practitioners monitor patients' progress during treatment and coordinate with specialists to ensure proper follow-up care and optimal outcome.
- Prevention strategies: general practitioners may provide guidance on preventive strategies for individuals at risk of tuberculosis, such as those with weakened immune systems.

Patients with both types of diabetes mellitus represent the one of the risk group for tuberculosis and should be annually screened

by the chest X ray. The association of the diabetes mellitus and pulmonary tuberculosis usually occurs in patients where diabetes was the previous diagnosed disease [51]. If both, tuberculosis and diabetes mellitus are detected simultaneously, diabetes worsens the tuberculosis outcome. One half of patients with diabetes mellitus develop tuberculosis in the first three years after the exposure to the infection [33]. Factors associated with the increased risk for tuberculosis are: disturbances of the innate resistance, dysfunction of alveolar macrophages, low cellular immunity response to the specific and non-specific infections, and reduced the capacity of the organism to produce antibodies, low levels of interferon gamma, microangyopathy (inclusive pulmonary) and micronutrient deficiency [46].

The first clinical signs of tuberculosis in patients with diabetes mellitus have a low specificity: increased weakness, decreased appetite, loss of the weight, and worsening of the diabetes symptoms [43]. The development of the chronic forms of tuberculosis – fibro-cavernous type, occurs when the organism's defenses are depleted [22]. The evolution of tuberculosis in diabetes mellitus is unfavorable due to disturbances of glucose metabolism [7]. Late detection and late onset of the therapy, dietary errors and inadequate treatment represent the causes of the worsening of tuberculosis process under the specific treatment. In diabetic patients, blood sugar levels increase, diuresis and glucosuria increase, acidosis may appear, patients

have the feeling of dry mouth, thirst, frequent urination and important weight loss [11].

Antidiabetic therapy in tuberculosis patients should be individualized and depends on the patient's state, the tuberculosis extensibility and the severity of diabetes [5]. Each patient with diabetes mellitus must be hospitalized. First of all, it is necessary to compensate the metabolic disorders with a physiological diet and optimal doses of antidiabetic drugs. Anti-tuberculosis therapy should be administrated with caution due to high rate of adverse reactions [39].To prevent possible side effects patients must be strictly monitored.

Although, most of the diabetic patients are misdiagnosed regarding tuberculosis, several factors are involved: low specificity of the clinical signs and atypical radiological aspects. The relevant localization of the pulmonary tuberculosis is upper and posterior segments of the lungs: I, II, VI and X. Although in diabetic patients tuberculosis is identified in segments III, IV and V. In patients with carbohydrate metabolism disorders predominated the inferior lobe involvement and may be revealed multiple c*avities* [7].

According to the WHO recommendation microbiological methods remain the golden standard for pulmonary tuberculosis diagnosis. Conventional microscopy for identification of acid-fast-bacilli is the first step in TB detection algorithm. The low sensibility of the conventional microscopy diminishes the detection efficiency of TB patients. The long duration of the culture methods delayed

of TB diagnoses. WHO recommends to use Xpert MTB/Rif assay in adults, children and persons living with HIV or other risk factors. Xpert MTB/Rif assay represents *in vitro* diagnostic medical device owned by Cepheid Company. Xpert MTB/Rif assay used with Cepheid Xpert MBT/Rif system is a semi-nested, quantitative, real-time polymerase chain reaction testing for the DNA detection of all *Mycobacterium tuberculosis (MBT) complex* species and rifampicin resistance mutations of the *rpoB* gene []. Several standard results must be known for appropriate interpretation of Xpert MBT/Rif system: 1. MTB detected & RIF resistance means that MTB target is present and mutation of *rpoB*gene is detected; 2. MTB detected & RIF susceptible means that MTB target is present and no mutation of *rpoB* gene has been detected; 3. MTB not detected – MTB target is not detected within the sample. Despite of clearly defined interpretations the test results must be always correlated with laboratory and clinical data of the investigated patient.

The aim of this study was to assess the risk factors for developing tuberculosis in patients with disorders of the glycemic disorders and the impact of the diabetes mellitus on the anti-tuberculosis treatment effectiveness and identifying the methods for improvement the disease outcome.

Material and methods. The research was cross-sectional, retrospective, selective, descriptive and conducted by including a contingent of 252 patients diagnosed with pulmonary tuberculosis

and diabetes mellitus during the period 1.1.2017-31.12.20123 in the RM.

The inclusion criteria of the patients in the study group DM&TB group were: age older 18 years, tuberculosis diagnosed by the pulmonologist, diabetes mellitus diagnosed by the endocrinologist and signed informed consent. The control group 1 – TB group included 318 patients without diabetes or glycemic disorders diagnosed with tuberculosis. The control group 2 – DM group included 352 patients only diabetes without any tuberculosis in anamnesis.

The diagnosis of pulmonary tuberculosis was established according to the criteria provided by the national tuberculosis policy. In every patient was done the sputum examination at Ziehl-Neelson staining, culture on the Lowenstein-Jensen and liquid BACTEC media, and chest X-ray investigations.

The protocol schedule included such data about the patient:

- Biological and social peculiarities: sex (male-female rate), age (distribution in age groups according to the WHO recommendations), demographic characteristics (urban/rural).
- Economical peculiarities: economical status (employed, unemployed, retired, disabled,) and health-insurance coverage (presence/lack of health insurance).
- Characteristics with high risk: social vulnerability such as homelessness, migration, history of detention, infectious contact.

