

RESEARCH STUDIES

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The impact of the molecular genetic test on the diagnosis delay and outcome in patients with pulmonary tuberculosis

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Abstract

Background: The aim of the study was the assessment of the impact of the molecular genetic Xpert MTB/Rif assay on the diagnostic delay and the treatment effectiveness in pulmonary tuberculosis, according to the results of the microbiological and molecular-genetic methods, in order to optimize the management of the drug resistant tuberculosis.

Material and methods: A retrospective selective study, which included 226 patients with multidrug-resistant pulmonary tuberculosis (study sample) and 78 patients with drug-susceptible pulmonary tuberculosis (control group), was performed. Patients were divided into 2 subgroups following the results of the molecular-genetic and bacteriological investigations. The study sample was divided in two groups: the 1st group included 85 patients enrolled in the treatment for drug resistant tuberculosis, according to the results of the Xpert MTB test and the 2nd group included 141 patients enrolled in the treatment DOTS plus, according to the results of the conventional cultures.

Results: The impact of the molecular genetic GeneXpert MTB/Rif assay was demonstrated on early diagnosis, adequate treatment according to the drug susceptibility to rifampicin, shorter duration of the treatment, low rate of the side events, optimal treatment compliance and higher treatment effectiveness. The conventional microbiological methods contributed to the late diagnosis of the patients, high rate of the severe and complicated tuberculosis, long duration of the treatment which were reflected on the low treatment effectiveness. However the conventional culture methods allowed the treatment individualization according to the drug susceptibility tests.

Conclusions: Target screening of the population with risks and compulsory investigation by GeneXpert MTB/Rif assay will diminish the rate of severe late detected forms of pulmonary tuberculosis and adapt the treatment according to the drug resistance to rifampicin.

Key words: MDR-TB, Xpert MTB/Rif assay, treatment.

Introduction

Tuberculosis is one of the most important challenges for any health care system [15, 38, 42, 45]. The World Health Organization (WHO) declared tuberculosis a global emergency in 1993. Actually the Republic of Moldova ranks among the 30 countries with the highest burden of multidrug-resistant tuberculosis (MDR-TB), defined as the infection with resistant strains of *Mycobacterium tuberculosis* to at least two most powerful anti-tuberculosis drugs: isoniazid and rifampicin [45]. In 2001 the Republic of Moldova started the national implementation of the DOT (Directly Observed Treatment) strategy in the tuberculosis patient's management recommended by the World Health Organization [10]. Its implementation determined the epidemic extension of primary drug-resistant infection in the population. The rate of the primary MDR-TB, established as an infection with drug resistant strains increased four times in our country during the period 2003-2017 (6.0% – 2003, 9.9% – 2004, 13.4% – 2005, 19.4% – 2006, 17.8% – 2007, 24.0% – 2008, 22.2% – 2009, 25.7% – 2010, 26.35% – 2011, 24% – 2012, 25% – 2013, 25% – 2014) and acquired drug-resistance two times (37.5% – 2003, 38% – 2004, 49.6% – 2005, 50.8% – 2006, 52.3% – 2007, 59.0% – 2008, 64.85% – 2009, 67.8% – 2010, 63.8% – 2011, 62% – 2012, 62% –

