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OPTIMISATION OF THE DIAGNOSIS AND IMPROVEMENT OF THE TREATMENT OF TUBERCULOSIS

321.07 - PHTHISIOPNEUMOLOGY

Summary of the habitation thesis in medical sciences

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The thesis and its summary can be found at the library of Nicolae Testemitanu State University of Medicine and Pharmacy and on the website of ANACEC (www.cnaa.md).

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CONCEPTUAL LANDMARKS OF THE RESEARCH

Relevance of the research

Tuberculosis (TB) is the infectious disease that account for a significant morbidity and the highest number of deaths caused by a single infectious agent globally each year [1]. As a result of this major impact on public health, tuberculosis is in view of the most recognized international institutions that prioritize policies and interventions aimed to reduce the burden and eventually eliminate this disease [2]. In this context, in the last decade, a series of important achievements have been registered both in diagnosis and treatment of tuberculosis.

In the field of tuberculosis diagnosis, several nucleic acid amplification-based tests have been developed, which in validation studies have demonstrated high accuracy in identifying *M. tuberculosis* that combined with a short turnaround time for test results [3]. As well, molecular-genetic technologies have offered the possibility of rapid assessment of susceptibility to basic anti-tuberculosis drugs. Among these tests a particularly wide implementation has been achieved in case of Xpert MTB/Rif (Xpert). Its technical characteristics (accuracy, speed, and operational simplicity) had inspired enthusiasm even from the first implementation reports. At the same time, it was clear that the effectiveness and real impact of using Xpert MTB/Rif technology in current clinical practice could be reduced by a number of factors conditioned by the particularities of local medical systems (high rate of TB/HIV co-infection, increased incidence of multidrug resistant strains, centralised healthcare delivery system etc.) [4] and patient characteristics (extrapulmonary forms of disease, immunocompromised status, children, etc.) [5]. Thus, assessment of the real impact of the use of Xpert technology in TB diagnosis in different healthcare systems has become one of the research priorities. Therefore, evaluation of the impact of Xpert MTB/Rif on therapeutic management in patients with MDR tuberculosis and on the microbiological confirmation of tuberculosis in patients with paucibacillary sputum, in clinical practice in the Republic of Moldova, are two of the objectives of the present work.

Along with the implementation of new diagnostic methods, the workload of TB microbiological laboratories has inevitably increased, both

because of direct testing using new technologies and secondary to testing performed as part of quality control in subordinate units. In this context, tools for automated performance and reading of classical diagnostic methods (microscopy and culture) became of great relevance. These technologies have the potential to replace or at least reduce the involvement of human staff and thereby "free" them for the supervision/performance of more complex tests. For some of these automated systems, there is a considerable amount of experimental and field data on their efficacy and cost-effectiveness [6], for others, the accuracy and feasibility of application in the context of different diagnostic scenarios has yet to be established. In this context assessment of the performance of the automated fluorescent staining microscopic smear reading platform (Fluorobot) is another objective of the present work [7].

Along with important achievements in the diagnosis of tuberculosis, there have also been some disappointments and unfulfilled hopes in recent years, in particular this is the case of latent tuberculosis diagnostic tests (LTBI) [8]. Thus, the development, over several decades, of IGRA (Interferon-Gamma Release Assay) tests has ended up with the conclusion of their low specificity in identifying persons with LTBI at increased risk of progression to active TB as well as in differentiating persons with LTBI from those with active TB [9]. As a result, numerous studies are currently conducted to identify new biomarkers (alternative or complementary to INF-γ assessed by IGRA) that would be more effective in differentiating various clinical statuses of TB infection. Thus, and one of the objectives of the present work was to evaluate the potential role of the assessment of the IL-2/INF-γ secretory profile of peripheral blood T lymphocytes in the differentiation of clinical statuses of *M. tuberculosis* infection [10].

The recent major advances in treatment of tuberculosis are related to the identification and introduction into clinical practice of new drugs and therapeutic regimens. Among these, the most notable are bedaquiline and delamanid, in line with short-course treatment regimens for MDR-TB. Although delamanid could be, at least in part, listed as an unmet expectation, bedaquiline has demonstrated its efficacy at all stages of clinical testing due

to which it has been included in the first-line battery of antituberculosis treatment [11, 12]. However, the efficacy reported in clinical trials may not be reproduced in real world practice especially in case of MDR-TB (administration in patients with more severe disease than those in clinical trial, lower adherence to long-term therapy, higher risk of adverse reactions due to co-administration with medication used for comorbidities, etc.). Thus, evaluation of the effectiveness of new drugs and regimens when applied in routine clinical practice, particularly in case of programmatic management of national TB response programmes, constituted a major practical research interest. The present paper describes the results of the evaluation of the effectiveness of including bedaquiline in antituberculosis treatment regimens in MDR-TB patients in the National Tuberculosis Response Programme in the Republic of Moldova.

Tuberculosis treatment monitoring and outcome assessment is an area of ongoing discussion. Currently used markers (mostly microbiological) for assessment of therapy efficacy and cure are indirect and of low specificity [13]. Thus, the research priorities in this area are directed towards the identification of more specific biomarkers that would allow individualized treatment duration and better cure prediction. The results obtained in this respect, to date, are promising, but still far from being applicable in current practice. Because of this there is a lively interest in identifying risk factors for unfavourable treatment outcome and for reviewing the criteria (microbiological and clinical) currently used to define antituberculosis treatment outcomes [14]. Considering that among the objectives of the present work is assessment of the impact of lung function on antituberculosis treatment outcomes and evaluation of the feasibility and impact of concluding on the outcome of antituberculosis therapy after one year follow-up.

Key words: TB, MDR, laboratory diagnostics, *M. tuberculosis*, antituberculosis treatment.

Area of research: Pneumophthisiology

Aim of the research

Evaluation of clinical and laboratory interventions with potential to optimise diagnosis and to improve treatment of tuberculosis patients.

Research objectives

1. To assess efficacy of determination of the interferon-γ and interleukin-2 peripheral cell response in differentiating the clinical statuses of *M. tuberculosis* infection.

2. To assess the diagnostic accuracy of automated reading by Fluorobot platform of the fluorescent stained smears in the diagnosis of pulmonary tuberculosis in adults.

3. To determine the impact of the implementation of GeneXpert MTB/Rif technique, on the therapeutic management of patients with mutlidrug-resistant pulmonary tuberculosis in the Republic of Moldova.

4. To determine the added value of the bronchial aspirate specimen testing in the diagnosis of pulmonary tuberculosis in patients with paucibacillary free expectorated sputum specimens.

5. To assess the effectiveness of including bedaquiline in multidrug-resistant tuberculosis treatment regimens in the conditions of programmatic management of tuberculosis patients.

6. To assess the rationality of including high-dose isoniazid in treatment regimens used in patients with multidrug-resistant tuberculosis in the Republic of Moldova.

7. To determine the impact of respiratory function, assessed at diagnosis, on treatment outcomes in patients with pulmonary tuberculosis.

8. To evaluate alternative approaches in assessment of treatment outcomes in tuberculosis patients.

Scientific novelty and originality.

The novelty of the research is given by the evaluation of the efficacy of applying new biomarkers (IL-2/INF-γ cytokine profile of peripheral blood lymphocytes), alternative specimens (bronchial aspirate) and original technological solutions (FluoroSpot, Fluorobot, Xpert MTB/Rif) for the diagnosis of tuberculosis in clinical practice. Similarly, the novelty of the study is ensured by the assessment of the effectiveness and/or rationality of the application in routine clinical conditions in the healthcare system of the Republic of Moldova of new drugs such as bedaquiline or high-dose isoniazid, together with the identification of new predictors of the effectiveness of antituberculosis treatment and the application of more

objective approaches, from a clinical point of view, in the assessment of antituberculosis treatment outcomes.

Results that led to the solution of a major scientific or applied problem.

- Fluorospot represents an alternative method for the diagnosis of latent tuberculosis infection with a diagnostic accuracy like that of EliSpot-IGRA techniques.
- Application of deep learning algorithm-based technologies such as Fluorobot allows automated reading of microscopic smears in fluorescent staining with an accuracy comparable to that obtained at manual reading.
- Nationwide application of the Xpert MTB/Rif technique in the diagnosis of tuberculosis has allowed a 6-fold reduction in therapeutic delay in patients with MDR pulmonary tuberculosis.
- The use of bronchial aspirate specimens tested by molecular-genetic tests (Xpert MTB/Rif) in patients with pulmonary tuberculosis with highly paucibacillary sputum (microscopy and Xpert MTB/Rif negative) allows additional microbiological confirmation in a quarter of these patients.
- Bedaquiline used in treatment regimens in patients with MDR tuberculosis, including those with previous treatment failure, under programmatic care delivery ensures a high cure rate and reduced mortality.
- Resistance to isoniazid in MDR tuberculosis patients in the Republic of Moldova is overwhelmingly due to mutations in the katG gene (associated with high levels of minimum inhibitory concentrations against this drug), which precludes empirical administration of high doses of isoniazid (15-20 mg/kg) in treatment regimens for these patients.
- Restrictive pattern of pulmonary function in patients with pulmonary tuberculosis, assessed at initiation of antituberculosis treatment, is a potentially modifiable predictor of negative treatment outcome in patients with pulmonary tuberculosis.
- Assessment of antituberculosis treatment outcome after a period of post-therapy surveillance is a more rational approach to antituberculosis treatment evaluation, feasible in conditions of programmatic care of tuberculosis patients.

Theoretical significance.

The research provides the rationale on optimisation of current diagnostic algorithms on diagnosis of *M. tuberculosis* infection by potentially including serological assays to assess INF-γ and IL-2 secreted by T lymphocytes, automated reading of microscopy of fluorescent stained smears and molecular-genetic testing of bronchial aspirate specimens in patients with paucibacillary sputum. Argumentation of the usefulness of the mentioned interventions is performed considering sensitivity, specificity, positive and negative likelihood ratios. Similarly, the impact of the nationwide implementation of the Xpert MTB/Rif technique has been argued by assessing the overall effect on reducing therapeutic delay in patients with MDR pulmonary tuberculosis. As part of the valuation of interventions for the improvement of tuberculosis treatment, was argued the effectiveness of including bedaquiline in treatment regimens under programmatic conditions in MDR-TB patients by demonstrating a higher cure rate and lower mortality when using this drug, and the ineffectiveness of empirical inclusion (without assessment of genetic determinants) of high-dose isoniazid in treatment regimens in MDR tuberculosis patients. In the same context, the rationality and feasibility of post-therapy surveillance in assessing TB treatment outcomes was argued.

Applicative value.

The application of the interventions evaluated in this work allows to reduce the diagnostic delay in patients with MDR pulmonary tuberculosis, significantly to increase the microbiological confirmation of pulmonary tuberculosis in patients with paucibacillary sputum, and to optimize the reading of fluorescent stained smears. Likewise, the use of the evaluated therapeutic solutions has been shown to be effective in increasing cure rate and reducing mortality rate in MDR tuberculosis patients, as well as avoiding irrational empirical use of high-dose isoniazid treatments with potential hepatotoxic effect in these patients. The use of treatment assessment after post-treatment surveillance have been shown to be more rational, from clinical point of view, for the assessment of therapeutic efficacy.