- Case-management peculiarities: health care seeking behavior and addressability, methods for the detection and medical staff involved in the case-management, comorbidities, complications and HIV status.
- Tuberculosis-related characteristics: localization (pulmonary / extrapulmonary) microbiological results (smear microscopy, culture on the conventional media, molecular-genetic tests and the drug susceptibility tests).
- Anti-tuberculosis and antidiabetic treatment and the final outcome.
- Glycemic control indicators: fasting venous plasma glucose (FVPG) and glycated hemoglobin (HbA1c) in patients with hyperglycemia.
- The research was approved by the bioethics committee of the State Medicine and Pharmacy University Nicolae Testemitanu.

Collection of primary material involved the extraction of data from medical record forms. The individual schedule included information about: anamnesis, clinical examination, results of radiological investigations (chest radiography, high resolution computer tomography), results of microbiological investigations (smear microscopy by Ziehl-Neelson coloration and culture on classic solid medium Lowenstein-Jensen or liquid medium). Investigations were performed according to the National Clinical Protocol – 123 Tuberculosis in adults. Statistical analysis methods used in the study were: comparative, synthesis and discriminant

analysis. Mathematic and statistical assessment was carried out by checking the quantitative and qualitative features. Accumulated material was tabled in simple and complex groups. Statistical study was performed using Microsoft Excel XP soft. The predictibility value of each involved factor was calculated using two by two table. Relative risk and confidence interval was calculated according to the established formula [7]. The interval of 1.2 to 1.6 was assessed as a low predictive factor, 1.6 to 2.4 – as a mild predictive factor, and more than 2.5 – as a high predictive factor.

The statistical analysis was performed using EpiInfo software. Data were appreciated as nominal or quantitative. The frequency and percentage were reported for nominal data, and the mean and standard deviation were reported for continuous data. The statistical analysis of the differences between normally distributed continuous variables was tested with the Student T-test. A p value of <0.05 was considered statistically significant. To estimate the strength of the association between the risk factors, the relative risk (RR) was used in the statistical analysis of the data.

Results. Distributing patients from the DM&TB group, which was defined as the study group, according to the type of the glycemic disorders was established that the type 1 diabetes was diagnosed in 74 (39%) patients and type 2 in 112 (61%) cases. In the group of patients diagnosed with DM without associated TB, type 1 was identified in 84 (24%) and type 2 in 264 (75%)

patients. The average duration of DM in DM&TB group was 12±4.5 years and in DM group 8±3.9 years. In 4 (2%) patients known with glucose metabolic disorders before TB was diagnosed DM type 2 during the ongoing anti-tuberculosis treatment. No one patient from the group with TB, defined as control group 1 had glucose metabolism disorders before or during the course of active tuberculosis. Comparatively in the group with DM, defined as control group 2 there were no patients with anamnesis of tuberculosis established.

In 139 (75%) patients from the DM&TB group were diagnosed different types of diabetes-related complications, including microvascular disease in 46 (33%) cases with foot lesions, chronic kidney disease (chronic pyelonephritis, litiasis) in 12 (8%), diabetic neuropathy in 89 (64%) cases with numbness, cardiovascular disease (arterial hypertension, ischemic cardiopathy, acute myocardial infarction in anamnesis) in 123 (89%) and diabetic retinopathy in 7 (5%).

In the groups with DM without associated TB, control group 1, the rate of patients with diabetes-related complications was 187 (58%). Among them, the highest rate of patients were diagnosed with cardiovascular disease (arterial hypertension, ischemic cardiopathy, acute myocardial infarction, left ventricular hypertrophy, arrhythmia) – 92 (49%). According to the share, followed diabetic neuropathy in 67 (36%), chronic kidney disease in 12 (6%) and diabetic neuropathy in 12 (6%) cases.

The fasting plasma glucose test (FPGT), which is the screening investigation for DM is the most rapid and simplest way for the detection of GMD. It consists in the fact that the patient should not eat or drink except water 8 – 12 hours before the blood collection. It was compulsory performed in all patients when they were investigated before the anti-tuberculosis treatment was recommended. The (FPGT exceeded the normal limit of 6 mmol/L in all patients of the DM&TB and in all patients from the control group 2. Reporting to the total number of patients with hyperglycemia in 71 (38%) cases, the FVPG was in the range between 6,1 and 8 mmol/L, in 75 (40%) cases between 8,1 and 12 mmol/l and more than 12 mmol/l was established in 40 (21%) patients. No one case from the TB was diagnosed with hyperglycemia and the FPGT was within normal values.

The concentration of the HbA1c included in the range between 4.8 - 5.6 % (normal values) were found in 45 (24%) from the patients of the study group - DM&TB. In the range between 5.7 - 6.4% (prediabetes values) the concentration of HbA1c was detected in 47 (25%) patients and in 85 (45%) cases the concentration of the HbA1c exceeded the 6,4%, which indicated a poorly managed DM or late detected DM. In the control group 2 - the rate of patients with the concentration of the HbA1c included in the range between 4.8 - 5.6% (normal values) were found in 67 (19%) patients, in the range between 5.7 - 6.4% (prediabetes values) in 84 (24%) cases and the concentration was above 6.4% in 200 (63%) patients.