2013, 63% – 2014) [10]. The extension of the drug resistance lowered the treatment effectiveness (2005 – 62%, 2006 – 62%, 2007 – 62%, 2008 – 58%, 2009 – 57%, 2010 – 52%, 2011 – 62%, 2012 – 62%, 2013 – 62%) which was lower in average by 20% compared with targeted 85% recommended by the WHO [4]. The low success rate was directly linked to the high rate of lost to follow-up patients: 2001 – 5.5%, 2002 – 15.6%, 2003 – 10.5%, 2004 – 10.4%, 2005 – 10.9%, 2006 – 11.7%, 2007 – 10.7%, 2008 – 7.4%, 2009 – 6.2%, 2010 – 7.9%, 2011 – 7.7%, 2012 – 5.2% [5]. International researches established high rate of drug resistance among HIV co-infected patients, which lowers treatment outcome and endangers the epidemiological situation [35]. Between 2011 and 2015 the rate of HIV infected among tuberculosis new cases patients increased by 2.6%: 2011 – 5.0%, 2012 – 5.0%, 2013 – 2.2%, 2014 – 5.3%, 2015 – 7.6% in Chisinau and 2011 – 5.1%, 2012 – 5.1%, 2013 – 5.2%, 2014 – 6.5% and 2015 – 7.7% in the Republic of Moldova [5]. The rate of HIV infection in new and relapsed cases increased evidently in Chisinau by 2.3%: 2011 – 4.6%, 2012 – 4.4%, 2013 – 2.9%, 2014 – 5.5% and 2015 – 6.9% and by 2.9% in the Republic of Moldova: 2011 – 5.2%, 2012 – 5.3%, 2013 – 5.9%, 2014 – 7.2% and 2015 – 8.1% [5]. The rate of TB-HIV co-infected cases among died patients showed an increased dynamic in Chisinau by 4.2%: 2011 – 11.9%, 2012 – 13.8%, 2013 – 9.2%,

2014 – 16,0% and 2015 – 16,1% as well as in the entire country by 5,3%: 2011 – 15,4%, 2012 – 12,8%, 2013 – 11,8%, 2014 – 20,8% and 2015 – 20,7% [5].

The diagnosis of MDR-TB, especially in children and HIV co-infected patients represents an important concern in many countries [12, 35, 36, 46]. No much technology is available for an accurate detection of the drug resistant strains of *Mycobacteria tuberculosis* worldwide [29]. So, detected deficiencies represent a major obstacle in the improvement of tuberculosis control and reduction of the global burden [11]. Microscopy alone, although inexpensive, misses one of three patients and detects only those with relatively advanced disease and severe lung destruction [8, 10, 32].

Only 28% of Moldovan patients were reported as smear positive cases in 2016 [5]. So, misdiagnosed cases increase the risk of infection transmission in the healthy population, endangering tuberculosis control at the regional level [11]. In the countries with a high burden of the HIV infection, the sensitivity of the smear microscopy is very low, which contributed to the increasing use of the rapid methods in the diagnosis of tuberculosis [35, 36]. According to the latest researches substantial efforts were made for strengthening the laboratory capacities for the diagnosis of smear-negative cases, TB-HIV co-infected cases and MDR-TB cases [10].

Conventional methods, such as smear microscopy, liquid and solid culture methods require qualified laboratories with important consequences: delayed diagnosis, worsening of the patient's condition, inadequate treatment which can generate additional drug resistance and continuous transmission of infection in the society [33]. In 2010 worldwide were implemented the first – and second – line probe tests which require extensive laboratory infrastructure and should be performed only in the reference facilities [33]. Starting from 2014 a real-time polymerase chain reaction Xpert MTB/RIF test for the *Mycobacterium tuberculosis* gene and the *rPob* gene mutation detection were implemented in the Republic of Moldova [8]. The Xpert MTB/RIF platform was developed by the Cepheid Company (Sunnyvale, USA). More than 50 devices and 12.000 cartridges were distributed in the Moldovan civilian specialized services, 2 in the penitentiary services and 2 in AIDS services. It contributed to an early detection of tuberculosis, precocious treatment, according to the rifampicin sensitivity, improvement of the infection control, increasing the treatment success rate, which are considered the most efficient tools for interrupting the epidemiological chain. [11]. Actual WHO recommendation regarding the Xpert MTB/RIF is to test all adults and children presumed to have pulmonary tuberculosis, with or without HIV co-infection. In addition, WHO established conditional recommendations to test extrapulmonary specimens of the adults and children presumed to have extrapulmonary tuberculosis [46]. The interpretation of the test results must be correlated with laboratory and clinical findings [9]. Published data established a high sensitivity of the Xpert MTB/RIF among culture positive specimens, in an average 97.3% and among smear positive patients – 99.5% [6]. The sensitivity of the Xpert MTB/RIF is slightly