Implementation of scientific results.

The theoretical and practical results of the study are used in training of medical students at the Department of Pulmonology and Allergology, as well as in the clinical departments of "Chiril Draganiuc" Phtisiopneumology Institute. There were registered 3 certificates of innovator and 3 acts of implementation of results.

The results of the thesis were reflected in 51 scientific publications including: 1 - nationally published single author monograph, 1 - chapter in an international monograph, 1 - contribution in medical textbook, 21 - articles in journals indexed in the Web of Science database, 1 - article in databases accepted by the ANACEC, 5 - articles in journals of the National Register of Journals, 14 - conferences abstracts, 7 - editorials and commentaries. As well, thesis results have been presented at 19 scientific meetings and events.

THESIS CONTENT

1. CURRENT DIAGNOSIS AND TREATMETN OF TUBERCULOSIS

The chapter reviews the literature on the diagnosis and treatment of tuberculosis. That is preceded by a description of the evolution of TB epidemiological indicators at local and international level, highlighting the major challenges that prevent TB elimination in the upcoming future. On the diagnostic options, the variety of molecular-genetic methods for confirming *M. tuberculosis* infection are described, alongside classical tests such as microscopy and culture are characterised. The diagnostic accuracy of the tests and the advantages of their application in clinical practice are comparatively analysed. The latest data on treatment regimens and new drugs introduced into clinical practice during the last decade are as well reviewed. The last part of the chapter is devoted to the drawbacks of current approaches in assessing TB treatment outcomes and potential opportunities for improvement.

2. SPECIFIC CYTOKINE RESPONSE IN DIFFERENTIATING CLINICAL STATUSES OF *M. TUBERCULOSIS* INFECTION

The chapter describes the results of the assessment of diagnostic accuracy of IL-2/INF-γ secretory profile of peripheral blood T lymphocytes assessed by the Fluorospot platform in differentiating latent tuberculosis infection (LTBI) from active TB (confirmed by culture or molecular-genetic test) and cured TB, using as a control group healthy individuals in whom LTBI was excluded (negative IGRA-Elispot result). For this purpose, 73 persons were enrolled, of which 4 patients, initially diagnosed with tuberculosis, were excluded due to subsequent confirmation of an alternative diagnosis. Of the 69 persons included in the final analysis 25 met the criteria for inclusion in the LTBI cohort, 18 - cohort of patients with active TB, 10 - cohort with cured TB, 16 - cohort of healthy persons.

FluoroSpot assessment of IL-2 and INF-γ response induced by ESAT-6

FluoroSpot detection of cytokines response induced by ESAT-6 stimulation revealed a higher number of IL-2-secreting PBMCs in TB patients and people with LTBI than in healthy individuals, i.e. median 9,5 spot forming cells (SFCs)/200.000 peripheral blood mononuclear cells (PBMCs) versus 2 SFCs/200.000 PBMCs, p=0.001 and respectively 4 SFCs/200.000 PBMCs versus 2 SFCs/200.000 PBMCs, p=0.02.

The number of INF-γ-secreting PBMCs was significantly different in TB patients and LTBI individuals compared to healthy individuals, i.e. median 18,5 SCFs/ 200.000 PBMCs versus 1 SFC/ 200.000 PBMCs, $p<0.0001$ and respectively 6 SFCs/ 200.000 PBMCs versus 1 SFC/ 200.000 PBMCs, p=0,0003.

The only subset of PBMCs for which significant differences between TB patients and LTBI individuals on stimulation with ESAT-6 were those with IL-2^- IFN-γ^+ secretory profile, median 13 SFCs/ 200.000 BPMCs versus 5 SFCs/ 200.000 BPMCs, p=0,0136, (**Figure 2.1 B**) Similarly, the number of IL-2^- IFN-γ^+ BPMCs is higher in TB patients compared to those with a history of cured TB, but without statistical significance, median 13 SFCs/ 200.000 BPMCs versus 6 SFCs/ 200.000 BPMCs, p=0,095.

In the case of secretory PBMCs of both cytokines IL-2^{+}IFN-γ^{+} significant differences were only revealed between individuals with a history of cured tuberculosis and healthy individuals (median 2,5 SFCs/200.000 PBMCs versus 0 SFC/200.000 PBMCs, p=0,007, **Figure 2.1 C**).

In the context of the differences in IL-2 and IFN-γ secretion profile between the analysed groups, it should be noted that the observed overlaps in the expression of all five cytokine patterns would not allow differentiation of clinical-immunological statuses of tuberculosis infection in the individual patient in clinical practice.

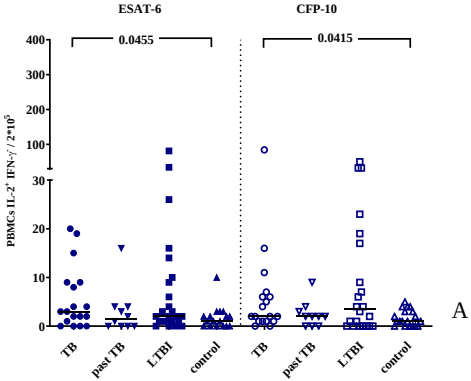
Comparison of the rate of each type of PBMCs with a specific cytokine secretion profile (IL-2^{+} IFN-γ^{-}, IL-2^{-} IFN-γ^{+} and IL-2^{+} IFN-γ^{+}) revealed no significant differences between TB patients and individuals with LTBI or those with a history of cured TB.

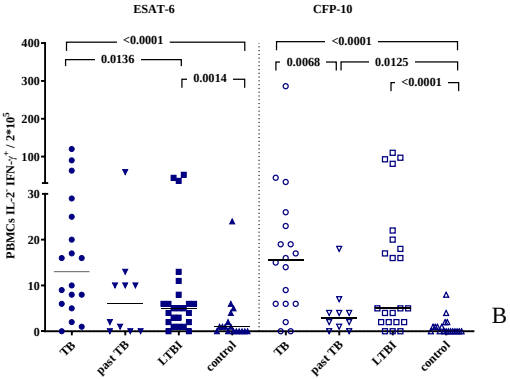
FluoroSpot assessment of IL-2 and INF-γ response induced by CFP-10

When assessing the cytokine response induced by CFP-10 stimulation, significant differences were observed in the number of PBMCs with IL-2^{-} IFN-γ^{+} secretory profile among individuals with history of cured tuberculosis compared to that in tuberculosis patients, (median 3 SFCs/200.000 PBMCs versus 15,5 SFCs/200.000 PBMCs, p=0.007) and that in healthy individuals (median 3 SFCs/ 200.000 PBMCs versus 0 SFCs/ 200.000 PBMCs, p=0,01, **Figure 2.1 B**).

The total number of INF-γ^{+} as well as IL-2^{+} IFN-γ^{+} and IL-2^{-} IFN-γ^{+} secreting cells in LTBI or healthy individuals was lower than in TB patients.

Similarly, the total number of IL-2^{+}-secreting PBMCs and that with IL-2^{+} IFN-γ^{-} secreting profile in healthy individuals was lower than in TB patients.





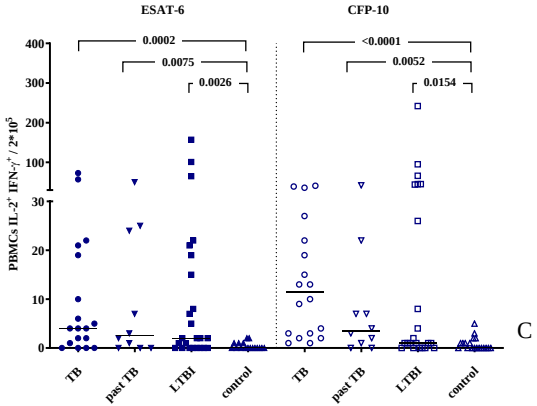


Figure 2.1 ESAT-6 and CFP-10- induced cytokine response in FluoroSpot. ESAT-6 induced cytokine immune response in 200.000 PBMCs/well in participants with active tuberculosis (TB, circle, n = 18), past tuberculosis (past TB, inverted triangle, n = 10), latent infection with M. tuberculosis (LTBI, square, ESAT-6-induced n = 24, CFP-10-induced n = 22), EliSpot-negative individuals (control, triangle, ESAT-6-induced n = 17, CFP-10-induced n = 19) was analysed. A p-value of <0,05 was considered significant.

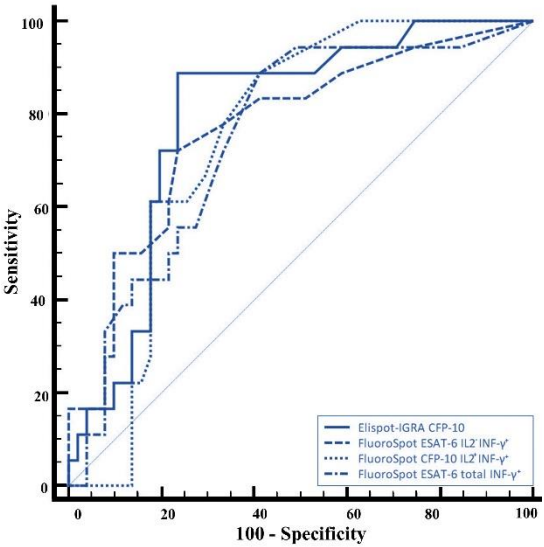


Figure 2.2 ROC plots of the FluoroSpot and EliSpot-IGRA tests with best discriminatory accuracy for subjects with active tuberculosis, assessed by AUC values.

Comparison of rates of PBMCs with various cytokine profiles shows a dominance of IL-2$^-$ IFN-γ^+ PBMCs in active tuberculosis, versus an approximately equal distribution of IL-2$^+$ IFN-γ^-, IL-2$^+$ IFN-γ^+ and IL-2$^-$ IFN-γ^+ in persons with history of tuberculosis, and increased rate of IL-2$^+$ IFN-γ^- PBMCs in persons with LTBI.

Overall diagnostic accuracy of IGRA-EliSpot and FluoroSpot tests

Comparison of the ROC curves of the IGRA-EliSpot and FluoroSpot assays revealed that the total INF-γ^+ response discriminates most effectively between the clinical states of *M. tuberculosis* infection - LTBI, active tuberculosis, cured tuberculosis and healthy (**Figure 2.2**). Similar results were observed for FluoroSpot at a threshold value greater than 5 SFCs/ 200.000 INF-γ^+ secreting PBMCs upon stimulation with ESAT-6 (AUC 0,751, 95%CI 0,632-0,847, sensitivity -59%, specificity - 89%) and at threshold value above 6 SFCs/ 200.000 PBMCs with IL-2$^-$IFN-γ^+ cytokine profile upon stimulation with ESAT-6 (AUC 0,77, 95%CI 0,653-0,862,

sensitivity 72%, specificity 76%). At a value greater than 12 SFCs/ 200.000, which is optimal for CFP-10-induced IL-2^+-secreting PBMCs, sensitivity and specificity were 82% and 67% (AUC 0,724, 95%CI 0,603-0,825).

