RESEARCH STUDIES

Molecular–genetic assay results and clinical, radiological features in patients with pulmonary tuberculosis

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Abstract

Background: Early diagnosis of smear-negative and multidrug-resistant tuberculosis represents a national priority, considering the fact that the Republic of Moldova ranks in the list of 30 high burden multidrug-resistance tuberculosis countries, with 26% rate of primary drug-resistance and 64% acquired drug-resistance. In April 2012 it was implemented the real-time fully automated diagnostic molecular test in 15 Moldovan districts and penitentiary institutions. The new assay leads to enhance the tuberculosis early diagnosis, especially multidrug resistant tuberculosis.

Material and methods: A retrospective, selective, descriptive and case-control study was performed using 361 new pulmonary tuberculosis patients, diagnosed and hospitalized in the Municipal Clinical Hospital of Phthysiopneumology of Chisinau city in the period of 01.01.2014-01.01.2015. The total sample was distributed in 2 groups: first group included 174 patients with pulmonary tuberculosis assessed by positive Xpert MTB/RIF assay and the second group included 187 patients with pulmonary tuberculosis assessed having negative Xpert MTB/RIF assay. Investigations were performed according to the National Clinical Protocols.

Results: The biggest rate of patients included in the research was detected by passive way, in the frame of examination of the symtomatic case: 111 (63.8%) patients of the 1st Group comparing with 87 (46.5%) in the 2nd Group, $p<0.001$.

Conclusions: The use of Xpert MTB/RIF technique must be improved in municipality Chisinau for achieving the international quality standards.

Key words: pulmonary tuberculosis, genetic assay results, Xpert MTB/RIF.

Introduction

Tuberculosis is the most important public health threat in the Republic of Moldova, the country ranking among 30 countries with the biggest burden of multidrug-resistance tuberculosis (MDR-TB) [1]. In overall, the primary MDR-TB increased four times in the Republic of Moldova in the period 2003-2011 (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) 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) [3]. MDR-TB represents a global concern and directly threatens disease-control efforts in many countries. Only 30.000 of nearly 500.000 new cases of MDR-TB every year are detected and reported [11]. The misdiagnosed cases contribute to thousands of deaths, nosocomial and community transmission.

No much technology is available for an accurate detection of tuberculosis and its drug resistant forms, this fact being a major obstacle for improvement of tuberculosis control and reduction of the global burden of disease [12]. Microscopy alone, although inexpensive, misses many patients and detects only those with relatively advanced disease. Presently, only 28% of registered tuberculosis cases are detected and reported as smear positive [3]. Undetected cases of tuberculosis increase the risk of disease transmission to the healthy population, endangering tuberculosis control. In low and middle income countries the high rate of HIV infection reduced the sensitivity of microscopy and increased the relevance of rapid diagnostic methods []. In recognition of these issues, substantial efforts were being made to strengthen laboratory capacities to diagnose smear-negative and MDR-TB cases, including the use of solid and liquid culture, conventional drug-susceptibility testing, and line-probe assays. Unfortunately, these tests require extensive laboratory infrastructure and cannot be done outside of reference facilities. Recently, a real-time PCR assay for *Mycobacterium tuberculosis* that simultaneously detects rifampicine resistance was developed on the GeneXpert platform (Cepheid, Sunnyvale, CA, USA), which integrates sample processing and greatly simplifies testing [2]. Expected outcomes ensured by the national implementation of the new Xpert MTB/RIF technique are: early detection of infectious cases, isolation and precocious treatment of early detected new case, especially of MDR-TB, as well as infection control and improving the treatment success rate, that are considered the most efficient tools for interrupting the epidemiological chain of infectious transmission [16]. Starting from December 2010 World Health Organization (WHO) strongly recommends the use of molecular-genetic Xpert MTB/RIF testing as the initial diagnostic test in adults and children presumed to have pulmonary MDR-TB, tuberculosis and HIV coinfection, or

TB meningitis [14]. In addition, WHO established conditional recommendations to use Xpert MTB/RIF test in adults and children presumed to have active TB (not especially MDR-TB), or for testing the non-respiratory specimens targeting the diagnosis of extra pulmonary TB [15]. Always interpretations of this test must be correlated with laboratory and clinical data of the investigated patient. Published data established a high sensitivity among culture positive specimens on average 97.3% and among smear positive patients – 99.5%. The specificity comparing with non-tuberculosis patients was determined to be at 97.9% [2]. Error rates vary from 3 to 4%. The sensitivity is slightly decreased in a single sputum sample. Assessing the threshold of analytical 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 [2]. In most of cases the detection of mycobacterial DNA 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 [2].