decreased in a single sputum sample [7]. Assessing the threshold of the estimated sensitivity it was demonstrated that the biological sample must contain at least 131 colony forming units (CFU) per ml of sample with a confidence interval ranging from 106.2 CFU to 176.4 CFU [6]. The sensitivity depends on the collection procedure, storage, transportation and technical errors. Insufficient volume of the specimen and insufficient concentration of the viable organisms are the most frequent causes of the negative results [9]. The test specificity compared with the non-tuberculosis patients are also high – 97.9% [6]. Despite of the higher sensitivity of the Xpert MTB/RIF test, more than one half of Moldovan patients were diagnosed through the clinical-radiological methods in 2016 [4]. Due to the low cost of the Lowenstein Jenson and BACTEC cultures, conventional methods remain the golden standard for *Mycobacterium tuberculosis* complex detection and drug susceptibility testing on the national standards, instead of highly recommended Xpert MTB/RIF test [10].

The standard treatment for drug-susceptible tuberculosis, according to WHO recommendations in the Republic of Moldova has been used since 2000 as a part of DOT strategy and lasts 6 months in new cases and 8 months in previously treated cases [11]. The treatment of the new drug-susceptible patients consists in a two phase regimen with four first-line anti-tuberculosis drugs: Isoniazid (H), Rifampicin (R), Ethambutol (E) and Pyrazinamide (Z) in the intensive phase and two drugs H and R in the continuation phase [39]. A regimen of eight months consisted of H, R, E, Z and Streptomycin (S) during the intensive phase and H, R and E in the continuation phase is used for the treatment of previously treated cases (relapses, failed and treatment for lost to follow-up cases) [39]. Multidrug-resistant patients are treated using the standard regimen for resistant tuberculosis that consists of second-line anti-tuberculosis drugs during 18-24 months [47]. Second-line anti-tuberculosis drugs used in the treatment of drug resistant tuberculosis are: Fluoroquinolones, Aminoglycosides, Thioamides, Oxazolidinones and new drugs such as Delamanid and Betaquiline. The major contributing factor of the treatment effectiveness represents the right combination of the drugs, according to the *Mycobacteria* susceptibility results [40]. Without an appropriate treatment tuberculosis-related death occurs in average within 2 years [24]. Due to high global epidemiological burden tuberculosis was placed on the 5th place in the top of the causes of death [45].

The major social determinants of tuberculosis and poor outcome are: social and economic inequalities, high levels of internal/external migration, rapid urbanization and population growth in urban areas [22, 25, 27]. Such determinants determine the polarization of the public health interventions, poor housing, low environmental conditions, malnutrition, geographic and cultural barriers in access to the health care [47]. There are several predictive factors for low treatment effectiveness. First of all – the infection with drug resistant and virulent mycobacteria [47]. The risk depends on the spread of the virulent mycobacteria in the community. The second group includes the factors which

increase the disease relapse or recrudescence of the latent tuberculosis infection: phtysiogenic ages (infants less than 5 years, teenagers, elders aged 65 and more), malnutrition, comorbid state and lack of the BCG vaccination [11]. The third group included the determinants of the patient's immune status: HIV infection, immune suppressive drugs (>15mg/day of prednisone for 1 month or more, immune modulators: TNF- α blockers or oral steroids, antineoplastic agents), diabetes, cancer, silicosis, chronic respiratory diseases, gastrointestinal diseases, underweight, phtysiogenic ages, harmful habits (tobacco smoking, alcohol abuse, illicit drug using) [27]. The fourth group includes: the accessibility of the sick people to the tuberculosis screening and health care, treatment compliance and patient's nursing [44]. In this group are also included social related barriers: lack of the social protection and medical insurance, geographic and economic barriers, cultural behaviour or stigma. The social determinants of the poor treatment outcome are strongly correlated with the psychotropic substance abuse: tobacco smoking, alcohol abuse and illicit drug using. Such behaviour habits contribute to multiple treatment interruptions and high rate of death.

