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CONTRIBUTION OF THE MOLECULAR-GENETIC METHOD IN THE DETECTION OF TUBERCULOSIS

***E. LESNIC*[1], *A. MALIC*[1], *A. NIGULEANU*[1], *V. POPA*[1], *A. USTIAN*[1], *A. JUCOV*[2], *A. TOMSA*[1], *T. BILEVICI*[3], *P. SÎRBU*[4], *C. IAVORSCHI*[1],**

[1]Department of Pneumophtysiology, Nicolae Testemiţanu State University of Medicine and Pharmacy,
[2]Department of Family Medicine, Nicolae Testemitanu SUMPh,
[3]Medical Territorial Association Botanica, Chisinau,
[4]Medical Territorial Association Buiucani, Chisinau

Summary

Tuberculosis detection represents the major challenge in actual health care system of the Republic of Moldova. The aim of the study was the assessment of the features and risk factors of 101 patients with pulmonary tuberculosis detected in the frame of the primary health care of Chisinau city and diagnosis confirmed by molecular genetic method GeneXpert MTB/Rif. The research results established that more frequently were detected patients with lack of health insurance and poor social economical state. The risk factors such as chronic alcoholism, drug consumption, and history of detention, migration and comorbid state were identified in a low proportion. Despite the fact that high risk groups such as persons with tuberculosis contact and HIV infected individuals must be detected by active screening, they were detected predominantly by passive way. Despite the fact, that severe and extensive clinical radiologic forms predominated in patients detected by addressing, the treatment success rate was without differences compared with patients detected by active screening.

Keywords: *tuberculosis, risk factors, detection, GeneXpert MTB/RIF*

Rezumat

Aportul metodei molecular-genetice în depistarea tuberculozei

Depistarea tuberculozei reprezintă o provocare majoră pentru sistemul actual de sănătate al Republici Moldova. Scopul cercetării a fost evaluarea particularităţilor şi a factorilor de risc a 101 pacienţi cu tuberculoză pulmonară, depistaţi în cadrul asistenţei medicale primare din municipiul Chişinău şi cu diagnostic confirmat prin metoda molecular-genetică GeneXpert MTB/RIF. Rezultatele studiului au confirmat că mai des au fost depistaţi pacienţi neasiguraţi, cu o stare socioeconomică precară. Factorii de risc precum alcoolismul cronic, consumul de droguri, istoricul de detenţie, migraţia şi comorbidităţile au fost identificaţi într-un număr redus. În pofida faptului că grupele cu risc înalt, precum persoanele cu contact tuberculos şi cele infectate HIV, trebuie să fie depistate prin screening activ, acestea au fost depistate mai frecvent pasiv. Deşi formele clinico-radiologice severe şi extinse au predominat la pacienţii depistaţi prin adresare, rata succesului terapeutic nu s-a diferenţiat de cea a pacienţilor depistaţi prin screening activ.

Cuvinte-cheie: *tuberculoză, factori de risc, depistare, GeneXpert MTB/RIF*

Резюме

Вклад молекулярно-генетического метода в выявление туберкулеза

Выявление туберкулеза является серьезной проблемой для нынешней системы здравоохранения Республики Молдова. Целью исследования было определить особенности и факторы риска 101 пациентов с туберкулезом легких, выявленных в рамках первичной медико-санитарной помощи города Кишинэу и с подтвержденным диагнозом молекулярно-генетическим методом GeneXpert MTB/Rif. Было установлено, что чаще всего были выявлены пациенты без медицинской страховки и со сниженным социально-экономическим статусом. Факторы риска, такие как хронический алкоголизм, употребление наркотиков, тюремное заключение, миграция, сопутствующие заболевания, были установлены у несколько случаев. Вопреки рекомендации активного выявления групп повышенного риска, включавшие людей с туберкулезным контактом и ВИЧ-инфицированные лица, они были обнаружены преимущественно пассивным путем. Несмотря на то, что тяжелые и обширные клинико-рентгенологические формы преобладали у пациентов, выявленных при обращении, доля успешно вылеченных было одинаково по сравнению с долей пациентов, выявленных активным путем.