The distribution of the patients, according to the sex established a statistical higher rate of man compared with women in the TB group (control group 1) - 2.9 compared with DM&TB (study group) which was 1.2 and a higher rate of women in the DM group (control group 2) - 1.5. Men with DM have 3 times higher risk of developing tuberculosis than women (Odds ratio=3.1; 95% CI for OR=2.1-4.9). Data are presented in the table 16.

Repartition of the patients, according to the age established that the young groups which were between 18 and 24 years old prevailed in the TB group (control group 1) - 20 (13%) vs. 11 (3%) in the DM (control group 2). There were no patients between 18 and 24 years old in the study group (DM&TB). The highest rate of patients constitued those integrated in the group between 45 and 54 years old which were 98 (31%) in the control group 1 and in the same proportion 24% in the SG and CG. In a similar proportion, almost 30% of the patients from the study group and the control group 2 were between 55 and 64 years old, which statistically predominated compared with the control group 1. Patients older 65 years old statistically predominated in the study group compared with the control group 1 and 2. Redistributing patients in two groups, younger 45 and older 45 years, was concluded the statistical predomination of the patients from the youngest group between 18 and 44 years old in the control group 1 - 80 (25 %) cases and control group 2 – 100 (28 %) compared with 38 (20 %) cases in the study group. Patients older 45 years

old predominated in the study group 148 (80 %) compared with the control group 1 – 161 (51 %) and in a similar propotion with the control group 2 – 251 (79 %). The average age of patients with DM&TB was 59±8 years, of patients from the DM 47±4 years and of patients with TB 38±5 years (Table 16).

Table 16

Distribution of patients with tuberculosis/diabetes comorbidity by gender and age groups (%)

Sex **Age groups**	**DM&TB** **N=186 *(P%)***	**TB** **N=318 *(P%)***	**DM** **N=351 (*P%*)**
Men	126 (67)*	236 (74)*	142 (40) ###
Women	60 (33)	82 (26)	209 (60)
18-24 years	0	20 (6)	11 (3)
25-34 years	8 (4)	60 (19) ###	22 (6)
35-44 years	30 (16)	81 (25) ##	67 (19)
45-54 years	42 (23)	98 (31) #	84 (24)
55-64 years	57 (30)	38 (12) ###	101 (29)
+65 years	49 (26)	21 (7) ###	66 (19)#

Note: Applied statistical test: paired simple T-test, P – probability;
*Absolute numbers and percentages per column (in brackets).
# p<0.05; ## p<0.01; ### p<0.001 at the comparison of the study group with the control group

When distributing patients according to the economic status, was established that employed persons, which were contributing in this way to the health budget by paying taxes, health insurance

policy and social taxes predominated in the control group 2 compared with study group and control group 1. Due to the predominance of patients older 55 years in the study group and control group 2 the rate of the retired patients statistically prevailed compared with the control group 1.

Because in every third patient of the study group and control group 2 were diagnosed different types of complications, the rate of disabled patients statistically predominated in the study group compared with both the control groups. So, the small conventional income associated with the free of charge health insurance obtained through the retirement and disability statistically predominated in the DM&TB. Health insurance represents the major condition for accessing health care and free of charge screening procedures. Due to a higher rate of retired and disabled patients in the DM&TB the health insurance coverage was established in a higher proportion in control group 2. The distribution of patients in economical groups established that the largest group was represented by the patients in the economical vulnerable state (unemployed, retired, disabled) in the DM&TB group (see table 17). Considering all above exposed data it was established the relevant risk factors for tuberculosis in patients with diabetes were economical vulnerable state which contributed with 18 times higher risk of developing tuberculosis than economical stable state with the same comorbid state (Odds ratio=18; 95% CI for OR=11.4-29.1).

Table I7

Distribution of patients with tuberculosis/diabetes comorbidity according to socio-economic data (%)

Economic and insurance state	DM&TB N=186 *(P%)*	TB N=318 *(P%)*	DM N=351 (*P%*)
Employed	26 (13)*	60 (19)* ###	119 (56)* ###
Disease disabled	54 (29)	13 (4) ###	31 (9) ###
Retired	106 (57)	59 (18) ###	167 (56)
Unemployed	123 (66)	246 (77) ###	34 (10) ###
Lack of health inssurance	123 (66)	246 (77) ###	38 (11) ###
Inssuranced	63 (34)	72 (23)	313 (89) ###

Note: Applied statistical test: paired simple T-test, P – probability; *Absolute numbers and percentages per column (in brackets).
# p<0.05; ## p<0.01; ### p<0.001 at the comparison of the study group with the control group.

Demographic distribution identified that every second patient from both groups came from urban localities of the RM. Patients residing in the rural areas of the country statistically predominated in the DM&TB 84 (45%) vs. 76 (24%) cases in the TB, but homeless or without a stable living residence predominated in the TB 68 (25%) vs 8 (4%) patients in the DM&TB. Patients living in

extreme poverty and without a stable place of living constituted one third of the TB and no one case in the DM&TB. Economic migrants, returned from abroad in the last 12 months were established in a similar proportion in both groups.

A couple of patients from the TB had a life history of detention. Closed TB contact was established in a low proportion in both group, however, more frequently in the TB, which showed the low quality of the infectious clusters investigation. Beside tuberculosis in two thirds of the DM&TB were diagnosed more than 2 associated diseases, among which one was due to glycemic disorders. In every 7th patient of the TB was diagnosed an associated with tuberculosis disease.