Thus, neither the assessment of the number of IL-2^+-secreting nor INF-γ^+-secreting cells allows differentiation with sufficient accuracy between active TB patients, persons with LTBI, those with a history of cured TB and healthy individuals.

3. OPTIMISATION OF MICROSCOPIC DIAGNOSIS OF TUBERCULOSIS

The chapter presents the results of the prospective assessment of the diagnostic accuracy of *M. tuberculosis* bacilli identification by automated reading of microscopic smears in fluorescent staining using the Fluorobot platform. The microscopic testing was performed for the diagnosis purpose of pulmonary tuberculosis. This was compared with manual microscopy reading by laboratory staff, using *M. tuberculosis* culture as the reference method. Diagnostic accuracy was assessed by determining sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), positive likelihood ratio (LR+) and negative likelihood ratio (LR-). ROC analysis was performed to quantify overall accuracy of the test.

As part of the study 588 lower airways specimens were collected. Of these, 136 were specimens other than sputum and were therefore not included in the study. In other 30 cases specimens were excluded due to contaminated cultures. Thus 422 sputum samples were included in the final analysis. *M. tuberculosis* culture was positive in 80 (18,9%) of the analysed samples.

At the manual reading of the smear microscopy 56 out of 80 culture-positive samples were graded as positive, corresponding to a sensitivity of 70,0% (95%CI, 58,7% - 79,7%). For culture-negative sputum samples 335 out of 342 samples were graded as negative by microscopy, corresponding to a specificity of 97,9% (95%CI, 95,8% - 99,2%).

The Fluorobot assessed 56 of 80 culture-positive samples as positive, corresponding to a sensitivity of 70,0% (95%CI, 58,7% - 79,7%). Out of 342 culture-negative samples, 280 samples were microscopically

negative when examined by the Fluorobot, that correspond to a specificity of 81,8% (95%CI, 77,4% - 85,8%).

The positive likelihood ratio (LR+) and the negative likelihood ratio (LR-) in case of manual reading was respectively 34,2 (95%CI, 16,2-72,2) and 0,4 (95%CI 0,2-0,4), and corepsonding values for Flurorobot examination - 3,9 (3,0-5,0) and 0,4 (0,3-0,5) respectively. Thus, a positive result by Fluorobot is 8,5 times less likely to confirm the diagnosis of tuberculosis than for manual examination of sputum smears in fluorescent staining.

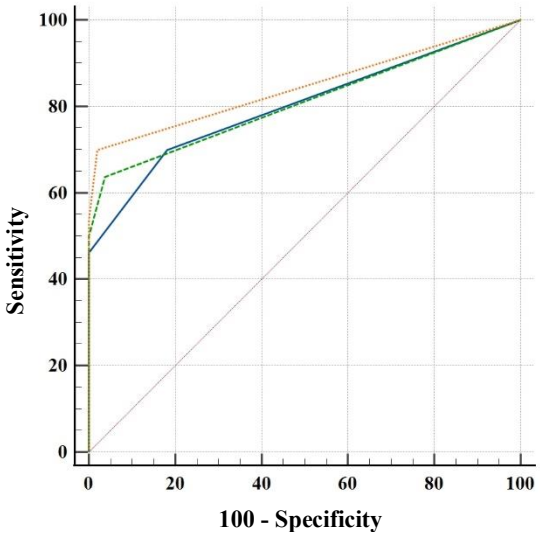


Figure 3.3 Diagnostic accuracy of Fluorobot examination of sputum smears fluorescently stained with auramine compared to manual examination by laboratory specialists, culture on solid media was considered as reference method, orange line - manual examination, green line - Fluorobot examination supplemented by manual re-examination of uncertain smears, blue line Fluorobot examination only.

Following our finding of lower specificity in Fluorobot examination, the grading of each smear by both methods was compared and 66 smears were identified as +/- by Fluorobot but negative by manual reading.

Thus, to reduce the rate of false positive results and to increase the specificity of Fluorobot examination, re-examination of some smears by manual examination was evaluated. To determine the minimum number of smears re-assessment of which could result in an optimal increase in specificity of Fluorobot examination, several options were examined depending on the number of bacilli identified in the smear. An acceptable result from the perspective of the number of smears re-examined versus the added value to specificity was obtained when re-examining smears with 2-4 bacilli in the visual field of interest. This subgroup constituted 63 (14,9%) smears out of the total smears analysed, re-examination of which resulted in a positive microscopy result in 51/80 of the culture-positive sputum samples (sensitivity 63,8%, 95%CI, 52,2 - 74,2) and a negative microscopy result in 329 out of 342 culture-negative sputum samples (specificity 96,2%, 95%CI, 93,6 - 98,0). The corresponding values of LR+ and LR- were 16,8 (9,6-29,3) and 0,38 (0,28-0,50) respectively. An even higher diagnostic accuracy can be obtained by re-examining a larger number of smears.

The AUC value for Fluorobot examination was 0,80 (95%CI 0,76 - 0,84), for Fluorobot examination and re-examination of a complementary set of smears (with 2 - 4 bacilli) - 0,81 (95%CI, 0,77-0,85), and for manual examination - 0,84 (95%CI 0,81 - 0,88) (**Figure 3.1**). Differences between AUC values are minimal and statistically insignificant, i.e., AUC_{manual} versus $AUC_{Fluorobot} = 0,044$, $p = 0,07$ and AUC_{manual} versus $AUC_{Fluorobot\ partially\ re-examined} = 0,036$, $p = 0,06$.

4. MOLECULAR DIAGNOSTIC TECHNIQUES AND ALTERNATIVE SPECIMENS

4.1 Impact of Xpert MTB/Rif testing on therapeutic delay in MDR-TB patients

The chapter describes the results of the retrospective pre-post analysis (2011-2015) of the impact of the nationwide implementation of the GeneXpert MTB/Rif test (Xpert MTB/Rif) in the Republic of Moldova. The study was conducted on the national cohort of patients who initiated treatment for MDR-TB in the Republic of Moldova during the study period, in which the dynamics of time to treatment initiation (therapeutic delay) and its correlation with the number of performed Xpert MTB/Rif tests were

analysed. For study purpose therapeutic delay was defined as time for diagnosis of TB to the initiation of the MDR-TB treatment, during which confirmation of MDR pattern and decision of treatment initiation should be taken Primary data were collected from SIME-TB (National Electronic System for Monitoring and Evaluation of Tuberculosis).

In the period 2011-2015, 74474 Xpert MTB/Rif tests were performed: year 2012 - 8273 tests, year 2013 - 16461, year 2014 - 23320, year 2015 - 262420 tests. The number of tests performed per time unit has been continuously increasing both due to the commissioning of new Xpert machines and increase in the number of tests performed per diagnostic unit. The number of tests performed per each diagnosed MDR-TB case has progressively increased from 15,1 tests/ MDR-TB case (confirmed by Xpert MTB/Rif) in 2012 to 27,3 tests/ MDR-TB case (confirmed by Xpert MTB/Rif) in 2015 (p=0,0001).

During the period under review there were 5507 cases of MDR-TB registered in the SIME TB. The analysis did not include cases with incomplete data on the date of TB diagnosis or the date of initiation of treatment for MDR-TB. Thus, 4353 cases were considered in the final calculation, i.e., 730 out of 1111 cases of MDR-TB patients diagnosed in 2011, 813 out of 1121 - in 2012, 920 out of 1162 - in 2013, 906 out of 1047 - in 2014, 984 out of 1066 - in 2015. Comparison of the time of therapeutic delay during the evaluated period showed a progressive reduction of it with the implementation of the Xpert MTB/Rif technique (**Figure 4.1 A, B**). Thus, in 2011 the median therapeutic delay time was 46,5 days (interquartile range (IQR) 14-89,25), 2012 -36 days (IQR 11-75), 2013- 24 days (IQR 7-63), 2014 - 10 (IQR 3-42), 2015 - 8 days (IQR 2-28). Similarly, the correlation between the number of Xpert tests performed and the time of therapeutic delay showed a strong inverse association between them ($r = - 0,88$ 95%CI - 0,95 − -0,73; $r^2 = 0,79$, $p < 0,0001$; **Figure 4.1 C**).

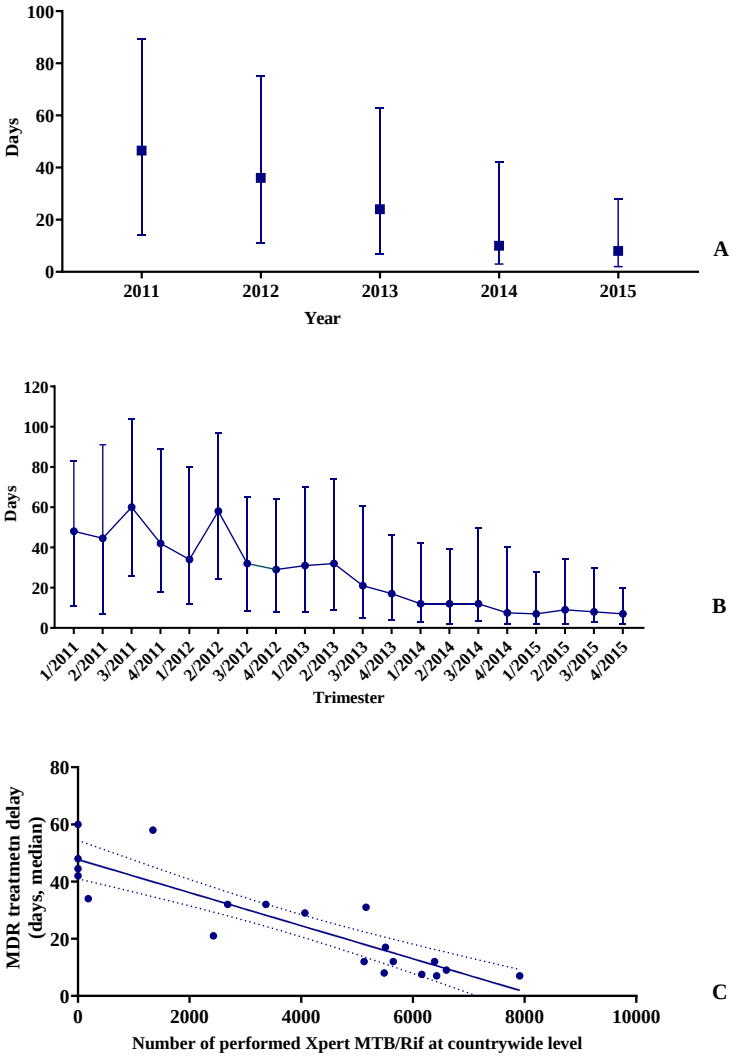


Figure 4.1 Treatment delay in MDR-TB patients in the Republic of Moldova in relation to countrywide implementation of Xpert MTB/RIF testing A) annual dynamics, B) monthly dynamics, C) correlational analysis between treatment delay and number of the performed Xpert MTB/Rif tests.

