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The Armenian SORT IT Course

Change in TB diagnostic profile after introduction of GeneXpert MTB/RIF assay in National TB Program of Armenia, 2013-2017

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Abstract

Introduction: In 2013, the National Tuberculosis (TB) Program of Armenia introduced GeneXpert MTB/RIF (Xpert) assay to address World Health Organization (WHO) target of 80% (2020) of notified new and relapse TB cases to be tested with WHO recommended rapid diagnostic methods. This study aimed to assess the change in laboratory diagnostic profile of Mycobacterium tuberculosis after introduction of the Xpert assay from 2013 to 2017.

Methodology: Retrospective cohort analysis of all presumptive TB patients' records retrieved from the National Reference Laboratory database was performed.

Results: This study showed increased trend of Xpert coverage for suspected TB cases from 25% in 2013 to 86% in 2017 which is in line with WHO TB global strategy's target of 80% in 2020. In 4.7% cases, Xpert tested positive while microscopy showed negative results. There was also an improved detection of Rifampicin resistance with increased concordance from 99.1% to 99.4% and decreased discordance from 6.7% to 1.4% between culture and Xpert results.

Conclusion: Armenia has achieved the 2020 target; in terms of utilizing the GeneXpert it is on track to achieve the End TB strategy target of 100% by 2025. The next step of this research will be assessment of the impact of GeneXpert and other TB tests utilization on the treatment outcomes in Armenia.

Key words: Tuberculosis; geneXpert MTB/RIF; diagnosis; NRL; laboratory.

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Introduction

Tuberculosis (TB) continues to be a major public health concern worldwide. The elimination of TB requires early detection and treatment. Laboratory methods conventionally used for active TB detection are sputum smear microscopy and culture-based tests [1]. Microscopy with Ziehl-Nielsen (ZN) staining is the most common and often the only laboratory technique used to diagnose TB in most developing countries [2]. It is rapid and inexpensive to conduct, however, has low sensitivity and positive predictive value frequently leading to misdiagnoses.

Currently, the gold standard of diagnosing TB is the culture growing [3–5]. However, the use of this method is limited due to lack of trained staff, biosafety requirements and long turnaround time (from 8 to 43 days after inoculation) [6,7]. Therefore, it was vital to improve and possibly replace microscopy with simpler, more affordable and more accurate diagnostic methods. To address this challenge, new molecular methods were developed, including GeneXpert (Xpert) [8].

Xpert combines TB case detection with rifampicin (RMP) susceptibility testing within 2 hours. Several studies conducted under programmatic conditions have shown that drug susceptibility testing with Xpert has dramatically improved access to multidrug resistant (MDR) TB (MDR-TB) diagnosis, with the number of MDR cases detected increasing by up to eight-fold and a two-fold increase in the rate of bacteriologically confirmed TB [8,2]. To increase access to rapid TB detection and to achieve the goals of the End TB Strategy, World Health Organization (WHO) has set a target of 80% in 2020 of notified new and relapse TB cases to be tested with WHO recommended rapid diagnostics which also includes Xpert assay and to reach the target of 100% in 2025 [9,10].

Before 2013, the main methods of TB detection in Armenia were ZN microscopy, culture-based testing and molecular Line Probe Assay (LPA) introduced and fully implemented only in late 2011. WHO-endorsed molecular method – Xpert MTB/Rif was successfully introduced and implemented in the National TB Reference Laboratory (NRL) of Armenia starting from 2013. The aim of this study was to assess the change in laboratory diagnostic profile of *Mycobacterium tuberculosis* after introduction of the WHO endorsed Xpert MTB/RIF molecular assay in Armenia from 2013 to 2017.

The specific objectives of this study were: (1) to determine the proportions and time-trends of patients tested for Xpert, (2) to compare Xpert performance with microscopy; and (3) to compare Xpert RMP resistance with conventional drug susceptibility tests (DST). All of these were observed among the presumptive TB cases registered in the NRL during the 2013-2017 in Armenia.

Methodology

Study design

This is a retrospective observational study that analyzed TB cases detected by both the Xpert MTB/RIF assay and microscopy. The data is drawn from the electronic registry of NRL.

Study setting

Armenia is one of the 18 high burden TB countries in the European Region of the WHO. It is divided into 11 administrative areas with different geographical characteristics.

TB Laboratory Network of Armenia

The TB laboratory service in Armenia aims to diagnose TB cases and monitor the treatment. It includes 1 NRL and 24 TB Microscopy Laboratories distributed throughout the country and the prison system. The NRL is the only facility that conducts culture testing, molecular diagnosis of the first line and the second line drug resistance, as well as non-tuberculosis mycobacterium speciation tests (with LPA and Xpert MTB/Rif) and DST.