Peculiarities, which statistically predominated in the DM&TB were assessed through the relative risk difference. The rural residence was identified with RR=1,7 (95%CI: 1,29-2,41) and associated diseases including the diabetes complications with RR=5,3 (95%CI: 3,56-7,99) (Table 18).

Table 18

Distribution of patients with tuberculosis/diabetes comorbidity by risk groups (*P %*)

Risks factors	**DM&TB**	**TB**	**DM**
	N=186 *(P %)*	**N=318 *(P %)***	**N=351 (*P %*)**
Urban residence	94 (50)*	164 (51)*	298 (85)* ###
Rural residence	84 (45)	115 (36) #	53 (15)

			###
Homelessness	8 (4)	39 (12)	0
Migration	14 (7)	16 (5)	12 (3)
History of detention	0	2 (0.6)	0
Closed contact	10 (5)	25 (8)	-
Co-morbid state	186 (100)	84 (26) ###	351 (100)

Note: Applied statistical test: paired simple T-test, P – probability; *Absolute numbers and percentages per column (in brackets).

# p<0.05; ## p<0.01; ### p<0.001 at the comparison of the study group with the control group

Studying the case-management before diagnosis of tuberculosis was established, it was identified that the general medical staff of the primary health care sector was involved in the detection in a higher proportion of patients from the TB 68 (43%) compared with the DM&TB 40 (21%). Only patients from the TB came directly to the specialized hospital, avoiding the primary health care sector. Active way performed by radiological screening of high risk groups was used in a higher proportion in the DM&TB, but the statistical threshold was achieved only regarding those detected by the specialist compared with the general practitioner. Assessing the anamnesis was established that previously treated patients statistically predominated in the DM&TB 64 (34%) compared with 62 (19%) patients in the TB. Following, the anti-TB in anamnesis was identified a risk factor for getting sick again with RR=1,67 (95% CI 1,13-2,14).

While assessing the laboratory features of the enrolled patients with involvement of the pulmonary parenchima it was identified a similar rate of patients, which were microscopic positive for acid-fast-bacilli (AFB) and positive bacteriological results (conventional culture on solid Lowenstein-Jensen either liquid MGIT BACTEC). The MDR-TB through GeneXpert MTB/Rif assay was established in every fourth patient from both groups and was confirmed through the conventional cultures (Table 19).

Table 19

Case-management characteristics of tuberculosis patients*(P %)*.

Health level	**Detection ways**	**DM&TB**	**TB**	**P value**
		N=186 *(P %)*	**N=318 *(P %)***	
Primary health care providers	Detected by GPs symptomatics	40 (21)*	136 (43)*	**<0.001**
	Detected by GPs screening of HRG	46 (25)	48 (15)	**<0.001**
Specialised healt care level	Detected by SP symptomatics	48 (26)	70 (22)	
	Detected by SP	32 (17)	20 (6)	**<0.001**

	screening of HRG			
	Addressed directly to the specialized hospital	20 (11)	44 (14)	**<0.001**
Case-type	New cases	122 (66)	256 (80)	**<0.001**
	Previously treated	64 (34)	62 (19)	**<0.001**
Microbiological results	Microscopic positive for AFB	76 (41)	158 (50)	>0.05
	Conventional cultures	92 (50)	196 (61)	>0.05
	MDR-TB	40 (21)	88 (28)	>0.05

Note: Applied statistical test: paired simple T-test, GP-general practitioner, SP-specialist, HRG-high risk group;

*Absolute numbers and percentages per column (in brackets).

Due to including criteria, all patients from both groups were diagnosed with pulmonary tuberculosis. A low number of patients from the DM&TB (24 (6%)) were diagnosed with an associated extrapulmonary localization and 2 (1%) case had a generalized form. No extrapulmonary or generalized forms were diagnosed in the TB. Distributing patients, according to the clinical, radiological criteria was obtained a similar rate diagnosed with pulmonary infiltrative tuberculosis. The statistical threshold was achieved regarding the chronic evaluating tuberculosis, such as fibrocavitary form, which predominated in the DM&TB and acute disseminated form, which predominated in the TB. All patients with fibrocavitary form were treated for tuberculosis in anamnesis.

Assessing the Body Mass Index (BMI) as an indicator of the nutritional state it was identified that every third case of the DM&TB 32 (34%) was malnourished (BMI<19 kg/m^2) and every second case - 90 (48%) was undernourished (BMI 19-21 kg/m^2). In the TB a higher rate of the patients were malnourished 148 (43%) and a lower proportion 32 (38%) cases were undernourished, however the statistical threshold was not achieved The rate of patients with normal weight was similar in both groups: 32 (17%) in the DM&TB vs. 56 (18%) in the TB. Before tuberculosis was diagnosed the BMI decreased under 21 kg/m^2 in 144 (83%) cases in the DM&TB and in 260 (81%) cases in the TB.

The standard treatment for the drug-susceptible tuberculosis, according to WHO recommendations in the RM is performed since 2000 [14]. All selected patients with drug susceptible tuberculosis were treated 6 months for new cases and 8 months for previously treated. The standard regimen for drug susceptible tuberculosis included the drugs: Isoniazid, Rifampicin, Pirazinamide and Streptomycin or Ethambutol with the dose adjusted to the weight assessed at the onset of the intensive phase and at the onset of the continuation phase of the treatment.