Low treatment adherence and high rate of the lost to follow-up patients contributed to the development of a shorter conventional MDR-TB regimen lasting less than 12 months with low costs (<1.000\$/patient) [47]. It showed promising results in selected MDR-TB patients and WHO updated its treatment guidelines in 2016 by including the recommendation to use the shorter MDR-TB regimen in patients with non-complicated tuberculosis (excluding extrapulmonary tuberculosis and pregnancy) [47]. Fluoroquinolones and Aminoglycosides are key drugs in the new MDR-TB regimen, however, surveillance of the cross resistance in five high burden countries: Azerbaijan, Bangladesh, Belarus, Pakistan and South Africa established that resistance of Rifampicin is frequently associated with resistance to Pyrazinamide, so the effectiveness of the short regimen might be lower than predicted [45]. Considering the WHO recommendations for aligning the Moldovan procedures for the international quality-assured standards, it was established the importance of the diagnosis algorithm improvement for increasing the treatment effectiveness.

The aim of the study consisted in the comparative assessment of the clinical aspects and the treatment effectiveness in patients with pulmonary tuberculosis diagnosed according to the results of the conventional microbiological methods (solid either liquid media) and molecular-genetic method Xpert MTB/RIF test (susceptible or resistant to Rifampicin forms).

Material and methods

A selective, descriptive and retrospective study was conducted according to a linear model, structured in several stages: purpose, sampling, investigation, data collection and interpretation. A preliminary documented research has been carried out on new cases of pulmonary tuberculosis investigated and treated in the Clinical Hospital of Phtisiopneumology of Chişinău between 2010 and 2014. The inves-

tigation methods were used according to the National Clinical Protocol. The patients were investigated by laboratory examinations: general blood and urine analysis, chest X-ray, sputum microscopy for acid fast bacilli, conventional microbiological investigations (smear microscopy, Lowenstein – Jensen culture, BACTEC assay) and innovative platform of the Xpert MTB/RIF test. The statistical processing of the results was done computerized through the Excel program of the Microsoft Office package. For estimating significant differences, the Student test was used (differences were true for $p < 0.05$; P – characteristic rate, ES – standard error).

Results and discussion

The study included 304 patients with pulmonary tuberculosis, of which 141 had proved multidrug-resistant tuberculosis (MDR-TB), according to the conventional drug susceptibility tests, 85 had Rifampicin resistance on the Xpert MTB/RIF test and 78 patients were Rifampicin susceptible of the Xpert MTB/RIF test. Distributing patients in groups according to the drug susceptibility definition, 226 were diagnosed with MDR-TB and 78 with sensible tuberculosis. The patients were divided into three groups according to the results of the molecular-genetic or bacteriological investigations: in the first group were included 85 patients enrolled in the DOTS plus cohort treated for MDR-TB according to the results of the Xpert MTB test and the second group included 141 patients treated for MDR-TB, according to the results of the drug susceptibility test on conventional microbiological methods (Lowenstein – Jensen culture, BACTEC assay). Both groups should be considered as a study sample constituted from 226 patients with MDR-TB. The third group, defined as the control group, included 78 patients with positive and sensible for Rifampicin Xpert MTB/RIF test and diagnosed with drug susceptible tuberculosis.

Assessing the groups according to the sex distribution was established the predominance of men in all groups. So, in the first group there were 61 men (71.7%) and 24 women (28.3%) cases with a male/female ratio = 2.5/1. In the second group men were more frequently identified than women and their rate was higher compared with the first group: 106 (75.1%) vs 35 (24.8%) women with male/female ratio=3/1. In the third (control group) there were 53 men (68%) cases and 25 women (32%) cases with a male/female ratio=2.1/1. The urban residence prevailed in all three groups compared with the rural one. So, in the first group there were 67 urban residents (78.8%), in the second group – 90 (70.2%) and in the third group – 60 (76.9%). Assessing the social vulnerability it was established that poor living conditions prevailed in the MDR-TB sample. In the first group there were 69 (81.2%) socially vulnerable patients, in the second group – 102 (81.2%), and only 28 (35.9%) patients in the third group. The rate of the unemployed patients was high in the first and second groups, 64 (75.3%) and 104 (73.7%) compared with the third group 47 (60.3%) patients. Unqualified workers were in a similar proportion in the first and second groups, 23 (27.1%) and 25 (24.1%) and higher compared with the third group 14 (17.9%) patients. Assessing the level of the school education it was established that completed general