Ключевые слова: *туберкулез, факторы риска, выявление, GeneXpert MTB/RIF*

Background

Tuberculosis (TB) represents a major threat to public health worldwide. The internationaly approved strategy in control of TB, defined as End TB Strategy combines health promotion, disease prevention, case detection and patient management at the first encounter [7, 8]. In the countries of Eastern Europe, Baltic States and the Commonwealth of Independent States (CSI) the diagnosis and treatment are performed in the specialized TB services. TB control system from those countries follows a three-tiered structure, incorporated at the national, regional and district level. The system of national TB control services consists of a network of TB facilities and health care institutions. However, for the successful disease control, entire health care sector must participate in TB control practices [5, 6]. Early detection and referral for out-patients treatment represent the frontline

where health care providers are involved, in a way reducing the burden of TB at the community level.

Primary health care provides (PHCP) are health workers: physicians-general practitioners (GPs), nurses, auxiliaries and community workers who serve frotline providers, responding to the health needs of the community [6, 7]. PHCP are the first to meet a TB patient, before the diagnosis is established [6]. Those providers represent the level where early detection must be performed and the efficacy of TB control activities has bigger impact (considering that a person with an undiagnosed active TB will infect in average 10-15 other people per year). Specific roles of PHCP differ according to regional or national guidelines, but they must accomplish the following functions established by the WHO Guide on TB Control for Primary Health Care Providers for countries localized in the WHO European Region with high and intermediate TB burden:

1. Suspect the disease in patients with symptoms suggestive of TB;

2. Ensure collection of sputum for microbiological examination for acid-fast bacilli from the patients with productive cough lasting more than 3 weeks;

3. Send the collected sputum to diagnostic laboratory for identifying acid-fast bacilli;

4. Order to perform chest X-ray examination of suspected patients;

5. Refer suspect individual to the specialized services for diagnosis and treatment;

6. Communicate to patients that the disease is curable and emphasize the importance of a regular and complete treatment;

7. Communicate with specialized organizations about the patients referred for diagnosis and treatment;

8. Perform screening of close TB contacts;

9. Educate general community about the signs and symptoms of TB;

10. Provide directly observed therapy till completion during the continuation phase;

11. Report complications, drug side effects, default of specific treatment to TB services;

12. Complete administrative forms and send them to the TB services;

13. Monitor high risk groups according to the national recommendations;

14. Perform BCG vaccination and tuberculin skin testation of children-closed contacts with TB patient [4, 5, 6].

Some specifique recommendations for Moldovan PHCP were included in the national guide for *Tuberculosis Control at the Primary Health Care Level*:

1. Ensure the chemo preventive treatment of contacts from 0-18 years old and HIV infected contacts;

2. Identify individuals at high TB risk according to the national guideline and to perform examination (chest X-ray examination in adults and tuberculin skin testation in children);

3. Perform collaborative activities with local NGOs in disease prevention;

4. Support TB patients in association with the community, public local authorities and governmental institutions.

The guide emphasizes some regulations of active screening to be performed by PHC sector:

1. Perform clinical examination of high risk groups of adults and children every 6 months;

2. Order a chest X-ray and microscopic examination of the sputum of individuals with suggestive signs and symptoms;

3. Order a chest X-ray in adults and tuberculin skin test in children from high risk groups at least one time in 12 months, even if there are no clinical signs suggestive for TB;

4. To perform the clinical examination annually to individuals included in the dangerous groups;

5. Perform a chest X-ray in patients from dangerous groups with suggestive signs and symptoms.

Perform a chest X-ray in individuals from contingency groups before the enrollment into the work field and one time per year [3]. Considering the exposed data it was performed a study with the aim of the assessment of the general, socio-economical and epidemiological risk factors of pulmonary TB patients detected by GPs according to the way of detection; 3. Evaluation of case-management, clinical aspects, radiological aspects and treatment outcome of pulmonary TB detected by GPs according to the way (passive and active) of detection.

Material and methods

It was performed a retrospective selective, descriptive study targeting social, demographic and economic peculiarities of 101 patients with pulmonary tuberculosis diagnosed in the Chisinau city in the period of 1.01.2015 – 31.12.2015. The investigational schedule included demographic, social and epidemiological data: sex (male/female ratio), age, urban/rural residence, country of patient's origin, educational level, socio-economic status (employed, unemployed, retired, disabled, student), health insurance status (uninsuranced, insuranced), migrational and detention history, presence of high risk (close contact, comorbidities: HIV-infection, psychiatric

diseases, immune suppressive treatment), type of infectious cluster, health care seeking behavior, way of the patient's detection. All selected patients were diagnosed and managed according to the National Clinical Protocol 123 *Tuberculosis in adults* [3]. Enrolled patients were distributed in two groups: 1st group constituted of 73 patients with pulmonary TB detected in the frame of primary health care sector by general practitioners as symptomatic patients and 2nd group constituted of 28 patients with pulmonary TB detected by general practitioners by active way of screening.