The standard treatment for MDR-TB included the 2^{nd} line antituberculosis drugs recommended according to the drug susceptibility test [10]. The standard treatment for MDR-TB used injectable antibiotics – aminoglycozides (Amikacin or Capreomycin) till 2021 and orally administrated anti-tuberculous

drugs: fluoroquinolones (Levofloxacin or Moxifloxacin), Ethionamide or Prothionamide, Paraaminosalicylic acid and Cycloserine [10].

New drugs such as Delamanid and Betaquilin were indicated in specific cases with MDR-TB. The standard treatment for drug susceptible tuberculosis received 73 (78%) cases in the DM&TB and 230 (72%) patients in the TB. The standard treatment for MDR-TB was applied in 40 (23%) patients of the DM&TB and 88 (28%) patients of the TB. The treatment success rate was statistically higher in the TB and the death rate was statistically higher in the DM&TB. A similar rate of patients in both groups was lost to follow-up or interrupted the treatment. All patients from DM&TB interrupted the anti-TB due to the toxic adverse drug effects.

Table 20

Microbiological features of tuberculosis patients *(P %)*.

Index	**Radiological features**	**DM&TB**	**TB**	**P value**
		N=186 *(P%)*	**N=318 *(P%)***	
Clinical forms of pulmonary TB	PIT	179 (91)*	288 (90)*	>0.05
	PDT	2 (1)	28 (9)	**<0.001**
	FCVT	14 (8)	2 (1)	**<0.001**
Treatment effectiveness	Treatment success	192 (71)	280 (88)	**<0.001**
	Treatment	14 (7)	12 (4)	>0.05

	failure			
	Lost to follow-up/interrupted the treatment	12 (6)	24 (7)	>0.05
	Died	28 (15)	2 (1)	**<0.001**

Note: Applied statistical test: paired simple T-test;

*Absolute numbers and percentages per column (in brackets).

The most used anti-diabetic treatment was the combination between short and medium acting insulin in 64 (34 %) patients, only medium acting insulin in 38 (20 %) cases and short acting insulin in 10 (5 %) cases. The association between the insulin and oral antidiabetic drugs (Metformin) was established in 24 (13 %) cases. In 50 (27 %) cases only oral antidiabetic treatment (monotherapy with Metformin or the combination between Metformin and sulfonylureas) was recommended. The FVPG was monitored weekly in all patients during the hospitalization period. In 44 (24 %) the FVPG exceeded the normal threshold despite the antidiabetic treatment and diabetic diet.

Patients were advised by the hospital dietitian about the methods of prevention of the metabolic imbalance, the type of diet with the amounts of carbohydrates determined according to age and body weight. It was recommended a diet based on slow glycemic released glucose foods such as non digestible fiber food: fresh vegetables, whole grains and beans [].

Patients were advised to decrease the consumption of saturated fats and increase the consumption of unsaturated fats

(vegetable oils), fish and white meat. They received the recommendations to decrease the consumption of salt, alcohol, smoking and refined sugar products. Patients were informed that the failure to follow the recommendations of the dietitian and endocrinologist could lead to the onset of acute complications and death.

Our research is among the few research works in the RM on an important topic related to the tuberculosis and diabetes. In our study more frequently was diagnosed type 2 diabetes, even the patients with type 1 were more vulnerable to tuberculosis, similar data were published in other studies [21].

We established that the patients older 55 years, residing in rural localities, in the economical vulnerable state, disabled had a higher risk for tuberculosis than those without glycemic disorders. The social, economical vulnerability was identified as a risk factor for tuberculosis in diabetic patients in multiple studies [6, 7, 11, 12, 13, 18].

The most relevant case-management features of diabetic patients were detection through the active screening using radiological investigation and positive microbiological assays in every third case. It is a consequence of the national policy recommendations and was confirmed by local papers [18, 22].

Risk factors were the anti-TB treatment in anamnesis and the TB contact, which were strongly linked with the sickness in multiple papers [6, 7, 11, 12, 13]. Severe, chronic evaluating

tuberculosis, determined by the late detection were identified as well in several papers [6, 7, 11, 12, 14].

Standard treatment for drug susceptible and MDR-TB was used in a similar proportion in both groups however the effectiveness was considerable lower in diabetic patients. Obtained results were similar with other studies, which identified a high rate of death and treatment failure [6, 7, 11, 12, 13, 14, 19, 23].

All diabetic patients received an individualized healthcare, however in every fourth patient the glycemic control was not achieved. Low treatment effectiveness was determined by late detection and progressive evolution of tuberculosis, side effects which contributed to the treatment interruptions, lack of the glycemic control under the antidiabetic treatment. Such results were confirmed in other international papers [23, 24].

Conclusion.

1. According to the research results the importance to improve the quality of diabetic case-management in the Republic of Moldova was confirmed.
2. A closed follow-up through the active screening for tuberculosis of all persons with disorders of carbohydrate metabolism should be done.
3. Fasting venous plasma glucose should be monitored regularly in patients with BMI higher than 24 kg/m^2 in order to prevent complications and including them in high risk groups for screening for tuberculosis.

4. For increasing the anti-TB treatment effectiveness the patient's compliance must be increased via the individualized treatment and adjusting the drugs dosage to the clinical tolerance avoiding the treatment interruption.
5. Patients should be informed about the role of the treatment compliance in prevention of complications related to tuberculosis and diabetes, and premature death.