studies presented a similar proportion of patients in the first and second groups 34 (40.1%) and 51 (36.2%) being higher compared with the third group of 14 (17.9%) patients. The professional studies prevailed in the third group – 39 (50.1%) compared with the first and second groups, 24 (28.2%) and 39 (27.7%) patients. Comparing the rate of patients with a low school education, which included graduated primary school, incomplete general school and secondary school it was determined their predomination in the first and second groups compared with the third group. The high level of the school education which included professional and superior studies predominated in the third group compared with the first and second groups. The civil status of the single state person prevailed in the first group 37 (43.5%) compared with the second and third groups, 36 (25.5%) and 26 (33.3%) patients. Married patients predominated in the second group 105 (74.5%) compared with the first and third groups, 48 (56.5%) and 52 (66.7%) patients. Lack of the health insurance predominated in the third group 43 (55.1%) compared with the first and second groups, 43 (50.6%) and 92 (65.2%) patients. Comparing the groups of patients was established a more evident social vulnerability of the MDR-TB patients from the study sample being appreciated through the rate of the unemployed persons, unqualified workers, patients with low level of the school education and lack of the health insurance (tab. 1).

Table 1

Social and economic state of patients with pulmonary tuberculosis

Economic Social state	1 st group n=85(P%)	2 nd group n=141(P%)	3 rd group n=78(P%)	P _{1/2}	P _{1/3}	P _{2/3}
Employed	21 (24,7)	37 (26,3)	31 (39,7)	>0,05	<0,05	<0,05
Unemployed	64 (75,3)	104 (73,7)	47 (60,3)	>0,05	<0,05	<0,05
Low level of education	53 (62,3)	92 (65,5)	23 (29,5)	>0,05	<0,05	<0,05
Optimal level	35 (37,7)	49 (34,5)	55 (70,5)	>0,05	<0,05	<0,05
Single civil state	37 (43,5)	36 (25,5)	26 (33,3)	>0,05	>0,05	>0,05
Married/ in couple	48 (56,5)	105 (74,5)	52 (66,7)	>0,05	>0,05	>0,05

Epidemiological risk factor, established during the household contact with a sick family member was identified in a similar proportion in the second and third groups, 68 (48.2%) and 39 (50.1%) compared with the first group, 25 (29.4%) patients. Migrants were identified in a higher proportion in the third group 12 (15.4%), compared with the first and second groups 10 (11.8%) and 14 (9.9%) patients. Former detainees were more numerous in the second group 28 (19.9%) compared with the first and third groups 13 (15.3%) and 11 (14.1%) patients. Harmful habits were detected in a high proportion in all groups. So, the active smoking prevailed in the second group 117 (83.1%) compared with the first group and third group 63 (74.1%) and 48 (61.5%) patients. Alcohol abusers prevailed in the second group 81 (57.4%) compared with the first group and third groups 38 (44.7%) and 32 (41.1%) patients. A limited num-

ber of drug users were diagnosed in all groups: 4 (4.7%) in the first group, 3 (2.4%) in the second group and 1 (1.3%) in the third group. Patients that form risk groups were distributed differently among selected samples. Even statistical difference was not established, contact with a sick family member predominated in the groups diagnosed with drug susceptible tuberculosis through the Xpert MTB/RIF and MDR-TB by conventional methods. No differences were established regarding harmful habits in the studied groups (tab. 2).

Table 2

Distribution of patients in risk groups

Risk groups	1 st group n=85 (P%)	2 nd group n=141 (P%)	3 rd group n=78 (P%)	P _{1/2}	P _{1/3}	P _{2/3}
TB contact	25(29,4)	68(48,2)	39(50)	<0,05	<0,05	>0,05
Migrants	10 (11,8)	14 (9,9)	12 (15,4)	>0,05	>0,05	>0,05
Former detainees	13 (15,3)	28 (19,9)	11 (14,1)	>0,05	>0,05	>0,05
Smokers	63 (74,1)	117 (83)	48 (61,5)	>0,05	<0,05	<0,05
Alcohol abuse	38 (44,7)	81 (57,4)	32 (41)	>0,05	>0,05	<0,05
Chronic alcoholism	9 (10,6)	17 (12,1)	0	>0,05	<0,05	<0,05
DIU	4 (4,7±2,3)	3 (2,1±1,2)	1 (1,3±1,3)	>0,05	>0,05	>0,05