Results and discussion

According to the published data by the Moldovan National Centre for Management in Health during the period 2011-2015 it was registered an important decline (with 23/100.000) of the new case incidence in Chisinau city from 76/100.000 in 2011 to 53,9/100.000 population in 2015. The total number of new pulmonary TB cases decreased from 602 in 2011 to 435 in 2015 [1]. By PHCP were detected 213 (48,96%) new pulmonary TB cases in 2015. In the frame of passive way of screening GPs detected 129 (60,5%) patients and through the active screening 484 (39,5%) cases. From the total number of 213 cases 101 (47,4%) were confirmed by the positive GeneXpert MTB/RIF assay.

Sex distribution established the predominance of men in comparison with women in both groups 48 (65,7%) in 1st group and 22 (78,5%) cases in the 2nd group, with male/female ratio=1,92/1 in 1st group and 3,67/1 in the 2nd group. Repartition of the patients into age groups according to the WHO recommendations, identified that the largest represented was 35-44 years old age group: 25 (34,2%) patients in the 1st group and 8 (28,6%) cases in the 2nd group, followed by the 45-54 years group 7 (25,0%) case in the 2nd group and 25-34 years group 18 (24,6%) patients in the 1st group. Redistributing patients in two age groups (young and >45 years) it was established the predominance of younger cases (55 (75,3%)) in the 1st group comparing with 17 (60,2%) patients in the 2nd group, and older patients in the 2nd group 11 (39,3%) comparing with 18 (24,6%) in the 1st group without achieving statistical threshold. So, stratifying patients according to the biological characteristics it was argued that men and older individuals (>45 years) are target more frequently by the screening methods and young persons, as well as women are more frequently detected and diagnosed through passive way of screening as symptomatic patients (*table 1*).

Table 1

Distribution of patients according to the demographic factors

Demographic factors		*SG, n=73*	*CG, n=28*	*P value*
		n (%)	*n (%)*	
Sex	Men	48 (65,7)	22 (78,5)	>0,05
	Women	25 (34,3)	6 (21,4)	>0,05
Young groups	18-24 years	12 (16,4)	3 (10,7)	>0,05
	25–34 years	18 (24,6)	6 (21,4)	>0,05
	35-44 years	25 (34,2)	8 (28,6)	>0,05
Older groups	45-54 years	13 (17,8)	7 (25,0)	>0,05
	>55years	5 (6,9)	4 (14,3)	>0,05
Residence	Urban	56 (76,7)	23 (82,1)	>0,05
	Rural	17 (23,4)	5 (17,9)	>0,05

Two thirds of patients were from urban area, and one third – from rural area. No homeless patients were identified among the selected cases. By distributing the patients according to the educational level, it was determined that individuals with low level of school education (primary and incomplete secondary school) were identified in a similar proportion in the 1st group and in 2nd group: 21 (28,8%) and 7 (25%) cases, respectively. By distributing the patients according to the socioeconomic status, it was established that the rate of patients with economic stability was higher in the 2nd than in the 1st group. So, one third of patients was employed in the 2nd group 10 (35,7%) patients comparing with 16 (21,9%) cases – in the 1st group. One half of both groups, (42 (57,53%) patients in 1st group and 13 (46,4%) patients in 2nd group) were unemployed. The totality of patients with low financial income (unemployed, retired and students) was in a similar proportion in both groups: 57 (78,1%) in 1st group vs 19 (67,9%) cases in 2nd group. Assessing the civil status it was identified the same rate of married persons in both groups: 1st group (46 (63,1%) cases and 13 (46,4%) cases in 2nd group. Patients living under the poverty line, with the income less than the minimum consumer basket were more frequently identified in the 1st group than in the 2nd group. Harmful social habits such as chronic alcoholism and drug use, as well as the history of imprisonment and migration during last year were identified in a small number of cases, without statistical differences between the groups. Epidemiological risk factor, such as a close contact and being a member of the family TB cluster statistically prevailed in the 1st group 17 (23,3%) vs the 2nd group 2 (7,1%) cases, although the active way of detection is oriented especially to close contacts. So, it was identified the unsatisfactory use of active way of TB screening in infectious clusters (*table 2*).