7.3.5. The risk factors for developing the MDR-TB among diabetic patients

The risk factors for developing the MDR-TB among diabetic patients: 34 patients with MDR-TB & DM and drug susceptible MDR-TB & DM. Assessing general, social and economical peculiarities it was established the statistical predominance of male *vs* female in both groups: 21 (61,8%) *vs* 13 (38,2%) in the 1st group and 61 (71,1%) *vs* 24 (28,3%) in the 2nd group. Comparing the groups it was established a moderate predomination of male in the 2nd group comparing with the 1st group, so male/female ratio=1,6/1 in the 1st group and 2,5/1 in the 2nd group.

Assessing the patients according to the age groups it was established the statistical predominance of the young patients (18-34 years) in the 2nd groups: 40 (47,1%) comparing with the 1st group 4 (11,8%) and older than 55 years in the 1st group 19 (55,8%) *vs* 15 (17,6%) in the 2nd group. Summing patients in two subgroups: under 44 and older than 44 years, it was identified a

statistical difference between the predominance of patients less than 44 years in the 2nd group 52 (61,7%) *vs* 6 (17,6%) in the 1st groups and older than 44 years in the 1st group 28 (82,3%) *vs* 25 (29,4%) patients in the 2nd group. Considering that old age represents the specific feature for the group with diabetes and MDR-TB it was assessed as a high risk factor for tuberculosis (OR=11,2 95% CI: 5,8-60). The data are presented in the table 21.

Table 21

Distribution in sex and age groups the MDR-TB among diabetic patients (P%)

Groups	**Indices**	**MDR-TB&DM**	**Drug susceptible TB&DM**	**p**
		n = 34 (P%)	**n = 85 (P%)**	
Sex	**Men**	24 (71)	64 (75)	>0,05
	Women	10 (29)	21 (25)	>0,05
18-44 years	**18-24**	1 (3)	0	**<0,05**
	25-34	6 (18)	6 (7)	**<0,05**
	35-44	11 (32)	18 (21)	>0,05
>44 years	**45-54**	12 (35)	23 (27)	>0,05
	55-64	4 (12)	33 (29)	**<0,05**
	>65 years	0	5 (6)	**<0,05**

Among patients of both groups a similar distribution from rural and urban areas was established, however homeless were

established only in the 2nd group – 6 (7,1%) patients. Distribution of patients by economic groups was relevant. Due to the old age of the patients with TB-MDR and DM, employed persons statistically predominated in the 1st group 14 (41,2%) *vs* 21 (24,7%) in the 2nd group. Social vulnerable patients were more frequently registered in the TB-MDR group: 52 (61,7%) unemployed patients *vs* 8 (23,%) in the 1st group. Low living conditions also predominated in the 2nd group 68 (80,1%) *vs* 21 (61,7%) in the 1st group due to high rate of unemployed patients. Retired patients predominated in the MDR-TB+DM group: 8 (23,5%) *vs* 8 (9,4%) in the 1st group; the same situation was determined for the persons with disabilities: 4 (11,8%) *vs* 7 (8,3%) patients, respectively (table 22).

Table 2

Main demographic, social and economical characteristics the MDR-TB among diabetic patients (P%)

Groups	Indices	MDR-TB&DM	MDR-TB	p
		n = 34 (P%)	n = 85 (P%)	
Demographic	Urban	17(50,1%)	41 (48,2%)	>0,05
	Rural	14(41,2%)	38 (44,7)	>0,05
	Homeless	0	6 (7,1%)	>0,05
Economic	Employed	14 (41,2%)	21 (24,7%)	>0,05

	Unemployed	8 (23,5%)	52 (61,7%)	**<0,01**
	Retired	8 (23,5%)	8 (9,4%)	>0,05
	Students	0	5 (5,9%)	>0,05
	Disease disability	4 (11,8%)	7 (8,3%)	>0,05
Life conditions	Low living condition	21 (61,7%)	68 (80,1%)	**<0,05**

The social risk groups with epidemiological role were evaluated. Migrants constituted a similar part in both groups, ex-detained were identified only in the MDR-TB group (13 (15,3%) cases). Exposure to tuberculosis infection (TB contact) statistically predominated in the 1st group 11 (52,9%) *vs* 25 (29,4%) in the 2nd group and was identified as a low risk factor (OR=1,1 CI 95% 0,5-2,7).

All patients in the 1st group and half of the 2nd group had associated diseases. So, the co-morbitities were established as a high risk factor (OR=45; CI 95% 42-48) for developing tuberculosis. In 15 (44,1%) patients of the 1st group diabetes mellitus was diagnosed in the same time as tuberculosis. Regarding the harmful habits, active tobacco smoking and alcohol drinking statistically prevailed in the 2nd group. Tobacco smokers were 63 (74,1%) in the 1st group *vs* 13 (39,2%) in the 2nd group and alcohol abusers were 38 (44,7%) in the 1st group *vs* 7 (20,6%) cases in the 2nd group (table 23).