WHO Global TB program provides worldwide leadership in strategic and technical aspects of TB control in order to eliminate TB. In 2011 the Stop TB Partnership started the ongoing of TB Xpert Project that provided 1.4 million Xpert MTB/RIF test cartridges and 220 Xpert instruments in 21 countries, the Republic of Moldova being one of them. Starting from April 2012, the Republic of Moldova received 25 units of Xpert MTB/RIF equipment and 12.000 cartridges. Civilian TB services acquired 21 units, penitentiary TB services – 2 and AIDS services – Xpert MTB/RIF units of equipment. Preliminary results established some problems. The most important is the low sensitivity of Xpert MTB/RIF Assay that is compared with the sensitivity of conventional microbiological methods. More than one half of patients with tuberculosis are diagnosed through clinical-radiological methods. Considering the WHO recommendations for aligning to international quality-assured standards it is necessary to improve the TB diagnostic algorithms.

Aim of the study consisted in evaluation of the Xpert MTB/RIF assay and diseases-related features (clinical and radiological) in patients with pulmonary tuberculosis.

Material and methods

It was performed a retrospective, selective, descriptive and case-control study targeting peculiarities of pulmonary tuberculosis of 361 patients, treated in the Municipal Clinical Hospital of Phthysiopneumology of Chisinau city between 01.01.2014 and 01.01.2015. The total number of cases was distributed in 2 groups: 1st Group (1st Group) included 174 patients with pulmonary tuberculosis assessed by positive Xpert MTB/RIF assay and second group (2nd Group) included 187 patients with pulmonary tuberculosis assessed being negative at Xpert MTB/RIF assay. Including criteria in the 1st group: age > 18 years old; patient with pulmonary tuberculosis was established as a new case; one smear positive GeneXpert MTB/RIF assay detecting MTB DNA; including criteria in the 2nd group: age > 18 years old; patient with pulmonary tuberculosis was established as a new case; one smear negative Xpert MTB/RIF assay. Collection of primary material involved the extraction of data from medical record forms. The individual schedule included information about: anamnesis, clinical examination, results of radiological investigations (chest radiography, plane tomography, and computer tomography), results of microbiological investigations (bacterioscopy of the smear with Ziehl-Neelson coloration) and bacteriological examinations (culture on classic solid medium Lowenstein-Jensen and liquid medium). Investigations were performed according to the national clinical protocol. Statistical analysis methods used in the study were: comparative, synthesis, discriminate analyses. Mathematical and statistical assessments were carried out by checking the quantitative and qualitative features. Accumulated material was tabled in simple and complex groups. Statistical survey was performed using Microsoft Excel XP soft. The predictability value of each involved factor was calculated using two by two tables. Relative risk and confidence interval were calculated according to the established formula [4].

Results and discussion

Considering the fact that 361 patients were investigated through Xpert MTB/RIF, all of them confirmed with pulmonary tuberculosis and only 174 had positive Xpert MTB/RIF. In the 1st group – 174 positive Xpert MTB/RIF cases included 103 microscopic positive at Ziehl-Neelson staining cases. So, the sensitivity of microscopic method compared with Xpert MTB/RIF assay represents 59.2±3.72%, which means two times lower. The same 1st Group – 174 positive Xpert MTB/RIF cases included 142 culture positive at Lowenstein-Jensen/BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960 media. So, the sensitivity of culture method compared with Xpert MTB/RIF assay represents 81.6±2.93%. Rapid detection of rifampicine resistance was established at 63 (36.2±3.64%) cases from 174 positive Xpert MTB/RIF assay cases. On the other hand, in the second group, that included 187 Xpert MTB/RIF negative cases, 9 (4.8±1.56%) cases of them were microscopic positive at Ziehl-Neelson and 28 (14.9±2.60%) were culture positive at Lowenstein-Jensen/BACTEC MGIT media. So, Xpert MTB/RIF assay can't replace conventional microbiological methods in actual epidemiological conditions of the Republic of Moldova.