Table 2

Distribution of patients according to the social economical factors

Demographic factors	SG, n=73		CG, n=28	P value
	n (%)		n (%)	
Educational status	Low (primary/ incomplete secondary)	21 (28,8)	7 (25,0)	>0,05
	Secondary	30 (41,09)	14 (50,0)	>0,05
	Professional	14 (19,2)	4 (14,3)	>0,05
	Superior	8 (10, 9)	1 (3,5)	>0,05
Economical status	Employed	16 (21,92)	10 (35,7)	>0,05
	Unemployed	42 (57,53)	13 (46,4)	>0,05
	Disabled	1 (1,37)	0	>0,05
	Student	8 (10,9)	2 (7,1)	>0,05
	Retired	6 (8,2)	4 (14,3)	>0,05
Social factors	Under minimum standard life	27 (36,9)	14 (50,0)	>0,05
	Migration	10 (13,67)	2 (7,1)	>0,05
	Alcohol abuse	2 (2,74)	1 (3,5)	>0,05
	Drug use	1 (1,37)	1 (3,5)	>0,05
	History of imprisonment	1 (1,37)	2 (7,1)	>0,05
	Family cluster of TB	17 (23,29)	2 (7,1)	<0,05

The rate of health uninsured patients was similar in both groups, as well as the rate of patients with comordities. So, two third of both groups were uninsured. One fifth of both groups were comorbid patients. Delayed diagnosis due to the insidious onset of the disease lasting more than 60 days statistically prevailed in the 1st group, 64 (87,7%) vs 8 (28,6%) cases in the 2nd group. Summing all patients form high risk groups it was established that persons with risk for TB predominated in 1st group 27 (36,9%) vs 6 (21,1%) patients from 2nd group. So, it can be concluded that passive way of screening contributes to delayed detection, diagnosis and treatment onset. By the other side the low rate of patients from high risk groups detected through the active way of screening demonstrates poor disease control performed by PHCP in the frame of high risk groups (*table 3*).

The rate of patients from high risk groups was statistically higher in the 1st group, 27 (36,9%) cases compared to the 2nd group – 6 (21,4%) cases. Being one of subgroups, HIV-infected individuals were more frequently in the 1st group 8 (29,6%) comparing with the 2nd group 2 (7,14%) cases. More evidently, TB contacts were more frequently identified in the 1st group – 17 (62,9%) cases comparing with the 2nd group of 2 (7,14%) patients. There were no differences in the rate of patients with immune suppressive treatment, and psychiatric diseases identified in both groups.

Table 3

Case-management of TB patients and high risk groups

Characteristics		SG, n=73	CG, n=28	P value
		n (%)	n (%)	
Case management	Lack of health insurance	47 (64,4)	17 (60,7)	>0,05
	Associated diseases	17 (22,3)	5 (17,6)	>0,05
	Late detected (>60 days)	64 (87,67)	8 (28,57)	<0,001
	High risk groups	27 (36,9)	6 (21,4)	>0,05
High risk groups		*SG, n=27*	*CG, n=6*	
	HIV coinfected	8 (29,6)	2 (7,14)	<0,001
	TB-contact	17 (62,9)	2 (7,14)	<0,001
	Immune suppressive treatment	1 (3,7)	2 (7,14)	>0,05
	Psychiatric disease	1 (3,7)	0	>0,05

When assessing the laboratory features of the enrolled new pulmonary TB cases, it was identified that one half of patients were microscopic positive for acid-fast-bacilli in the 1st group and only one third – in the 2nd group. The same rate was identified in the repartition of patients according to the positive bacteriological results (culture on solid Lowenstein-Jensen either liquid MGIT BACTEC). The sensibility to the rifampicine through GeneXpert MTB/Rif assay was established more frequently in the 1st group than in the 2nd group, without achieving the statistical threshold (*table 3*).

Assessing radiological features of investigated patients it was established a high degree of difference between the groups regarding the extensibility of infiltrative processes. More frequently were patients with 1 lung involved in the 2nd group, 26 (92,8%), and with both lung affected in the 1st group, 63 (86,3%) cases. Lung infiltrates were complicated with destructions in two third of 1st group, 49 (67,1%), and only in a couple of patients from the 2nd group (*table 4*). This fact is due to the late detection of new TB cases in the 1st group and earlier detection in the 2nd group.