WHO-recommended rapid diagnostic tests for TB have been introduced in 2010 and 2013 for Genotype MTBDR*plus* and Xpert MTB/Rif respectively and are in use in the country [11]. Also, in line with the WHO recommendation to decentralize rapid diagnostic tests for early detection and treatment of TB cases and after the optimization of the regional TB microscopy laboratory network 11 Xpert II modules machines were installed in 2017 [12].

TB diagnosis is based on the approved National TB diagnostic algorithm and performed through smear microscopy, Xpert MTB/Rif for smear-negative cases and LPA-Genotype MTBDRplus v2.0 for smear-positive cases, confirmed by culture inoculated in both solid (Lowenstein-Jensen) and liquid (MGIT) media. Though, the National TB diagnostic algorithm was not yet been updated to reflect the WHO most recent recommendation to perform Xpert assay as the initial test for TB diagnosis regardless of smear microscopy results, the peripheral laboratories are already performing it on both smear-negative and smear positive cases [12]. In case of identifying of isoniazid and/or RMP resistant cases, testing is continued by Genotype MTBDRsl v2.0 kit to obtain resistance/sensitivity to fluoroquinolones and/or aminoglycosides/cyclic peptides. Study population/Participants and time periods

Data on all presumptive TB patients were collected and extracted to Microsoft Excel spreadsheet from from the NRL database for the period of 2013-2017.

Data variables collected

This included data on the following variables: year of testing, patient ID, gender and region of patient, microscopy results, Xpert results and Culture DST results of presumptive TB patients.

Analysis and statistics

Data cleaning and statistical analysis was conducted using the web-based statistical application Easystat available at https://easystat.app/.

Mainly descriptive statistics (means, medians, standard deviations, frequencies) was performed for this study.

Ethics Approval

The study was approved by the Center for Medical Genetics and Primary Health Care. Only secondary data was used for this study.

Results

General data

A total of 34746 samples were evaluated in final analysis which represented 17,896 cases. The duplicate entries were removed.

Among the presumptive TB cases registered in NRL from 2013 to 2017, 89% were males. In 88% cases, pulmonary specimen was used for laboratory analysis, in 8% extra-pulmonary and in 4% cases both

Table 1. Characteristics of presumptive TB cases registered in NRL and proportion of tested for GeneXpert for the period of 2013-2017.

Factor	Category	Presumptive TB patients registered N = 17896 *		Xpert MTB/RIF performed N = 10424 (58%) **	
Gender	Female	1745	(11)	1139	(65)
	Male	16150	(89)	9285	(58)
Region	Aragatsotn	708	(4)	471	(67)
	Ararat	1338	(7)	808	(60)
	Armavir	1851	(10)	1135	(61)
	Gegarkunik	1092	(6)	697	(64)
	Kotayk	1995	(11)	1234	(62)
	Lori	1086	(6)	492	(45)
	Shirak	1424	(8)	846	(59)
	Syunik	898	(5)	607	(68)
	Tavush	660	(4)	399	(60)
	Vayots dzor	201	(1)	103	(51)
	Yerevan	6069	(34)	3289	(54)
	NKR	397	(2)	243	(61)
	Other countries***	92	(1)	35	(38)
Age	< 16	518	(3)	336	(65)
	16-55	10767	(60)	6129	(57)
	> 55	6610	(37)	3959	(60)
Specimen	Pulmonary	15762	(88)	9059	(57)
	Extrapulmonary	1370	(8)	772	(56)
	Both	763	(4)	593	(78)

* All results are presented as n (%) unless otherwise is noted in the table; ** The proportion shows row percentages; *** This includes both foreigners and Armenian residents having foreign citizenship.

pulmonary and ex-pulmonary specimens were examined. The majority of presumptive TB cases were registered in Yerevan (34%), Kotayk (11%) and Armavir (10%). Cumulative coverage of the Xpert MTB/RIF for the time period of 2013-2017 was 58%. The highest Xpert coverage was in Syunik – 68% and the lowest in Lori - 45%. This study found that the age group with the highest TB infection rate was between 16 and 55 years. The age group of below 16 years had the least amount of people with TB infection. Detailed information on characteristics of presumptive TB cases registered in NRL and GeneXpert coverage is presented in the Table 1.

The time distribution and comparison of microscopy and GeneXpert results are shown in the Table 2. The coverage of microscopy increased from 93.3% in 2013 to 99.6% in 2017. There is a positive trend in Xpert coverage: in 2013 it was 25% increasing to 86% in 2017. Proportion of patients tested TB positive with GeneXpert testing increased from 6.1% in 2013 to 9.4% in 2017.