4.2 Role of the examination of bronchoscopic specimens for microbiological confirmation of tuberculosis.

The chapter describes results of the evaluation of diagnostic accuracy of the Xpert MTB/Rif test in bronchial aspirate specimens in patients with paucibacillary sputum (AFB smear microscopy and Xpert MTB/Rif both negative), as well it includes assessment of added value of the examination of the bronchial aspirate specimens for microbiological confirmation of pulmonary tuberculosis. Diagnostic accuracy was assessed considering test sensitivity, specificity, PPV, NPV, LR+, LR-, having *M. tuberculosis* culture as reference standard.

During the study period, 705 patients with paired respiratory samples (sputum and bronchial aspirate) examined at the National Reference Laboratory in Mycobacteriology (NRL) from the clinical wards of the Chiril Draganiuc Institute of Phthysiopneumology, were identified.

In the analysed patients the positive culture result was recorded in 5,5% (39/705) cases, including 23 cases where the culture was positive only from bronchial aspirate, 9 cases - from bronchial aspirate and sputum and in another 3 cases only from sputum specimens. AFB smear microscopy was positive in bronchial aspirate in 13 (1,8%) of the analysed cases, and Xpert MTB/Rif (also in bronchial aspirate) - 30 (4,2%) cases. Considering culture as the reference standard, the following sensitivity and specificity values were obtained in bronchial aspirate for Xpert MTB/Rif: 46,1% (95%CI 31,6% - 61,4%) and 98,2% (95%CI 96,9% - 99,0%) and for AFB microscopy 20,5% (95%CI 10,8% - 3,5%) and 99,2% (95%CI 98,5% - 99,7%). Similarly, the positive and negative likelihood ratios were for Xpert MTB/Rif 14,8 (95%CI 8,6 - 25,5) and 0,56 (95%CI 0,42 - 0,74), respectively and for AFB microscopy 27,3 (95%CI 9,4-79,6) and 0,8 (95%CI 0,68 - 0,94), respectively.

Added value of bronchial aspirate examination in the diagnosis of pulmonary tuberculosis

The diagnosis of tuberculosis was established in 91 of the patients included in the study, subsequently in 11 of them tuberculosis was refuted (after initiation of treatment). Thus, in patients with definite diagnosis of pulmonary tuberculosis (n=80), culture was positive in 30 cases, inclusively:

3 cases (3,7%) only in sputum, 8 (10%) - in sputum and bronchial aspirate, 19 (23,7%) - only in bronchial aspirate (**Figure 4.2**). Similarly, Xpert MTB/Rif was positive in 19/80 cases (23,7%), and microscopy in 8 cases (all Xpert MTB/Rif positive). Thus, examination of bronchial aspirate specimens allowed additional microbiological confirmation in one-fourth of patients with advanced paucibacillary pulmonary tuberculosis (both AFB microscopy and Xpert MTB/Rif negative in freely expectorated sputum samples).

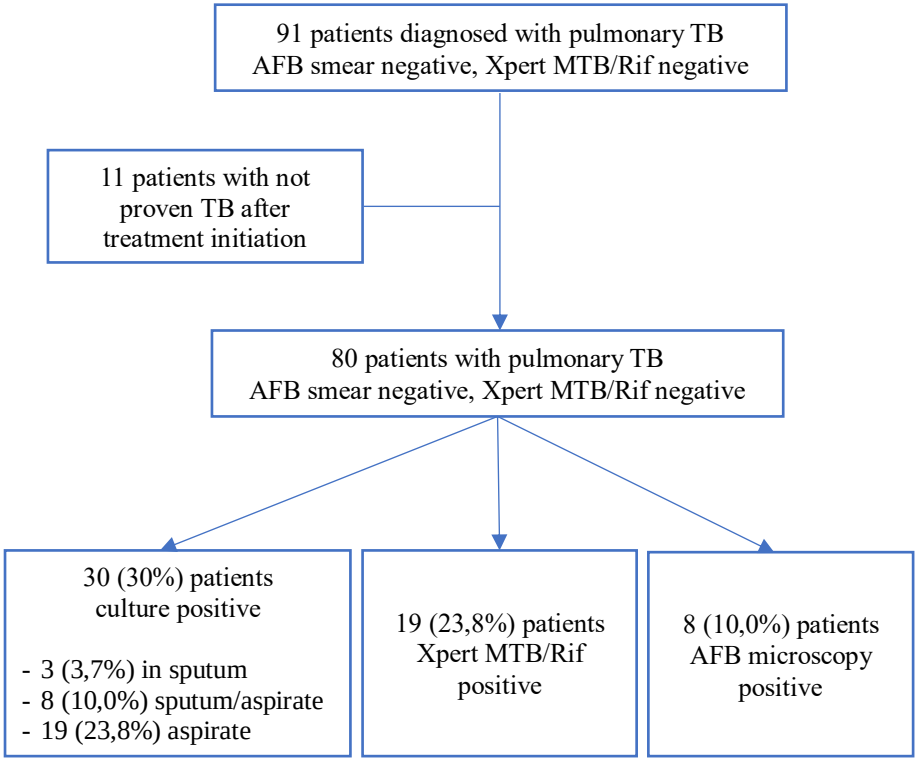


Figure 4.2 Rate of microbiological confirmation of pulmonary tuberculosis by evaluation of the bronchial aspirate in patients with negative sputum microscopy and Xpert MTB/Rif.

5. INTERVENTIONS FOR THE IMPROVMENT OF TUBERCULOSIS TREATMENT

5.1 Impact on treatment outcomes of including bedaquiline in treatment regimens in MDR-TB patients

In this section are presented results of the retrospective case-control study that assess treatment outcomes in patients with MDR-TB whose treatment regimen included bedaquiline (bedaquiline cohort) versus those without bedaquiline (non-bedaquiline cohort) who initiated treatment in the Republic of Moldova during 2016-2018. Cohort matching by clinical, demographic and treatment parameters was performed based on propensity score. Cohorts were compared by time and culture conversion rate and final treatment outcomes (2014 WHO criteria) at the end of therapy and over one-year post-therapy (simplified TB-NET criteria).

During the reference period, 2069 MDR-TB patients were identified. Of these 115 were assigned to the bedaquiline cohort and 1954 to the non-bedaquiline cohort. After propensity score matching 114 patients were included in each cohort. As result between the matched cohorts there were no significant differences in age, gender, living environment, positive AFB smear microscopy at treatment initiation, presence of cavitary lesions, fluoroquinolone resistance, history of tuberculosis and given drugs (except bedaquiline).

The sputum conversion rate at 6 months after MDR treatment initiation in the bedaquiline cohort was significantly higher compared to the non-bedaquiline cohort (66,7% versus 40,3%, $p < 0,001$, **Figure 5.1 A**). Similarly, sputum conversion in the bedaquiline cohort was achieved significantly faster compared to the non-bedaquiline cohort (less than 1 month [0-1] versus 1 [0-3] month, $p < 0,001$, **Figure 5.1 B**).

When assessing treatment outcomes by WHO criteria in the bedaquiline cohort a higher rate of successful treatment and a lower mortality rate was found, compared to the non-bedaquiline cohort (55,3% versus 24,6%, $p < 0,001$ and 8,8% versus 20,2% respectively, $p < 0,001$, **Figure 5.1 C**).

Assessment of final treatment outcomes in the studied cohorts by TBNET criteria was possible in 92/114 (80,7%) of patients in the bedaquiline

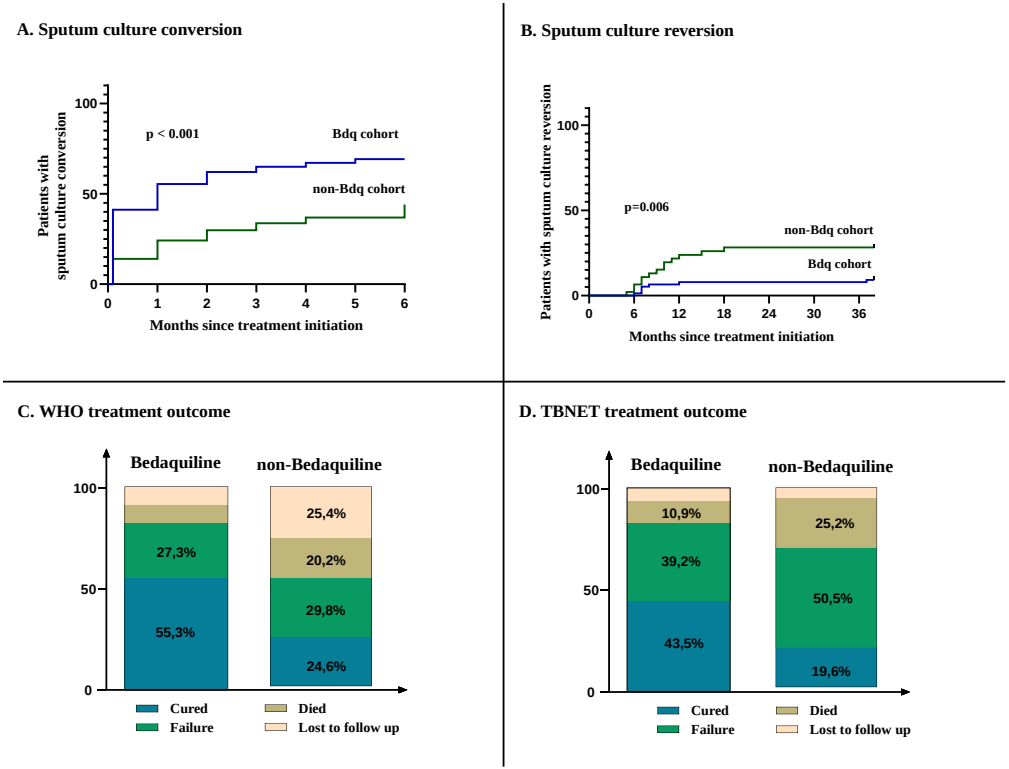


Figure 5.1 Treatment outcome in MDR-TB patients with and without bedaquiline in the treatment regimen

cohort and 107/114 (88,6%) patients in the non-bedaquiline cohort. Thus, patients in the bedaquiline cohort had a higher cure rate and lower death rate, respectively 43,5% versus 19,6% (p=0,004) and 10,9% versus 25,2% (p=0,01, **Figure 5.1 D**).

5.2 Use of isoniazid in treatment of patients with multidrug-resistant tuberculosis

A descriptive analysis of the frequency of mutations associated with isoniazid resistance in MDR-TB strains from the Republic of Moldova was performed. These were determined using a commercial variant of Line Probe Assay - LPA (Genotype MTBDR plus ver. 1) as well as Next Generation Sequencing (NGS).

Results of assessment by Line Probe Assay

The study analysed 3625 positive LPA test results (one patient one result). Mutations associated with resistance to both isoniazid and rifampicin (MDR strains) were identified in 2012 analysed strains. In 1980/2012 (98,4%) of the strains the mutation was present in the katG gene at position 315, associated with a high level of resistance to isoniazid. Only in 17/2012 (0,8%) of the analysed strains the resistant mutations were identified in the promoter of the inhA gene (without concomitant mutation in katG). In 15/2012 (0,7%) of the strains the location of the mutation could not be determined by LPA (**Table 5.1**).