Table 23

Distrbution in risk groups the MDR-TB among diabetic patients (P%)

High risk groups	**Indices**	**MDR-TB&DM**	**MDR-TB**	**p**
		n = 34 (P%)	**n = 85 (P%)**	
High risk groups	Migrants	6 (17,6%)	16 (18,8%)	>0,05
	Ex-detained	0	13 (15,3%)	**<0,05**
	TB contact	11(52,9%)	25 (29,4%)	**<0,01**
	HIV infection	1 (2,9%)	2(2,6%)	>0,05
	Associated diseases	34 (100%)	36 (42,4%)	**<0,05**
	Psichiatric disorders	1 (2,9%)	8(9,4%)	>0,05
Associated harmful habits	Tobacco smoking	13 (39,2%)	63 (74,1%)	**<0,001**
	Alcohol abusers	7 (20,6%)	38 (44,7%)	**<0,05**

By studing the civil status it was identified a statistical higher rate of married patients in the 1st group: 16 (47,1%) *vs* 15 (17,6%) patients in the 2nd group and of the divorced and widows persons: 10 (29,4%) *vs* 22 (7,1%), respectively.

Single persons predominated in the 2nd group due to young age of most of the patients. When assessing the educational level

it was established that one half of both groups graduated general school.

The incomplete general educational level was more frequently identified in the patients from the 2nd group: 24 (28,2%) *vs* 6 (17,6%) in the 1st group. Higher education level was established in a limited number of cases in both groups.

No statistical differences were established between the groups of tuberculosis patients (table 24).

Table 24

Main social characteristics the MDR-TB among diabetic patients (P %)

Status	**Indices**	**MDR-TB&DM**	**MDR-TB**	**P**
		n = 34 (P%)	**n = 85 (P%)**	
Marital	Single	8 (23,5%)	48 (56,5%)	<0,01
	Married	16 (47,1%)	15 (17,6%)	<0,001
	Divorced/widow	10 (29,4%)	22 (7,1%)	<0,05
Education	Primary/illiteracy	2 (5,7%)	8 (9,4%)	>0,05
	Incomplete secondary	6 (17,6%)	24 (28,2%)	>0,05
	General	16 (47,1%)	34 (40,1%)	>0,05
	Professional	6 (16,6%)	17 (20%)	>0,05
	Superior	4 (11,7%)	2 (2,4%)	>0,05

Studying case-management it was identified that the most of the patients from the 1st group comparing with the 2nd group were detected by high risk group screening, as recommended by the national protocol: 22 (64,1%) *vs* 13 (15,3%), respectively.

As a symptomatic cases the patients were detected more often in the 2nd group: 60 (70,6%) *vs* 12 (35,3%) in the 1st group. By direct addressing were detected 12 (14,2%) patients in the 2nd group. Microscopic smear positive results were established in more than two third of patients of both groups: 22 (64,1%) in the 1st group and 61 (71,6%) in the 2nd group.

Positive culture results were 24 (70,6%) patients in the 1st group and 76 (89,4%) in the 1st group. Positive results of Xpert MTB/Rif assay were more frequently identified in patients from the 2nd group: 76 (89,4%) *vs* 26 (76,5%) in the 1st group (table 25).

Table 25

Case-finding detection, microbiological characteristics and treatment outcomes the MDR-TB among diabetic patients (P %)

Characteristics	**Indices**	**MDR-TB&DM**	**MDR-TB**	**p**
		n = 34 (P%)	**n = 85 (P%)**	
Case-finding	Detected as	12	60 (70,6%)	**<0,001**

	symptomatic case	(35,3%)		
	Detected by active screening	22 (64,1%)	13 (15,3%)	**<0,001**
	Direct addressing to the specialized hospital	0	12 (14,2%)	**<0,05**
Microbiological positive results	Microscopy	22 (64,1%)	61 (71,6%)	>0,05
	Culture	24 (70,6%)	64 (75,3%)	>0,05
	Xpert MTB/Rif	26 (76,5%)	76 (89,4%)	>0,05

When identifying the radiological characteristics of pulmonary tuberculosis patients it was established lung parenchymal destruction in both groups: 25 (73,6%) in the 1st group and 61 (71,7%) cases in the 2nd group.

Disseminated opacities were established in a similar rate in patients of both groups: 21 (61,7%) in the 1st group and 58 (68,2%) in the 2nd group. Both lungs were affected more frequently in patients of the 1st group: 24 (70,6%) *vs* 49 (57,6%) in the 2nd group, but the statistical threshold was not achieved.

More than three affected segments had 65 (76,5%) patients in the 2nd group and 22 (64,7%) in the 1st group. Radiologic evolution under the specific treatment with second line anti-tuberculosis drugs assessed as partial resorption was determined in a similar proportion in both groups: 28 (82,3%) in the 1st group and 76 (89,4%) in the 2nd group.

Lung infiltrates progression was established in higher proportion in the 1st group: 7 (20,6%) *vs* 6 (7,1%) in the 2nd group, which contributed to the high rate of died patients.

Although infiltrative TB form was diagnosed in the majority of patients, the severest forms such as disseminated TB and fibro-cavernous TB were diagnosed more frequently in the 1st group: 6 (17,6%) *vs* 9 (10,6%) in the 2nd group. Data are exposed in the table 26.