Performance of Xpert and Microscopy

In the Table 3 results of Xpert and microscopy and time trend are presented among those patients who had both tests. In 2013, 0.6% of the examined samples were microscopy and GeneXpert positive while in 2017 it increased to 3.7%, microscopy positive and GeneXpert negatives were 0.4% in both 2013 and 2017, microscopy negative and GeneXpert positive cases decreased from 6% in 2013 to 4.6% in 2017, and both tests results were negative in 93.0% of cases in 2013 and 91.3% in 2017.

Comparing culture vs Xpert rifampicin-resistance test results

The data of RMP resistance comparing culture versus Xpert test results is presented in the Table 4.

Table 2. Presumptive TB patient's microscopy and GeneXpert testing performance in Armenia over the years 2013-2017*.

Year	Presumptive TB patients N = 17895	Microscopy tested N = 17161	Microscopy AFB identified N = 924		Xpert tested N = 10424	Xpert MTBC identified N = 765	
2013	3331	3069 (93.3)	194	(6.3)	836 (25.1)	51	(6.1)
2014	3742	3573 (96.6)	159	(4.5)	1860 (49.7)	144	(7.7)
2015	3678	3541 (97.1)	160	(4.5)	2182 (59.3)	117	(5.4)
2016	3789	3654 (97.6)	203	(5.6)	2661 (70.2)	181	(6.8)
2017	3355	3324 (99.6)	208	(6.3)	2885 (86.0)	272	(9.4)

*All results are presented as n (%) unless otherwise is noted in the table.

Table 3. Comparison of the microscopy and GeneXpert performed specimens' results of presumptive TB patients from 2013 to 2017*.

	2013		2014		2015		2016		2017		Total	
$M^+ X^+$	5	(0.6)	65	(3.1)	20	(0.8)	46	(0.6)	133	(3.7)	269	(2.3)
$M^+ X^-$	3	(0.4)	7	(0.3)	13	(0.5)	11	(0.4)	13	(0.4)	47	(0.4)
$M^- X^+$	47	(6.0)	97	(4.6)	94	(3.9)	155	(5.1)	164	(4.6)	557	(4.7)
$M^- X^-$	726	(93.0)	1924	(91.9)	2311	(94.8)	2830	(93.0)	3261	(91.3)	11052	(92.7)
Total	**781**		**2093**		**2438**		**3042**		**3571**		**11925**	

*All results are presented as n (%) unless otherwise is noted in the table; M^+X^+ - Microscopy positive and Xpert MTBC identified; M^+X^- - Microscopy positive and Xpert MTBC not identified; M^-X^+ - Microscopy negative and Xpert MTBC identified; M^-X^- - Microscopy negative and Xpert MTBC not identified; There are the samples that have both smear and Xpert results only.

86.4% cases in 2013 and 78.4% in 2017 tested susceptible using culture and Xpert. Culture susceptible and GeneXpert resistant cases decreased from 4.5% in 2013 to 1.4% in 2017, Culture resistant and GeneXpert susceptible cases decreased from 4.5% in 2013 to 0.5% in 2017, and both tests results showed resistance of 4.5% in 2013 and 19.7% in 2017.

Discussion

This study shows that there is an increased trend of GeneXpert and microscopy coverage performed in Armenia from 2013 to 2017 which is consistent with WHO TB global strategy. In 2017, Armenia has already reached the WHO 2020 target for GeneXpert coverage. Though there is a positive trend in the proportion of patients tested TB positive with Xpert assay (from 6.1% in 2013 to 9.4% in 2017) still 9 out of 10 presumptive TB cases are tested TB negative with Xpert assay. Hence, the National TB Control Center should continue efforts in implementation of proper case finding algorithms to ensure the further increase in the proportion of patients tested TB positive with Xpert assay and ultimately its cost effectiveness.

This study found that TB infection is not evenly distributed throughout provinces of Armenia, with males considerably more infected than females, which may be associated with the high rates of labor migration of males to TB high-burden countries. Yerevan which is a densely populated capital city had the highest number of presumptive TB cases, whereas Vayots Dzor had the lowest numbers of TB cases.

There is a steady positive trend in Xpert coverage. It increased from 25% in 2013 to 86% in 2017 (Figure 1) reaching the WHO target of 80% for 2020 [9]. Proportion of patients tested TB positive with Xpert also increased, which may evidence to improvement of the laboratory performance (Table 2). Comparison of Xpert and microscopy results showed good concordance of the tests results. Only 0.4% of cases tested positive for microscopy and negative for Xpert which mainly described by the presence of non-tuberculosis mycobacteria in the specimen.