Note: DIU – drug injection use;

Assessing the associated diseases, it was established that the comorbid state predominated in the first group 69 (81.1%) compared with the second and third groups, 69 (48.9%) and 58 (74.3%) patients. Distributing patients, according to the nosological groups of the associated diseases was determined the predomination of the gastrointestinal disorders in the first group 36 (42.4%), followed by the second and third groups, 45 (31.9%) and 28 (35.9%) patients. Chronic respiratory diseases were diagnosed in a similar proportion in all groups: 31 (36.5%) in the first group, 39 (27.7%) in the second group and 35 (44.9%) patients in the third group. Diabetes mellitus was diagnosed more frequently in the first group 12 (14.1%) followed by the third and the second groups 9 (11.5%) and 7 (5.1%) patients. Co-infected TB/HIV has been encountered frequently in the first group 4 (4.7%) followed by the third group 2 (2.7%) and 1 (0.7%) patient in the second one.

Detected particularities established the predominance of the passive case-finding in the first and second groups, 68 (80.1%) and 106 (75.2%) patients compared with 38 (49.7%) patients in the third group. Active screening of the risk groups was most frequently used to detect patients in the third group 40 (51.3%) compared with 17 (20.1%) and 35 (24.8%) patients from the first and second groups. Distributing patients according, to the diagnostic delay, it was identified that each fourth patient in the first and second group were diagnosed in more than three months after the disease onset. The early diagnosis till 30 days after the disease onset was established in each fifth patient in the third group 7 (18.4%). Pulmonary infiltrative tuberculosis was

diagnosed in most of the patients in all groups: 79 (93.1%) in the first group, 132 (93.6%) in the second group and 72 (92.3%) patients in the third group. Severe forms in terms of disseminated and fibro-cavitary tuberculosis were diagnosed in a similar proportion in all groups: 6 (7.1%) in the first group, 7 (4.9%) in the second group and 6 (7.7%) in the third group. Complicated tuberculosis was diagnosed in a similar proportion in the first group and third groups, 28 (32.9%) and 24 (30.7%) patients, compared with 24 (17.1%) patients of the second group. Among complications predominated pleurisy in a similar proportion in all groups: 14 (16.5%) in the first group, 21 (14.9%) in the second group and 9 (11.5%) patients in the third group. Hemoptysis predominated in the second group 25 (17.8%), followed by the first and third groups 9 (10.6%) and 9 (11.6%) patients. The most severe complication defined as tuberculosis meningitis was diagnosed in 3 (1.4%) patients of the second group. No such complication was diagnosed in other groups. Lung parenchyma destruction predominated in the second group 137 (97.2%), followed by a similar proportion in the first and third groups, 67 (78.9%) and 58 (74.4%) patients. More than three pulmonary segments were affected by tuberculosis in the majority of patients from all groups: 80 (94.1%) in the first group, 127 (90.1%) in the second group and 48 (61.5%) patients in the third group. Both lungs were involved in two thirds of the first and second groups, 74 (87.1%) and 124 (87.9%) and in each second patient of the third group – 51 (65.4%) cases. So, passive way of finding of symptomatic cases predominated in the MDR-TB sample and active screening of the risk groups in the drug susceptible sample. Even the pulmonary infiltrative tuberculosis was diagnosed in most of the cases, severe forms, involvement of both lungs and complicated tuberculosis were more frequently diagnosed in the sample diagnosed by microbiological conventional methods.