Table 5.1 Genotypic resistance to isoniazid and rifampicin in MDR *M. tuberculosis* strains from the Republic of Moldova assessed by LPA

LPA results	n	%	95%CI
Resistance to isoniazid in MDR strains (n=2012)			
katG315T without known mutations in inhA	766	38,1	35,9-40,2
inhA8C or 8A or15T or16G without known mutations in katG	17	0,8	0,5-1,3
katG315T+ inhA8C or 8A or 15T or 16G	1214	60,3	58,2-62,4
MDR without known mutations in katG or inhA	15	0,7	0,4-1,2

Results of assessment by Next Generation Sequencing

When the spectrum of mutations associated with isoniazid resistance was assessed by genome sequencing, in most of the cases (99,7%) there were a mutation in the katG gene (either alone or in combination with mutations in inhA), whereas isoniazid resistance caused exclusively by mutations in the inhA gene was present in only one strain among those analysed (**Table 5.2.**). Note that in this case the classical mutation in the inhA gene promoter (15C-T) was also accompanied by mutation in the coding region of the inhA gene (I194T).

Table 5.2 Genotypic resistance to isoniazid and rifampicin in MDR *M. tuberculosis* strains from the Republic of Moldova assessed by Next Generation Sequencing

Sequencing results	n	%	95%CI
Resistance to isoniazid in MDR strains (n=288)			
katG315T without known mutations inhA	122	42,4	36,7-48,1
inhA8C or 8A or 15T sor 16G without known mutations in katG	1	0,3	0,01-1,9
katG315T+ inhA 15T or 17T	165	57,3	51,5-62,8

6. ANTITUBERCULOSIS TREATMENT OUTCOMES - PROGNOSTIC FACTORS AND ASSESSMENT OPTIONS

6.1 Impact of lung function on treatment outcomes in tuberculosis patients

The chapter describes the comparative analysis of treatment outcomes in TB patients with normal lung function versus those with impaired lung function (stratified into three patterns, obstructive, restrictive and mixed) assessed at diagnosis (initiation of therapy). The study cohort was collected at the Anti-Tuberculosis Dispensary No. 1 in the city of Harkiv, Ukraine, were all patients with culture-confirmed pulmonary tuberculosis were enrolled. The independent association between pulmonary dysfunction and antituberculosis treatment outcomes was assessed in a multiple logistic regression model.

In the reference year for the present study (2016) 516 patients with pulmonary tuberculosis were admitted to the Anti-Tuberculosis Dispensary No.1 in Harkiv. In 18,9% (106/561) of them the diagnosis was not confirmed by culture, therefore they were not included in the final analysis. Other 62 patients were excluded from the study due to lack of spirography results, and another 115 due to lack of information on sputum conversion at 6 months. Thus, 278 patients were included in the final analysis.

The treatment success rate was higher in patients with normal spirometry than in those with restrictive (87,8% versus 68,8%, p = 0,003) or mixed (87,8% versus 62,5%, p = 0,04) dysfunction. At the same time, the treatment success rate in patients with normal spirometry was not significantly different from that in patients with obstructive changes (87,8% versus 83,3%, p = 0,07) (**Figure 6.1**). Note the small number of patients with obstructive dysfunction (n=12) identified in the study cohort. Similarly, no statistically significant differences were observed in the rate of successful treatment between groups of patients with different types of lung function.

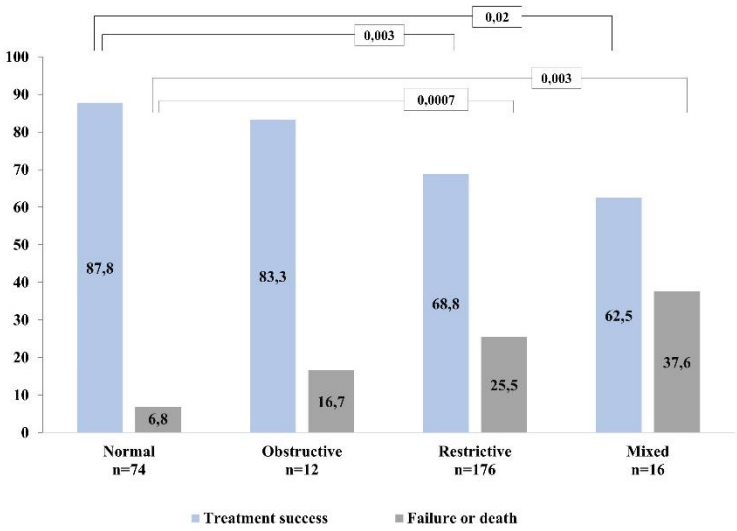


Figure 6.1 Treatment outcome and lung function pattern in patients with tuberculosis (p-values greater than 0,05 are not shown)

The rate of antituberculosis treatment failure in patients with normal spirometry was significantly lower than in any of the three lung dysfunction groups: restrictive - 5,4% versus 17,0%, p=0,01; obstructive - 5,4% versus 16,7%, p=0,02; or mixed - 5,4% versus 31,3%, p=0,008.

The mortality rate in patients with normal spirometry was not significantly different from that in patients with restrictive (1,4% versus 8,5%, p = 0,07), mixed (1,4% versus 6,3%, p = 0,6) or obstructive (1,4% versus 0%, p = 0,07) changes. No statistically significant differences were observed between groups of patients with different types of lung dysfunction (restrictive versus obstructive, p = 0,6; mixed versus obstructive, p = 0,04; restrictive versus mixed, p = 0,7).

The rate of negative outcome of antituberculosis treatment (failure and death) was higher in patients with restrictive or mixed pulmonary dysfunction compared to those with normal spirometry (25,6% versus 6,8%, p = 0,0007 and 37,5% versus 6,8%, p = 0,003, respectively). At the same time, the differences in the rates of negative outcome of antituberculosis treatment in patients with obstructive dysfunction compared to those with normal spirometry were not significant (16,7% versus 6,8%, p = 0,5) (**Figure 6.1**).

When stratifying patients by degree of severity of FVC decline (as marker of severity of restriction) patients with FVC between 59% - 40% had a lower rate of successful treatment than those with FVC between 79% - 60% or those with FVC ≥ 80%, respectively, 41,2% versus 80,0%, p = 0,0001 and 41,2% versus 87,2%, p = 0,2 (**Figure 6.2**). The rate of negative treatment outcomes (failure and death) was lower in patients with FVC ≥ 80% and those with FVC between 79% - 60% compared to patients with FVC 59% - 40%, (respectively, 8,1% versus 40,0%, p = 0,0003 and 16,5% versus 40,0%, p = 0,008) and those with FVC < 40% (8,1% versus 47,1%, p = 0,0001 and 16,5% versus 47,1%, p = 0,0009, respectively) (**Figure 6.2**).

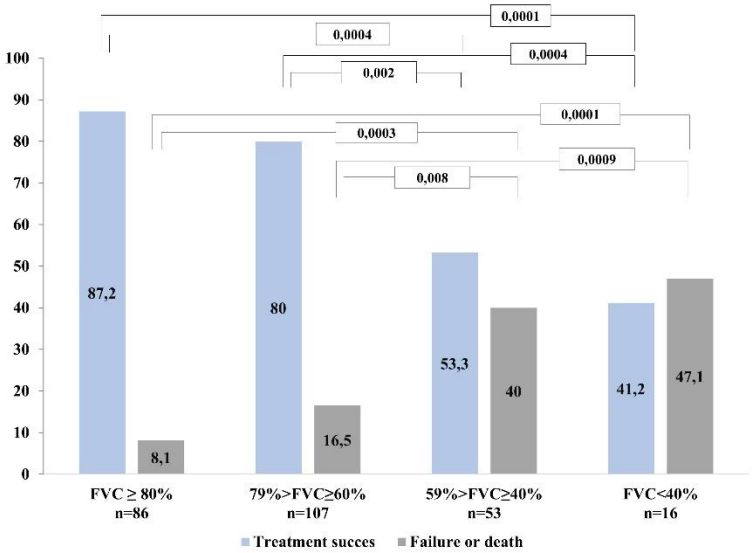


Figure 6.2 Treatment results stratified by severity of restriction (p-values greater than 0,05 are not shown)

Association between type of pulmonary dysfunction and outcomes of anti-tuberculosis treatment

Besides differences in pulmonary function parameters, patients who experienced successful treatment outcome differed from those with negative treatment outcome also by such variables as: age, BMI, positive sputum microscopy status, multidrug resistance pattern and history of tuberculosis. To test the independent association between these factors and TB treatment outcome, a multiple logistic regression model was generated, that showed the association between restrictive (OR 4,19; 95%CI 1,60-13,28, p=0,007) and mixed (OR 5,46; 95%CI 1,28-24,44, p=0,02) lung dysfunction with negative TB treatment outcome **(Figure 6.3).** As well, the negative treatment outcome was associated with such factors as MDR pattern of *M. tuberculosis* strain (OR 2,56; 95%CI 1,26-5,28, p=0,01) and disease relapse (OR 2,54; 95%CI 1,12-5,70, p=0,02).

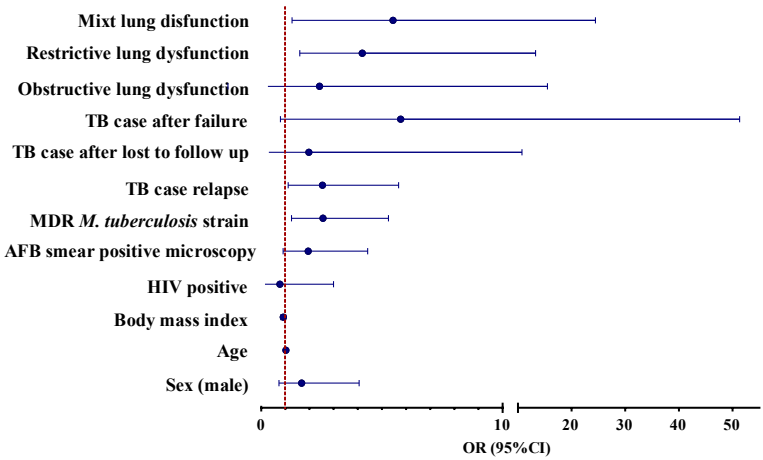


Figure 6.3 Association between clinical and lung function parameters with negative treatment outcome in patients with pulmonary tuberculosis (results of multiple logistic regression).

6.2 Assessment of multidrug-resistant tuberculosis treatment outcomes

In this section of the thesis, two cohorts of tuberculosis patients were analysed, the first - a nationwide cohort of MDR-TB patients, who initiated antituberculosis treatment during two consecutive years (2013-2014) in the Republic of Moldova (identified through National Tuberculosis Register, SIME-TB) and the second consisting of all patients who initiated treatment for MDR-TB at the Anti-tuberculosis Dispensary in the city of Harkiv, Ukraine, during 2014. In the mentioned cohorts, retrospectively, treatment outcomes were described applying two sets of definitions, the currently used one (in line with WHO recommendations) and the second one involving pot-therapeutic surveillance (simplified definitions by TBNET), potential advantages of the latter approach were evaluated when applied in in these two TB health care systems from Eastern Europe.