All patients were treated during intensive phase in the Municipal Hospital of Pneumophtysiology from Chisinau. By assessing the final treatment outcome, it was identified a similar rate of healed patients treated with standard regimen for new TB case in both groups, 30 (41,1%) cases in 1st group and 10 (35,7%) cases in 2nd group. As well as, completed the specific treatment a similar proportion of patients in both groups, 8 (10,9%) cases in 1st group comparing with 5 (17,85%) cases in the 2nd group. Died only 3 (4,1%) patients from the 1st group, comparing with

no registered deaths in the 2nd group. Almost one half of patients (13 (46,24%) from the 2nd group are still continuing an individualized regimen. A similar rate of patients from both groups is performing the treatment for MDR-TB.

Table 4

Microbiological, radiological features and treatment outcome

Characteristics		*SG, n=73*	*CG, n=28*	*P value*
		n (%)	*n (%)*	
Microbiological	Microscopic positive	38 (52.01)	10 (35,7)	<0,001
	Culture positive	40 (54,8)	11 (39,3)	>0,05
	GeneXpert MTB/Rif sensible	47 (64,4)	20 (71,4)	>0,05
	GeneXpert MTB/Rif resistant	26 (35,6)	8 (28,6)	>0,05
Radiological	1 lung involved	10 (13,7)	26 (92,8)	<0,001
	2 lungs involved	63 (86,3)	2 (7,14)	<0,001
	Lung destructions	49 (67,1)	2 (7,14)	<0,001
	Infiltrative TB	67 (91,8)	25 (89,2)	>0,05
	Disseminated TB	6 (8,2)	3 (10,7)	>0,05
Treatment outcome	Healed	30 (41,09)	10 (35,7)	>0,05
	Treatment completed	8 (10,9)	5 (17,85)	>0,05
	Dead	3 (4,1)	0	>0,05
	Continuing TB treatment (individualized regimen)	0	13 (46,24)	
	Continuing DOTS plus treatment	16 (21,9)	5 (17,8)	>0,05
	Hospitalized during intensive phase	73 (100)	28 (100)	>0,05

Conclusion

In Chisinau, primary health care providers identified only 101 new pulmonary TB cases with positive GeneXpert MTB/RIF assay from a total number of 213 cases, 78 cases as symptomatic patients and only 23 patients by active screening.

By distributing patients according to the sex, it was identified the predominance of men in comparison with women in both groups, but the male/female ratio was 2 times higher in the group detected by active screening.

All patients were originary from the Republic of Moldova. No homelessness were registered. Two third of patients were from urban area of Chisinau. Data confirm the inaccessible primary health care for immigrants, homessness and low accessibility for rural population.

Educational and civil status was similar in both groups of selected patients.

Economic status was higher in the group detected by active screening. By the other side the unemployment and rate of patients living under the poverty line was higher in the group of patients detected as symptomatic cases.

Two third of patients from both groups were uninsured, demonstrating their low accessibility to health care.

Chronic alcoholism, drug use, history of imprisonment, migration were established in a small number of cases from both groups. Comorbidities were identified more frequently in the symptomatic group of patients.

Close contact with infectious source, as well as HIV infection prevailed in the group detected as symptomatics, despite the fact that those patients might be detected by active screening.

High statistical difference between groups was established comparing microbiological and radiological features. Positive bacillary state, extensive process and lung destruction were more frequently in the group of symptomatics.

The same rate of successfully finished the treatment in both groups contrasted with death rate registered only in the groups of symptomatics.

References

1. Centrul Național de Management în Sănătate. Chișinău, 2015.
2. Rasanathan K., Sivasankara K., Jaramilllo E., et al. *The social determinants of health: key to global tuberculosis control*. In: Inter. Journal Tuberculosis Lung Diseases, 2011, p. 30-36.
3. Vilc V., Alexandru S., Crudu V., et al. *Controlul tuberculozei la nivelul asistenței medicale primare*. Chișinău, 2015.
4. World Health Organization. *Brief Guide on Tuberculosis Control for Primary Health Care Providers*. Geneva, 2004.
5. World Health Organization. *The global plan to stop TB 2011-2015: transforming the fight towards elimination of tuberculosis*. Geneva, 2011.
6. World Health Organization. *Systematic screening for active tuberculosis*. Geneva, 2013.
7. World Health Organization. *End TB Strategy*. Geneva, 2014.
8. World Health Organization. *Tuberculosis report 2016*. Geneva, 2016.

Evelina Lesnic, asist. univ.,
Catedra *Pneumoftiziologie*,
IP USMF *Nicolae Testemițanu*,
tel. 069883302
e-mail: evelina.lesnic@usmf.md