Due to wider use of Xpert there is an improved detection of RMP resistance among the presumptive TB cases. Both tests discordance decreased from 6.7% in 2014 to 1.4% in 2017 which shows improved laboratory performance. There is increase of cases when both tests showed resistance due to increased Xpert coverage. That has tremendous implication for early detection and

Figure 1. Presumptive TB patient's microscopy and GeneXpert testing performance in Armenia over the years 2013-2017.

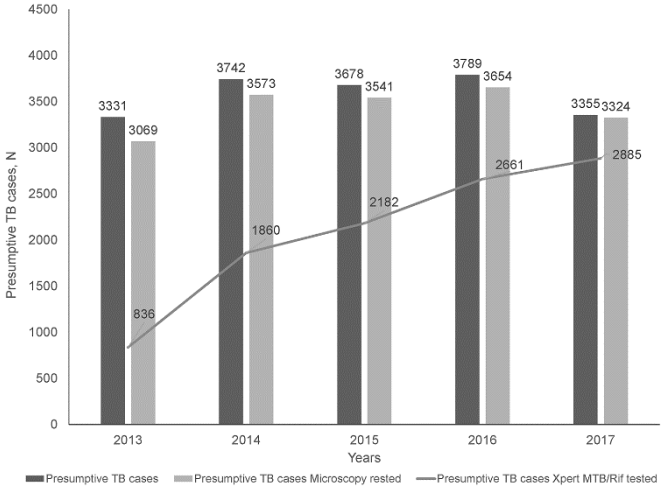

Table 4. Comparison of the culture and Xpert Rifampicin resistance specimens' results of presumptive TB patients from 2013 to 2017.

	2013	2014	2015	2016	2017	Total
$C^S X^S$	19 (86.4)	116 (77.3)	90 (81.1)	114 (79.7)	167 (78.4)	506 (79.2)
$C^S X^R$	1 (4.5)	10 (6.7)	5 (4.5)	5 (3.5)	3 (1.4)	24 (3.8)
$C^R X^S$	1 (4.5)	0 (0)	1 (0.9)	1 (0.7)	1 (0.5)	4 (0.6)
$C^R X^R$	1 (4.5)	24 (16.0)	15 (13.5)	23 (16.1)	42 (19.7)	105 (16.4)
Total	**22**	**150**	**111**	**143**	**213**	**639**

*All results are presented as n (%) unless otherwise is noted in the table; $C^S X^S$ - Culture R Susceptible and GeneXpert R Susceptible; $C^S X^R$ - Culture R Susceptible and GeneXpert R Resistant; $C^R X^S$ - Culture R Resistant and GeneXpert R Susceptible; $C^R X^R$ - Culture R Resistant and GeneXpert R Resistant.

treatment of MDR cases, which directly impacts the treatment outcome.

The strengths of the study included the large sample size with all presumptive TB cases extracted from NRL database from 2013 to 2017. Also, this was the first national level review of the program after the introduction of Xpert in Armenia.

The limitation of the study was the fact that not all patients' specimen reached the NRL because of the low quality of samples and almost half of TB cases were diagnosed clinically. Other limitation of the study was the fact that microscopy negative specimens were predominantly tested with GeneXpert as per the current TB laboratory algorithm. However, Xpert is now recommended as the initial test for TB diagnosis regardless of smear microscopy results [13] and Armenia is in the process of updating the National TB Diagnostic Algorithm accordingly.

Xpert® MTB/RIF assay provides accurate results in a short time period (2 hours) and is more affordable than culture. Thus, it is more appropriate for use in low- and middle-income countries [14]. Our study supports the findings from other studies directed to evaluate performance of Xpert and to compare its results with microscopy. Particularly Mavenyengwa *et al*. [1] indicated that smear microscopy detected TB in fewer patients compared with Xpert® MTB/RIF assay. Smear microscopy might miss specimens with low bacilli/ml thus Xpert coverage needs to become universal [15].

Conclusion

The findings of the study further strengthen high importance of wide use of rapid diagnostic techniques for early detection and treatment of MTB. Armenia has achieved the 2020 target and is on track to achieve the End TB strategy target of 100% by 2025. The National TB Program needs to strive for universal GeneXpert coverage which is especially critical for early detection and treatment of MDR and extremely drug resistant cases. Also, there are scarce data on impact of rapid tests utilization on patients' treatments outcome. The next step should be evaluation of the use of different TB tests on the treatment outcomes in Armenia.

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