Treatment outcomes in the Republic of Moldova cohort

During 2013-2014 in the Republic of Moldova were registered 2012 patients who started treatment for MDR-TB. Of these, 1864 were

included in the study, being excluded patients with missing data on sputum culture status at 6 months of treatment (n=78), paediatric cases (n=19) and extrapulmonary cases (n=17).

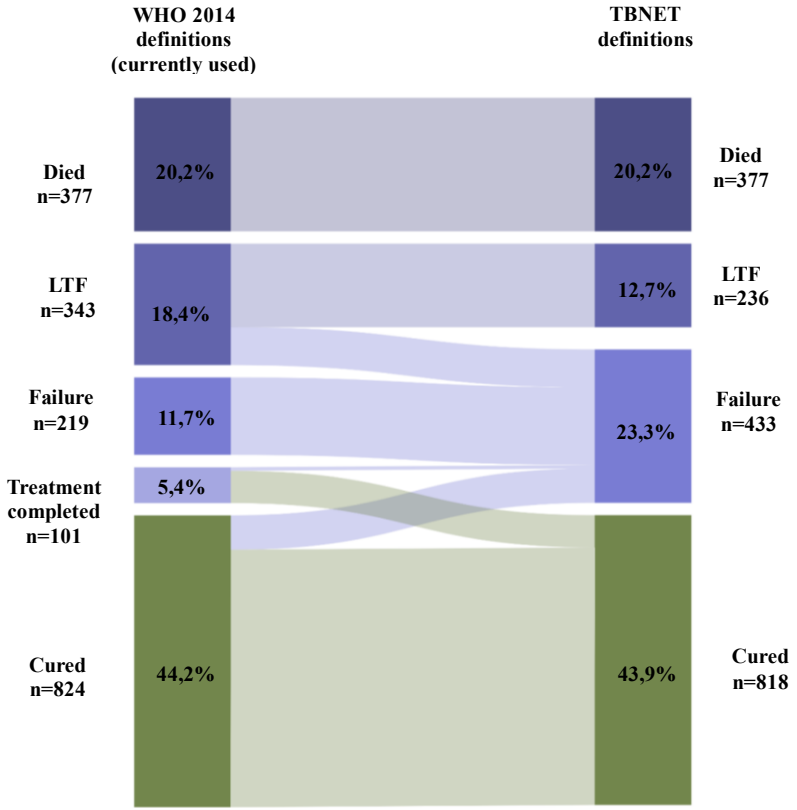


Figure 6.4 MDR-TB treatment outcomes in the Republic of Moldova cohort assessed according to WHO definitions (currently applied) and simplified TBNET definitions (with post-therapy surveillance). (LTF- lost to follow up)

Comparison of treatment outcomes assessed by the two sets of definitions revealed a twofold increase in the rate of failure cases when post-treatment surveillance was applied compared to the end-of-treatment (currently used) assessment (i.e. 23,2% vs. 11,7%). This increase is on account of 97 cases that in the current assessment were considered cured

(11,8% of all cases rated as cured in the current assessment) and 107 cases initially considered lost to follow up (19,5% of all cases lost to follow up based on the current assessment). Reclassification of the 97 cases from cured to failure was based on lack of sputum culture conversion at 6 months after the start of therapy (n=24, 2,9% of all cases initially rated as cured) or reversion to positive sputum culture after 6 months of therapy (n=73, 8,8% of all cured cases at current assessment). Cases lost to follow up were reclassified as failures due to positive sputum culture status at the 6-month time point after treatment initiation (n=64, 18,6% of all lost to follow up cases in current assessment) or due to positive sputum culture result at reassessment during the first year after treatment discontinuation (n=43, 12,5% of all lost to follow up cases at current assessment). The cure rate was similar when applying the two sets of treatment outcome criteria (43,9% versus 44,2%, **Figure 6.4**).

Treatment outcomes in the Harkiv cohort

This cohort included 169 tuberculosis cases. Most patients included in the cohort (n=104, 61,5%) were new TB cases. As in the Moldovan cohort, the application of the TBNET criteria led to a significant redistribution of patients according to treatment outcome, the trend of these changes being like that in the Moldovan cohort (**Figure 6.5**).

Thus, 24/42 (57,1%) of patients assessed as lost to follow up according to the current treatment evaluation criteria were classified as cured when applying the simplified TBNET criteria. Of the patients lost to follow up 6 were reclassified as failure, 6 undeclared and 2 remained in the same category (lost to follow up). Similarly, all patients who were assessed as treatment completed according to the current assessment criteria did not qualify for the cure criteria according to the simplified definitions and were considered undeclared. At the same time, 17 (26,1%) of the 65 cured patients according to the WHO criteria were considered as failures according to the simplified criteria.

Beside the differences in the treatment outcome observe by use of different sets of criteria, analysis of both these cohorts illustrated the feasibility of posttreatment follow-up (one year) in case of programmatic management of the TB patients in Eastern European settings.

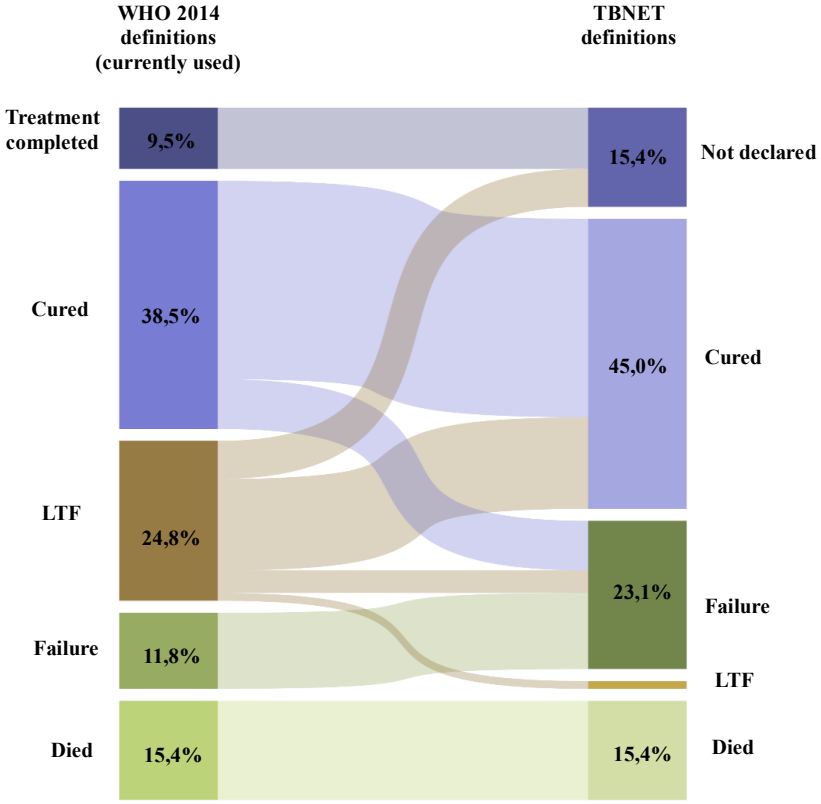


Figure 6.4 MDR-TB treatment outcomes in Harkiv cohort assessed according to WHO definitions (currently applied) and simplified TBNET definitions (with post-therapy surveillance). (LTF- lost to follow up)

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. The level of T lymphocytes with IL-2$^-$IFN-γ^+ cytokine profile is increased in patients with active TB compared to those with LTBI or those with cured TB, a parameter that can be easily determined by FluoroSpot, although this biomarker does not provide the required accuracy for the application in clinical practice.
2. Automated reading of microscopic smears in fluorescent staining with auramine using the Fluorobot device possesses sensitivity and specificity comparable to those obtained at human reading by an experienced

microscopist and can be successfully applied to optimise the work of laboratory staff, especially in specialised laboratories with high sample workflow.

3. The nationwide implementation of Xpert MTB/Rif in the Republic of Moldova has contributed to a significant reduction of treatment delay in adult patients with multidrug-resistant pulmonary tuberculosis, which is now 6 times lower than before implementation.
4. The use of bronchial aspirate specimens in patients with pulmonary tuberculosis with paucibacillary sputum (AFB smear microscopy and Xpert MTB/Rif negative) allows additional microbiological confirmation of the diagnosis in one fourth of these patients.
5. The inclusion of bedaquiline in treatment regimens administered under programmatic management of multidrug-resistant tuberculosis patients, including those with previous treatment failure, is associated with a higher and faster sputum conversion rate, a higher rate of successful treatment and lower mortality.
6. Resistance to isoniazid of multidrug-resistant strains of *M. tuberculosis* circulating in the Republic of Moldova is caused by mutations in the katG gene, alone or in combination with mutations in the inhA promoter, associated with a high level of resistance to this drug, which jeopardizes the empirical administration of high-dose isoniazid as part of treatment regimens in multidrug-resistant tuberculosis in this setting.
7. Restrictive pulmonary dysfunction established at the time of disease diagnosis in adult patients with pulmonary tuberculosis is an independent risk factor associated with negative outcomes (failure or death) of antituberculosis treatment, with an impact comparable to that of multidrug resistance.
8. Post-treatment follow up, also under programmatic management of multidrug-resistant patients, allows a more clinically objective assessment of tuberculosis outcomes.

Recommendations

1. Assessment of IL-2^{-}INF-γ^{+}-secreting peripheral blood T lymphocytes is not recommended for use in clinical practice for the diagnosis of active tuberculosis or the differentiation of latent tuberculosis infection from active tuberculosis.
2. For microscopic examination of sputum smears collected for diagnostic purposes from persons suspected of having pulmonary tuberculosis in laboratories with a high workflow of examined samples (regional or national reference laboratories), the use of automated reading of microscopic smears using robotic Fluorobot devices is recommended.
3. In patients with suspected pulmonary tuberculosis with paucibacillary sputum (AFB smear microscopy and Xpert MTB/Rif negative) for microbiological confirmation of pulmonary tuberculosis microbiological examination by (Xpert MTB/Rif and culture) of bronchial aspirate samples is recommended.
4. In order to ensure timely initiation of antituberculosis treatment in patients with multidrug-resistant tuberculosis, it is recommended that supplies for molecular-genetic testing for rapid diagnosis of rifampicin-resistant and multidrug-resistant tuberculosis be made available on a sustainable basis.
5. Inclusion of bedaquiline in individualised treatment regimens for multidrug-resistant tuberculosis, with assuring of treatment adherence, is recommended for all patients without proven resistance to this drug.
6. Testing for *M. tuberculosis* genotype mutations associated with resistance to isoniazid, prior to inclusion in treatment regimens of high-dose isoniazid, is recommended in all patients with multidrug-resistant tuberculosis who are potentially to receive isoniazid in high therapeutic doses.
7. Assessment of pulmonary function by spirometry in patients with pulmonary tuberculosis, with appropriate infection control measures, is recommended to identify patients at increased risk of treatment failure who could potentially benefit from respiratory rehabilitation interventions.