Case-management of patients with pulmonary tuberculosis

According to the National Tuberculosis Program at least 70% of all new tuberculosis cases must be detected by passive way, through microscopic smear examination of the symptomatic patient. Active way of detection is reserved only to high risk groups, annually investigated by chest Xray examination and also dangerous groups of 3 professional fields: health care staff, public service employees as well as educational staff. National TB Policy approved an examination algorithm of different groups of population (patients, adults and children with TB symptoms, HIV positive patients with TB symptoms,

vulnerable groups – homeless, drug-users, immunosupressed, medical staff, patients with suspected relapse, patients with clinical signs of extrapulmonary TB) for TB diagnosis targeting the appropriate use of Xpert MTB/RIF assay. So, according to this, the sputum smear negative samples in highlighted groups of patients with clinical signs of TB (pulmonary TB or extra pulmonary TB) must be compulsorily investigated by Xpert MTB/RIF.

The biggest rate of patients included in the research was detected by passive way, in the frame of examination of the symptomatic case: 111 (63.8±3.64%) patients of the 1st Group comparing with 87 (46.5±3.64%) in the 2nd Group, p<0.001. By active way of screening (targeted radiological investigation) were detected more frequently the patients from the 2nd Group 100 (53.5±3.64%) comparing with 63 (36.2±3.64%) cases of the 1st Group, p<0.001. However, by passive way were detected more frequently the patients of the 1st Group 111 (63.8±3.64%) comparing with the 2nd Group 87 (46.5±3.64%), p<0.001. Data are shown in the table 1.

Table 1

Distribution of detectional case-management

Screening way	1st Group n=174		2nd Group n=187		P
	n	M± m (%)	n	M ± m (%)	
Passive	111	63.8±3.64	87	46.5±3.64	<0.001
Active	63	36.2±3.64	100	53.5±3.64	<0.001

In one third of the cases the clinical diagnosis of pulmonary tuberculosis was erroneously omitted due to similar aspects with other pathologies. Distributing patients by clinical onset masks, it was identified that pneumonia mask is more frequently involved in both groups, being more prevalent in the 1st Group 34 (26.4±3.87%) comparing with the 2nd Group 14 (11.2± 2.82%), p<0.05. No statistical differences were identified according to other type of TB masks (table 2).

Table 2

Distribution according to the clinical onset masks

Diagnosis	1st Group n=174		2nd Group n=187		P
	n	M± m (%)	n	M ± m (%)	
Pneumonia	34	26.4± 3.87	14	11.2± 2.82	<0.05
Bronchitis	6	4.7± 1.85	2	1.6± 1.12	>0.05
Laringitis	6	4.7± 1.85	1	0.8± 0.79	>0.05
Pleuresy	8	6.2± 2.12	1	0.8± 0.79	>0.05
Hemoptysis	4	3.1± 1.52	1	0.8± 0.79	>0.05

Note: LIT - limited pulmonary infiltrative tuberculosis, EIT – extensive pulmonary infiltrative tuberculosis, DT – disseminated pulmonary tuberculosis. FCv – fibro-cavitary pulmonary tuberculosis;

The period of time from the clinical onset till the confirmation of diagnosis was established similar distribution of patients in both groups. The period of one to three months between the clinical onset and Xpert MTB/RIF testing (establishing of TB diagnosis) was more prevalent in both groups: 96 (55.2±3.77%) cases in the 1st Group and 108 (57.8±3.61%) - in the 2nd Group. Other delayed periods from the onset till the confirmation of TB diagnosis were similarly distributed within the groups (table 3).