Microscopic smear positive was more frequently distinguished in patients from the second group 96 (68.1%), followed by the first and third groups, 53 (62.3%) and 51 (66.7%) patients. The delay between the diagnosis by Xpert/RIF examination and the beginning of the treatment was less than 10 days in the first and third groups. In one third of the second group the delay between culture examination and initiation of the treatment, according to the drug susceptibility test constituted in average 2 months, however, in 3 till 5 months the therapy was started in 64 (45.4%) patients and more than 5 months in 46 (32.6%) patients. Each second patient from the study sample with MDR-TB was identified resistant against all first-line anti-TB drugs: Isoniazid, Rifampicin, Ethambutol and Streptomycin: 40 (47.1%) in the first group and 67 (47.5%) patients in the second group. No drug resistant patients were diagnosed by culture methods in the third group, which confirmed the high specificity of the Xpert/RIF test. Sputum conversion under the anti-tuberculosis treatment assessed by microscopic method during the first three months of the treatment for MDR-TB (DOTS-Plus) was distinguished in a higher proportion in the first group compared with the second group: 31 (58.5%) vs 21 (21.2%) patients. In most of the cases with drug sus-

ceptible tuberculosis included in the third group, 43 (84.2%) patients, the sputum conversion was obtained in the first three months. Sputum conversion during the first three months of the treatment assessed by culture methods was determined in the highest proportion in the third group, 68 (87.2%) patients. In a lower proportion was established the sputum conversion in patients from MDR-TB groups. Comparing the first with the second group, the sputum conversion predominated in the first group 55 (64.8%) vs. 30 (21.3%) patients from the second group. A high proportion of the non-assessed patients, which included died and lost to follow-up patients were established in the second group, due to delayed diagnosis and longer duration of the treatment compared with the first group (tab. 3).

Table 3

Conversion of sputum by culture methods

Sputum conversion	1 st group	2 nd group	p
	n=85 (P%)	n=141 (P%)	
Till 3 months	55 (64.8)	30 (21.3)	<0,001
3-6 months	39 (45.5)	64 (54.7)	<0,05
6-12 months	2 (2.4)	13 (4.7)	>0,05
More than 12 months	0	1 (0.7)	>0,05
Non-assessed patients	9 (10.6)	45 (31.9)	>0,05

Obtained data confirmed the impact of molecular genetic test for early diagnosis, interruption of the epidemiological chain and the appropriate treatment according to the drug resistance against Rifampicin. The early sputum conversion assessed through the microscope and conventional culture methods had a high relevance in groups diagnosed by the Xpert MTB/RIF assay. It was due to a lower rate of severe forms, lung destruction and an appropriate treatment, according to the drug resistance test to Rifampicin.

The anti-tuberculosis treatment duration in the first group was 24 months in 54 (63.52%) patients and 18 months in 16 (18.8%) patients. A limited number were treated less than 6 months due to a precocious death. So, the Xpert MTB/RIF assay offers the opportunity of an early diagnosis and rapid beginning of the appropriate treatment for drug resistant tuberculosis. In the second group the duration of the anti-tuberculosis treatment was long due to a high rate of patients with severe and complicated tuberculosis. So, during 24 months were treated 37 (62.2%) and more than 24 months 76 (53.9%) patients. Treatment in out-patient settings was performed by the every third patient in the first and third groups, 27 (31.8%) and 26 (33.1%) cases. First-line anti-tuberculosis drugs received all patients with drug susceptible tuberculosis from the third group. A few patients from the first and second groups were treated for a limited duration with first-line therapy till obtaining the results of the drug susceptibility test. The standard treatment for MDR-TB was initiated in the first and second groups 83 (97.6%) and 113 (80.1%). However, in every fifth patient of the second group it was individualized according to the drug resistant susceptibility obtained through the conventional culture methods. Data are shown in the table 4.