8. Post-treatment monitoring for assessment of final treatment outcomes is recommended in tuberculosis patients treated under the National Programme of Response to Tuberculosis.

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1.1. Specialist books single author

1.1.1. CHESOV, D. *Tuberculoza diagnostic și tratament în era multidrog rezistenței*. Chișinău: Tip. „Print-Caro". 2022. 224 p. ISBN 978-9975-82-302-9.

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1.2. Specialist books multiple authors

1.2.1. CHESOV, D., BOTNARU, V., Imaging for diagnosis and management of tuberculosis. In: *Tuberculosis (ERS Monograph)*. Sheffield: Editura „European Respiratory Society". 2018. pp. 116–136. ISBN 978-1-84984-100-9.

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SUMMARY

Chesov Dumitru

"Optimisation of the diagnosis and improvement of the treatment of tuberculosis"

Thesis of doctor habilitattus in medicine, Chisinau, 2023

Thesis structure: introduction, six chapters, conclusions, bibliography containing 307 sources, 29 figures, 17 tables, results of the study were published in 51 scientific papers.

Keywords: TB, MDR, laboratory diagnosis, *M. tuberculosis*, antituberculosis treatment.

Field of study: General medicine. Pulmonology.

Research aim: Evaluation of clinical and laboratory interventions with the potential to optimise diagnosis and to improve treatment of tuberculosis patients.

Research objectives: To assess efficacy of determination of the interferon-γ and interleukin-2 peripheral cell response in differentiating the clinical statuses of *M. tuberculosis* infection; To assess the diagnostic accuracy of automated reading by Fluorobot platform of the fluorescent stained smears in the diagnosis of pulmonary tuberculosis in adults; To determine the impact of the implementation of GeneXpert MTB/Rif technique, on the therapeutic management of patients with mutlidrug-resistant pulmonary tuberculosis in the Republic of Moldova; To determine the added value of the bronchial aspirate specimen testing in the diagnosis of pulmonary tuberculosis in patients with paucibacillary free expectorated sputum specimens; To assess the effectiveness of including bedaquiline in multidrug-resistant tuberculosis treatment regimens in the conditions of programmatic management of tuberculosis patients; To assess the rationality of including high-dose isoniazid in treatment regimens used in patients with multidrug-resistant tuberculosis in the Republic of Moldova; To determine the impact of respiratory function, assessed at diagnosis, on treatment outcomes in patients with pulmonary tuberculosis; To evaluate alternative approaches in assessment of treatment outcomes in tuberculosis patients.

Scientific novelty and originality.

The novelty of the research is given by the evaluation of the efficacy of applying new biomarkers (IL-2/INF-γ cytokine profile of peripheral blood lymphocytes), alternative specimens (bronchial aspirate) and original technological solutions (FluoroSpot, Fluorobot, Xpert MTB/Rif) for the diagnosis of tuberculosis in clinical practice. Similarly, the novelty of the study is ensured by the assessment of the effectiveness and/or rationality of the application in routine clinical conditions

in the healthcare system of the Republic of Moldova of new drugs such as bedaquiline or high-dose isoniazid, together with the identification of new predictors of the effectiveness of antituberculosis treatment and the application of more objective approaches, from a clinical point of view, in the assessment of antituberculosis treatment outcomes.

Results that led to the solution of a major scientific or applied problem. Fluorospot represents an alternative method for the diagnosis of latent tuberculosis infection with a diagnostic accuracy like that of EliSpot-IGRA techniques; Application of deep learning algorithm-based technologies such as Fluorobot allows automated reading of microscopic smears in fluorescent staining with an accuracy comparable to that obtained at manual reading; Nationwide application of the Xpert MTB/Rif technique in the diagnosis of tuberculosis has allowed a 6-fold reduction in therapeutic delay in patients with MDR pulmonary tuberculosis; The use of bronchial aspirate specimens tested by molecular-genetic tests (Xpert MTB/Rif) in patients with pulmonary tuberculosis with highly paucibacillary sputum (microscopy and Xpert MTB/Rif negative) allows additional microbiological confirmation in a quarter of these patients; Bedaquiline used in treatment regimens in patients with MDR tuberculosis, including those with previous treatment failure, under programmatic care delivery ensures a high cure rate and reduced mortality; Resistance to isoniazid in MDR tuberculosis patients in the Republic of Moldova is overwhelmingly due to mutations in the katG gene (associated with high levels of minimum inhibitory concentrations against this drug), which precludes empirical administration of high doses of isoniazid (15-20 mg/kg) in treatment regimens for these patients; Restrictive pattern of pulmonary function in patients with pulmonary tuberculosis, assessed at initiation of antituberculosis treatment, is a potentially modifiable predictor of negative treatment outcome in patients with pulmonary tuberculosis; Assessment of antituberculosis treatment outcome after a period of post-therapy surveillance is a more rational approach to antituberculosis treatment evaluation, feasible in conditions of programmatic care of tuberculosis patients.

Theoretical significance.

The research provides the rationale on optimisation of current diagnostic algorithms on diagnosis of *M. tuberculosis* infection by potentially including serological assays to assess INF-γ and IL-2 secreted by T lymphocytes, automated reading of microscopy of fluorescent stained smears and molecular-genetic testing of bronchial aspirate specimens in patients with paucibacillary sputum. Argumentation of the usefulness of the mentioned interventions is performed considering sensitivity, specificity, positive and negative likelihood ratios. Similarly,

the impact of the nationwide implementation of the Xpert MTB/Rif technique has been argued by assessing the overall effect on reducing therapeutic delay in patients with MDR pulmonary tuberculosis. As part of the valuation of interventions for the improvement of tuberculosis treatment, was argued the effectiveness of including bedaquiline in treatment regimens under programmatic conditions in MDR-TB patients by demonstrating a higher cure rate and lower mortality when using this drug, and the ineffectiveness of empirical inclusion (without assessment of genetic determinants) of high-dose isoniazid in treatment regimens in MDR tuberculosis patients. In the same context, the rationality and feasibility of post-therapy surveillance in assessing TB treatment outcomes was argued.

Applicative value.

The application of the interventions evaluated in this work allows to reduce the diagnostic delay in patients with MDR pulmonary tuberculosis, significantly to increase the microbiological confirmation of pulmonary tuberculosis in patients with paucibacillary sputum, and to optimize the reading of fluorescent stained smears. Likewise, the use of the evaluated therapeutic solutions has been shown to be effective in increasing cure rate and reducing mortality rate in MDR tuberculosis patients, as well as avoiding irrational empirical use of high-dose isoniazid treatments with potential hepatotoxic effect in these patients. The use of treatment assessment after post-treatment surveillance have been shown to be more rational, from clinical point of view, for the assessment of therapeutic efficacy.

Implementation of scientific results.

The theoretical and practical results of the study are used in training of medical students at the Department of Pulmonology and Allergology, as well as in the clinical departments of "Chiril Draganiuc" Phtisiopneumology Institute. There were registered 3 certificates of innovator and 3 acts of implementation of results.

ADNOTARE

Chesov Dumitru

”Optimizarea diagnosticului și eficientizarea tratamentului tuberculozei”

Teză de doctor habilitat în medicină, Chișinău, 2023

Structura tezei: introducere, șase capitole, concluzii, bibliografia din 307 surse, 29 de figuri, 17 de tabele, Rezultatele studiului au fost publicate în 51 de lucrări științifice.

Cuvinte cheie: TB, MDR, diagnostic de laborator, *M. tuberculosis*, tratament antituberculos.

Domeniul de studiu: Medicină generală. Pneumoftiziologie.

Scopul cercetării: Evaluarea intervențiilor clinice și de laborator cu potențial de optimizare a diagnosticului și de eficientizare a tratamentului bolnavilor de tuberculoză.

Obiectivele cercetării: Aprecierea eficacității determinării răspunsului celular periferic specific prin interferon-γ și interleukina-2 în diferențierea statutelor clinice ale infecției prin *M. tuberculosis;* Evaluarea acurateței diagnostice a citirii automatizate a frotiurilor de microscopie în colorație fluorescentă prin platforma Fluorobot în diagnosticul tuberculozei pulmonare la adulți; Determinarea impactului implementării tehnicii GeneXpert MTB/Rif asupra managementului terapeutic la bolnavii cu tuberculoză pulmonară mutlidrog rezistentă în Republica Moldova; Determinarea rolului evaluării specimenelor de aspirat bronșic în diagnosticul tuberculozei pulmonare la pacienții cu specimene paucibacilare ale sputei liber expectorate; Aprecierea eficacității includerii bedaquilinei în schemele de tratament al tuberculozei multidrog rezistente în condițiile managementului programatic al bolnavilor de tuberculoză; Evaluarea raționalității includerii izoniazidei în doze mari în schemele terapeutice utilizate la bolnavii cu tuberculoza multidrog rezistentă; Aprecierea impactului funcției respiratorii la momentul diagnosticului asupra rezultatelor tratamentului la bolnavii cu tuberculoză pulmonară; Evaluarea opțiunilor de apreciere a rezultatelor tratamentului la bolnavii de tuberculoză.

Noutatea și originalitatea științifică. Noutatea cercetării este dată de evaluarea eficacității aplicării de noi biomarkeri (profilul citokinic IL-2/INF-γ al limfocitelor din sângele periferic), specimene alternative (aspirat bronșic) și soluții tehnologice originale (FluoroSpot, Fluorobot, Xpert MTB/Rif) în scopul diagnosticării infecției tuberculoase în practica clinică. La fel, noutatea studiului este asigurată de aprecierea eficacității și/sau raționalității aplicării în condițiile clinice de rutină din cadrul sistemului de sănătate din Republica Moldova a unor medicamente noi precum bedaquilina sau dozelor mari de izoniazidă, de rând cu identificarea de noi predictori ai eficacității tratamentului antituberculos și aplicării

unor abordări mai obiective, din punct de vedere clinic, în aprecierea rezultatelor tratamentului antituberculos.