Table 26

Case-management characteristics and imagistic features the MDR-TB among diabetic patients (P %)

Characteristics	**Indices**	**MDR-TB&DM**	**MDR-TB**	**p**
		n = 34 (P%)	**n = 85 (P%)**	
Imagistic	Destruction	25 (73,6%)	61 (71,7%)	>0,05

	Dissemination	21 (61,7%)	58 (68,2%)	>0,05
	Both lungs	24 (70,6%)	49 (57,6%)	>0,05
	Extensive TB	22 (64,7%)	65 (76,5%)	>0,05
	Partial resorption	28 (82,3%)	67 (78,8%)	>0,05
	Progression	7 (20,6%)	6 (7,1%)	>0,05
Clinical radiological forms	Infiltrative	28 (82,3%)	76 (89,4%)	>0,05
	Disseminated	4 (11,7%)	8 (9,4%)	>0,05
	Fibro-cavernous	2 (5,9%)	1 (1,2%)	>0,05

Treatment outcome was assessed using the standardized indices. The success rate was lower than recommended by WHO (85%) in both groups. The lowest success rate was registered in the 1st group: 20 (58,8%) *vs* the 2nd group 66 (77,6%).

Poor outcomes predominated in the 1st group: 14 (41,2%) *vs* the 2nd group 11 (12,9%). The highest rate of died patients was identified in the 1st group: 6 (17,5%) comparing with the 2nd group 3 (3,5%) (table 27).

Table 27

Treatment outcome types the MDR-TB among diabetic patients (P %)

Outcome	MDR-TB&DM	MDR-TB	p
	n = 34 (P%)	n = 85 (P%)	
Successfully treated	20 (58,8%)	66 (77,6%)	**<0,05**
Died	6 (17,5%)	3 (3,5%)	>0,05
Lost to follow-up	5 (14,7%)	8 (9,4%)	>0,05
Failure	3 (8,8%)	5 (5,8%)	>0,05

Considering all above exposed data it was established that the most relevant general and biological characteristics of the pulmonary MDR-TB patients associated with diabetes mellitus were old age and comorbid state.

They were more frequently divorced or widow and were detected by active screening according to the national recommendations (table 28).

Table 28

Odds Ratio assessing factors associated with diabetes mellitus and MDR-TB in patients with Xpert MBT/Rif resistant results

F**actors**	**Odds Ratio**
Old age (more than 55)	11,2 (95% CI: 5,8-60), p**<0.001**
Comorbid state	45 (95% CI: 42-48), p<0,001

Detected by active screening	11,2 (95% CI:11,7-11,4) p<0,05
Divorced or widow	1,9 (95% CI: 1,16-1,3), p<0,05

The most relevant social-economic characteristics of the pulmonary MDR-TB patients were economical disadvantaged state, low living condition, single civil state and life history of imprisonment. They were more frequently detected as symptomatic cases and were directly addressed to the specialised hospital. Despite the low social-economic state and late detection associated with the passive way of detection they had a high treatment success, demonstrating the strong impact on the disease outcome of the diabetes mellitus (Table 29)

Table 29

Odds Ratio assessing factors associated with MDR-TB in patients with Xpert MBT/Rif resistant results

Factors	**Relative Risk**
History of imprisonment	5,6 (95% CI: 5,3-5,9), p<0,05
Economicaly disadvantaged state (unemployment)	5,12 (95% CI: 2,9-13,6), p<0.001
Low living conditions	2,5 (95% CI: 1,5-3,8), p<0.001
Single-civil state	3,9 (95% CI: 3,7-4,1), p<0.01
Detected as symptomatic case	4,2 (95% CI: 4,1-4,3),

	p<0.001
Detected by addressing to the specialized hospital	5,3 (95% CI: 4,9-5,7), p<0.05
Successfully treated	1,3 (95% CI: 1,25-1,37), p<0.05

Discutions. Association of tuberculosis and diabetes represents an epidemiological challenge and important problem for the health system in the Republic of Moldova. It was established that the tuberculosis prevalence rate among patients with diabetes is 1.8–9.5 times higher than in the general population [48].

In the Republic of Moldova 12,3% of the population have diabetes or reduced tolerance to glucose and 409 patients died due to diabetic complications in 2015 [7]. Since the incidence of the Republic of Moldova slowly decreased, the rate of MDR-TB is increased. MDR-TB represent another serious threat to the global disease control.

In clinical studies established a strong association between the risk factors and MDR-TB. There are several risk factors which increase the risk for MDR-TB in patients with diabetes: previous treatment, young age, HIV associated infection, smoking , alcohol and other substance abuse [14]. Some clinical studies denoted a high rate (10-23%) of MDR-TB among patients with diabetes [15, 20, 40, 42]. Other cited factors were: HIV co-infection, age older than 45, overweight, and male sex [13, 20].

If tuberculosis is detected earlier a more favorable outcome can be achieved. A severe course of tuberculosis with a tendency to the rapid progression and lung parenchyma destruction occurs mainly in patients with untreated diabetes mellitus or in late detected tuberculosis [6].

Our study demonstrated a strong influence of diabetes on tuberculosis outcome. Obtained results were similar to other studies, which determined a high rate of failure and death among patients with tuberculosis and diabetes [5, 10, 24, 29, 38].

Poor treatment outcomes could be explained by the co-morbidities such as diabetes, HIV infection, and social determinants of health (unemployment, educational level, income distribution, social vulnerability, health services accessibility) [10]. Nowadays, in the Republic of Moldova the global prevalence of tuberculosis among patients with diabetes is high and reflects the general epidemiological situation.

Conclusion. The treatment success rate amoung patients with drug resistant tuberculosis and diabetes was low due to folowing contributing factors: old age and comorbid state.

More freaquvently patients with drug resistant tuberculosis and diabetes were detected by active screening and had a civile unfavorable state (divorsed and widow), as associated with old age.

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