Table 4

Anti-tuberculosis treatment duration in patients from the study sample

Duration	1 st group	2 nd group	P
	n= 85 (P%)	n=141 (P%)	
6 months	7 (8,2)	14 (9,9)	>0,05
18 months	16 (18,8)	21 (14,9)	>0,05
24 months	54 (63,5)	37 (26,2)	<0,001
24-30 months	2 (2,3)	74 (52,5)	<0,001
>30 months	0	2 (1,4)	>0,05

Multiple treatment interruptions were established more frequently in the second group 42 (29.9%) followed by the first and third groups 18 (21.2%) and 8 (10.3%) patients. So, it was assessed that long treatment duration diminished the treatment compliance of patients with MDR-TB patients, especially those diagnosed through the conventional microbiological methods. Major adverse drug events were diagnosed in every third patient of the second group 43 (30.5%), followed by the first group and third group 19 (22.4%) and 4 (5.1%) patients. Treatment success more frequently was established in the third group 67 (85.9%), followed by the first group 59 (69.4%) and second group 84 (59.6%). Were lost to follow-up more frequently patients from the second group 21 (14.9%) which was due to multiple treatment interruptions. The rate of patients lost to follow-up in the first and third groups was similar, 9 (10.7%) and 8 (10.3%). Died more frequently in the second group 22 (15.6%), followed by the first group 12 (14.1%) and the third group 1 (1.3%). So, the highest rate of lost to follow-up patients was explained by the long treatment duration and the higher death rates due to severe tuberculosis and high rate of major adverse drug events. Exposed data are presented in the table 5.

Table 5

Anti-tuberculosis treatment outcome in the study sample

Treatment outcomes	1 st group	2 nd group	p
	n= 85 (P%)	n=141 (P%)	
Treatment success	59 (69,4)	84 (59,6)	>0,05
Including cured	54(63,5)	82(58,1)	>0,05
Including completed the treatment	5(5,9)	2(1,4)	>0,05
Treatment failure	5(5,9)	14(9,9)	>0,05
Lost to follow-up	9(10,6)	21(14,9)	>0,05
Died	12(14,1)	22(15,6)	>0,05

Assessing all related data it was established the predominance of men in all selected groups, which confirmed their social vulnerability. Urban residence identified in two thirds of each group established that the level of the epidemiological control is lower in cities. The social vulnerability was higher in the MDR-TB patients than in drug susceptible cases, demonstrated by the rate of the unemployed persons, with the lack of the social and medical insurance. Epidemiological risk in terms of the tuberculosis contact was more relevant in patients with drug resistant tuberculosis. Such risk

conditions as migration, former detention, and harmful habits had no prevalence in drug resistant or susceptible groups. Passive finding of the symptomatic cases predominated in the MDR-TB sample and active screening in the drug susceptible sample. Pulmonary infiltrative tuberculosis was diagnosed in most of the cases, but severe forms, involvement of both lungs and complicated tuberculosis were more frequently identified in the sample diagnosed by microbiological conventional methods. Early diagnosis, infection control and the adequate treatment according to the drug susceptibility to Rifampicin were possible in the groups diagnosed by the Xpert MTB/RIF assay. It permitted the sputum conversion in the first three months in most of the patients. Patients diagnosed through culture methods had more frequently extended radiological processes, both lungs involvement, which contributed to a long duration of treatment, high rate of the adverse drug events and low treatment outcome. The treatment success rate for drug susceptible tuberculosis reached the WHO recommended level 85%. The treatment success level for drug-resistant tuberculosis was low, mostly in the group diagnosed through the conventional microbiological methods. Long duration till the sputum conversion of patients treated for drug resistant tuberculosis demonstrated their epidemiological burden on the healthy population. The low rate of the lost to follow-up patients and died due to the progression of pulmonary tuberculosis in groups diagnosed through the Xpert MTB/RIF assay was due to a shorter period of the anti-tuberculosis treatment.

Conclusions

The molecular genetic Xpert MTB/Rif assay demonstrated an important impact on the diagnosis of pulmonary tuberculosis in terms of earlier diagnosis, adequate treatment according to the drug resistance to Rifampicin, shorter duration of the treatment, low rate of the side events, optimal compliance and higher treatment effectiveness.

The conventional microbiological methods contributed to the late diagnosis of the patients, high rate of the severe and complicated tuberculosis and long duration of the treatment which were reflected on the low treatment effectiveness. However, the conventional culture methods allowed the individualization of the treatment, according to the drug susceptibility tests.

Target screening of the population with risks and compulsory investigation by Xpert MTB/Rif assay will diminish the rate of severe late detected forms of pulmonary tuberculosis and adapt the treatment according to the drug resistance to Rifampicin.

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