Rezultatele noi pentru știință și practică: Fluorospot - ul reprezintă o metodă alternativă pentru diagnosticul infecției tuberculoase latente cu acuratețe diagnostică similară cu cea a tehnicilor EliSpot-IGRA; Aplicarea tehnologiilor pe bază de deep learning algorithm precum Fluorobotul permite citirea automatizată a frotiurilor microscopice în colorație fluorescentă cu o acuratețea comparabilă cu cea obținută la citirea manuală; Aplicarea la scară națională a tehnicii Xpert MTB/Rif în diagnosticul tuberculozei a permis reducerea întârzierii terapeutice de 6 ori la bolnavii cu tuberculoză pulmonară MDR; Utilizarea specimenelor de aspirat bronșic testate prin teste molecular-genetice (Xpert MTB/Rif) la bolnavii cu tuberculoză pulmonară cu paucibacilaritate înaltă (microscopie și Xpert MTB/Rif negative în probele de spută liber expectorată) permite confirmarea microbiologică adițională la o pătrime dintre acești bolnavi.; Bedaquilina utilizată în schemele de tratament la bolnavii cu tuberculoză MDR, inclusiv la cei cu eșec a tratamentelor anterioare, în condițiile oferirii îngrijirilor medicale în mod programatic asigură o rată înaltă de vindecare și reducerea mortalității; Rezistența față de izoniazidă la bolnavii cu tuberculoză MDR în Republica Moldova este în majoritatea covârșitoare a cazurilor determinată de mutații în gena katG (asociate cu niveluri înalte a concentrațiilor minime inhibitorii față de acest medicament), fapt care exclude administrarea empirică a dozelor mari de izoniazidă (15-20 mg/kg) în schemele de tratament la acești bolnavi; Pattern-ul restrictiv al funcției pulmonare la bolnavii cu tuberculoză pulmonară, apreciat la inițierea tratamentului antituberculos, este un predictor negativ, potențial modificabil, al rezultatelor tratamentului la bolnavii de tuberculoză; Aprecierea rezultatelor tratamentului antituberculos după o perioadă de supraveghere posterapie reprezintă o abordare mai rațională din punct de vedere clinic de evaluare a tratamentului antituberculos, fezabilă în condițiile de îngrijire programatică a bolnavilor de tuberculoză.

Semnificația teoretică. Cercetarea oferă argumentarea teoretică necesară luării deciziilor de optimizare a algoritmelor diagnostice curente de confirmare a infecției tuberculoase prin includerea potențială în acestea a testelor serologice de apreciere a INF-γ și IL-2 secretate de limfocitele T, citirii automatizate a frotiurilor de microscopie în colorație fluorescentă și testării prin teste molecular-genetice a specimenelor de aspirat bronșic la bolnavii cu spută paucibacilară. Argumentarea intervențiilor menționate poate fi efectuată din perspectiva sensibilității, specificității, raportului de probabilitate pozitivă și cel de probabilitate negativă demonstrate în prezenta lucrare. La fel, a fost argumentat impactul implementării la nivel național a tehnicii Xpert MTB/Rif prin aprecierea efectul global asupra

reducerii întârzierii terapeutice la bolnavii cu tuberculoză pulmonară MDR. În cardul evaluării intervențiilor de eficientizare a tratamentului antituberculos a fost argumentată eficacitatea includerii în schemele de tratament în condiții programatice la bolnavii cu tuberculoză MDR a bedaquilinei prin demonstrarea unei rate de vindecarea mai mare și a mortalității mai reduse la utilizarea acestui medicament precum și ineficacitatea includerii empirice (fără aprecierea determinatei genetice) a izoniazidei în doze mari în schemele de tratament la bolnavii cu tuberculoză MDR. În același context, a fost argumentată raționalitatea și fezabilitatea supravegherii postterapeutice în evaluarea rezultatelor tratamentului antituberculos.

Valoarea aplicativă. Aplicarea intervențiilor evaluate în cadrul lucrării permite reducerea întârzierii diagnostice la bolnavii cu tuberculoză pulmonară MDR, creșterea semnificativă a confirmării microbiologice a tuberculozei pulmonare la bolnavii cu spută paucibacilară, precum și optimizarea citirii frotiurilor de microscopie în colorație fluorescentă. La fel, utilizarea soluțiilor terapeutice evaluate sunt eficiente în creșterea vindecării și reducerea ratei de mortalitate la bolnavii cu tuberculoză MDR, precum și evitarea tratamentelor neargumentate cu doze mari de izoniazidă cu potențial efect hepatotoxic la acești bolnavi. Utilizarea aprecierii eficacității tratamentului după supravegherea post-tratament oferă o evaluare mai obiectivă din punct de vedere clinic al eficacității terapeutice.

Implementarea rezultatelor științifice. Recomandările studiului sunt utilizate în procesul didactic de pregătire a cadrelor medicale la Disciplina de Pneumologie și Alergologie, precum și în secțiile de ftiziopneumologie a IMSP IFP "Chiril Draganiuc". Au fost înregistrate 3 certificate de inovator și 3 acte de implementare a rezultatelor.

АННОТАЦИЯ

Кесов Думитру

"Оптимизация диагностики и повышение эффективности лечения туберкулеза"

Диссертация доктора наук в области медицины, Кишинев, 2023 г.

Структура диссертации: введение, шесть глав, выводы, библиография из 307 источников, 29 рисунков, 17 таблиц, результаты исследования опубликованы в 51 научной работе.

Ключевые слова: ТБ, МЛУ, лабораторная диагностика, *M. tuberculosis*, противотуберкулезное лечение.

Область исследования: Общая медицина. Фтизиопульмонология.

Цель исследования: Оценка клинических и лабораторных вмешательств, способных оптимизировать диагностику и улучшить лечение больных туберкулезом.

Задачи исследования: Оценить эффективность определения специфического ответа интерферона-γ и интерлейкина-2 периферических клеток в дифференциации клинического статуса *M. tuberculosis*; Оценить диагностическую точность автоматизированного чтения мазков в флуоресцентном окрашивании с помощью платформы Fluorobot в диагностике туберкулеза легких у взрослых; Определить влияние внедрения методики Xpert MTB/Rif на ведение пациентов с множественно лекарственно устойчивым туберкулезом легких в Республике Молдова; Определить роль оценки образцов бронхиального аспирата в диагностике туберкулеза легких у пациентов с малобациллярной мокротой; Оценка эффективности включения бедакиллина в схемы лечения туберкулеза с множественной лекарственной устойчивостью в контексте программного ведения больных туберкулезом; Оценка рациональности включения высоких доз изониазида в схемы лечения пациентов с туберкулезом с множественной лекарственной устойчивостью; Оценка влияния параметров респираторной функции на результаты лечения пациентов с туберкулезом легких; Оценка разных подходов определения результатов лечения у пациентов с туберкулезом.

Научная новизна и оригинальность. Новизна исследования заключается в оценке эффективности применения новых биомаркеров (IL-2/INF-γ цитокиновый профиль лимфоцитов периферической крови), альтернативных образцов (бронхиальный аспират) и оригинальных технологических решений (FluoroSpot, Fluorobot, Xpert MTB/Rif) для диагностики туберкулезной инфекции в клинической практике. Также новизна исследования заключается в оценке эффективности и/или рациональности применения в повседневных клинических условиях в рамках системы здравоохранения Республики Молдова новых препаратов, таких как бедакилин или изониазида в высоких дозах. В том числе новизна обусловлена выявлением новых предикторов эффективности противотуберкулезного лечения и применением более объективных, с клинической точки зрения, подходов в оценке результатов противотуберкулезного лечения.

Новые результаты для науки и практики: Fluorospot представляет собой альтернативный метод диагностики латентной туберкулезной инфекции с диагностической точностью, сходной с точностью методов EliSpot-IGRA; Применение технологий на основе алгоритмов глубокого обучения, таких как Fluorobot, позволяет автоматизированное чтение микроскопических мазков в

флуоресцентном окрашивании с точностью, сравнимой с точностью, получаемой при ручном чтении; Общенациональное внедрение метода Xpert MTB/Rif в диагностике туберкулеза позволило в 6 раз сократить задержку терапии у пациентов с МЛУ туберкулезом легких; Использование образцов бронхиального аспирата, протестированных молекулярно-генетическими тестами (Xpert MTB/Rif) у пациентов с туберкулезом легких малобацилярной мокротой (микроскопия и Xpert MTB/Rif отрицательны) позволяет получить дополнительное микробиологическое подтверждение заболевания у четверти этих пациентов; Использование бедакилина в схемах лечения пациентов с МЛУ ТБ, включая тех, кто ранее не получал лечения, при программном оказании помощи обеспечивает высокий процент излечения и снижение смертности; Резистентность к изониазиду у больных МЛУ ТБ в Республике Молдова в подавляющем большинстве случаев обусловлена мутациями в гене katG (ассоциированными с высокими уровнями минимальных ингибирующими концентрациями этого препарата), что исключает эмпирическое назначение высоких доз изониазида (15-20 мг/кг) в схемах лечения этих пациентов; Рестриктивный паттерн функции легких у пациентов с туберкулезом легких, оцененный в начале противотуберкулезного лечения, является потенциально модифицируемым негативным предиктором результатов лечения пациентов с туберкулезом; Оценка результатов противотуберкулезного лечения после периода постлечебнаго наблюдения является более клинически рациональным подходом в оценке противотуберкулезного лечения, осуществимым в условиях программного ведения пациентов с туберкулезом.

Теоретическая значимость. Исследование дает теоретическое обоснование для принятия решений по оптимизации текущих диагностических алгоритмов подтверждения туберкулезной инфекции путем потенциального включения серологических анализов для оценки INF-γ и IL-2, секретируемых Т-лимфоцитами, автоматизированного чтения микроскопии мазков в флуоресцентной окраски, и молекулярно-генетического тестирования образцов бронхиального аспирата у пациентов с малобациллярной мокротой. В представленной работе аргументация указанных вмешательств проведена с точки зрения чувствительности, специфичности, отношениях правдоподобия положительного и отрицательного результатов. Также, в диссертации было доказанно положительный эффект общенационального внедрения метода Xpert MTB/Rif на ведение больных с туберкулезом путем оценки общего влияния на снижение задержки терапии у пациентов с МЛУ туберкулезом. При оценке мероприятий по повышению эффективности лечения туберкулёза

эффективность таких как включения бедакилина в схемы лечения в программных условиях у больных с МЛУ туберкулезом были доказанны более высокая частота излечения и более низкая смертность при использовании этого препарата, а также неэффективность, эмпирического включения (без оценки генетических детерминант), высоких доз изониазида в схемы лечения больных МЛУ туберкулёзом. В этом же контексте была аргументирована рациональность и целесообразность постлечебного наблюдения при оценке результатов лечения туберкулёза.

Практическое значимость. Внедрение мероприятий, исследованных в данной работе, позволяет сократить диагностическую задержку у пациентов с МЛУ туберкулезом легких, значительно увеличить микробиологическое подтверждение туберкулеза легких у пациентов с малобациллярной мокротой и оптимизировать чтение микроскопии мазков в флуоресцентной окраске. Также, использование исследованных терапевтических вмешательств эффективно для увеличения излечения и снижения смертности у пациентов с МЛУ туберкулёзом, а также позволяет избежать неоправданнаго лечения высокими дозами изониазида с потенциальным гепатотоксическим эффектом у больных туберкулёзом. Использование оценки эффективности лечения после постлечебного наблюдения обеспечивает более объективную, с клинической точки зрения, оценку эффективности лечения.

Внедрение результатов. Научно-практические результаты исследования используются в учебном процессе на Кафедре Пульмонологии и Аллергологии ГУМФ «Николае Тестемицану», а также в отделениях Института фтизиопульмонологии "Кирилл Драганюк". По результатам работы зарегистрировано 3 сертификата рационализации и 3 акта внедрения результатов.

DUMITRU CHESOV

OPTIMISATION OF THE DIAGNOSIS AND IMPROVEMENT OF THE TREATMENT OF TUBERCULOSIS"

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