Table 3

Distribution according to the delayed period till TB confirmation

Diagnosis	1st Group		2nd Group		P
	n	M± m(%)	n	M ± m (%)	
14 days	29	16.7±2.82	24	12.8±2.44	>0.05
1 – 3 months	96	55.2±3.77	108	57.8±3.61	>0.05
3 – 6 months	38	21.8±3.13	43	22.9±3.07	>0.05
>6 months	11	6.3±1.84	12	6.4±1.79	>0.05

All above related data were directly linked to the established clinical-radiological diagnosis of pulmonary tuberculosis. So, pulmonary infiltrative tuberculosis was prevalent form in both groups: 159 (91.4%) of the 1st Group and 180 (96.3%) cases in the 2nd Group. Caseous pneumonia, the severest form of the pulmonary infiltrative tuberculosis was diagnosed in 5 (2.9%) cases of the 1st Group. Disseminated tuberculosis was established in several cases of both groups - 14 (8.0%) cases of the 1st Group compared with 3 (1.6%) cases in the 2nd Group. Less diagnosed were the rest of forms (table 4).

Table 4

Clinical radiological forms of pulmonary tuberculosis

Diagnosis	1st Group n=174		2nd Group n=187		P
	n	M± m (%)	n	M ± m (%)	
PIT	159	91.4±2.12	180	96.3±1.38	>0.05
PDT	14	8.0±2.06	3	1.6±0.91	>0.05
NT	0	0	4	2.1±1.05	>0.05
FCv	1	0.6±0.57	0	0	>0.05

Note: PIT - pulmonary infiltrative tuberculosis, PDT – disseminated pulmonary tuberculosis. FCv –pulmonary fibrocavitary tuberculosis, NT – pulmonary nodular tuberculosis;

While assessing radio-morphological features of pulmonary tuberculosis it was clearly identified the predomination of the destructive compound of the parenchyma in the 1st Group -58 (33.3%) cases compared with the 2nd Group - 18 (9.6%) cases. The localization of the infectious process in both lungs was also predominated in the 1st group: 105 (60.3%) cases compared with 29 (15.5%) cases in the 2nd Group. The extensibility in more than three segments was argued more often in the 1st Group 104 (59.8%) compared with the 2nd Group 21 (11.2%) (table 5).

Table 5

Morpho-radiological features of pulmonary tuberculosis

Diagnosis	1st Group n=174		2nd Group n=187		P
	n	M± m (%)	n	M ± m (%)	
Destructions	58	33.3±3.57	18	9.6±2.15	<0.001
Dissemination	11	6.3±1.84	4	2.1±1.05	>0.05
Unilateral	69	39.7±3.70	158	84.5±2.64	<0.001
Bilateral	105	60.3±3.70	29	15.5±2.64	<0.001
Limited	70	40.2±3.71	166	88.8±2.30	<0.001
Extended	104	59.8±3.71	21	11.2±2.30	<0.001

Note: LIT – limited pulmonary infiltrative tuberculosis, EIT – extensive pulmonary infiltrative tuberculosis, DT – disseminated pulmonary tuberculosis. FCv – fibrocavitary pulmonary tuberculosis.

Impact of disease related determinants on MTB detection by the positive Xpert MTB/RIF testing

Assessing statistically all above exposed data expressing case management, clinical-radiological characteristics and morpho-radiological features of patients with pulmonary tuberculosis with positive/negative results at the detection of MTB by Xpert MTB/RIF testing through multivariate logistic regression model was identified that all disease related factors have high impact on the positivity of MBT DNA detection: extensive infectious process (involving three and more segments), tuberculosis in both lungs, lung parenchyma destructions. Case management by passive way as well as pneumonia mask were revealed as factors with medium value (table 6).

Table 6

Relative risk forTB factors Xpert MTB/RIF assay positive patients

Factors	Relative Risk	95% CI
Passive way of detection	1.45	1.15-1.82
Pneumonia mask	1.56	1.25-1.94
Lung destruction	1.87	1.55-2.26
Localisation in both lungs	2.47	1.71-3.56
Extensive pulmonary process	5.32	3.49-8.11

Conclusions

Passive case management and pneumonia mask were identified as factors with medium impact on Xpert MTB/RIF assay positive patients.

Extensive tuberculosis (involving three and more segments, localization of pathological changes in both lungs), parenchyma destructions have high impact in Xpert MTB/RIF assay positive patients.

Xpert MTB/RIF assay leads to rapid identifing of possible multidrug-resistant TB (MDR TB) and early starting of effective treatment much sooner than waiting for results from other types of drug susceptibility testing.

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