

Performance of real-time PCR Xpert[®] MTB/RIF in diagnosing extrapulmonary tuberculosis

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SUMMARY

The real time PCR Xpert[®] MTB/RIF is fundamental for rapid diagnosis in paucibacillary respiratory samples and for the detection of multidrug-resistant TB cases. This paper aimed to determine its performance on different extrapulmonary samples. We determined sensitivity, specificity, positive and negative predictive value on respiratory and non-respiratory samples collected from January 2010 to June 2014. The protocol for the Xpert[®] MTB/RIF PCR suggested by Cepheid was strictly followed for all specimens. In 12257 respiratory samples we observed a sensitivity of 87.1% and a specificity of 99.9%. There were 2818 extrapulmonary specimens, of which 250 were followed by a positive culture for *Mycobacterium tuberculosis* complex, whereas 72 samples were cul-

ture-negative: tuberculosis was clinically confirmed in 71 of them and was excluded for one sample. The sensitivity of the test on urine, pus and CSF samples was 88.2%, 95.6% and 100% respectively. In contrast, the sensitivity of gastric aspirates and biopsies was 81.8% and 83.6% respectively, whereas results of total cavitory fluids were significantly worse than expected (53.7% sensitivity). Our experience shows that Xpert MTB/RIF assay is an accurate, sensitive, and specific test for the rapid detection of pulmonary and extra-pulmonary TB with the only exception of cavitory fluids.

Keywords: Xpert MTB/RIF, PCR, Real Time, diagnosis, extrapulmonary tuberculosis.

INTRODUCTION

Mycobacterium tuberculosis is still one of the most serious diseases caused by an infectious agent. It remains a great challenge the timely and accurate diagnosis of pulmonary (PTB) and extra-pulmonary tuberculosis (EPTB), especially in cases of paucibacillary disease [1-4]. Traditionally, the culture of mycobacteria and the phenotypic test of sensitivity to anti-tuberculosis drugs is necessary for the laboratory diagnosis of tuberculosis (TB). This approach depends by the laboratory equipment that must be relatively advanced, high labor-intensive, and requires also

2-3 months before results are available. Early diagnosis of active TB is essential to limit mortality and disease transmission [5].

The World Health Organization (WHO) has endorsed GeneXpert for use in patients with suspected pulmonary TB in areas where HIV incidence is high or drug resistance present. It has been quoted as having similar sensitivity and specificity to conventional testing methods, such as TB culture [6-9].

In the last 10 years, the non-respiratory forms represent between 10% and 42% of the TB manifestations, with a greater proportion among children and patients with immunodeficiency by HIV infection. The diagnosis is strongly influenced in extrapulmonary samples from atypical presentation and low bacterial load, where the disease may occur silent even for a long period of time [10,11]. Various gene targets such as *IS6110*, 16S rRNA

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gene, 65 kDa protein gene, MPB-64/MPT-64 protein gene, 38 kDa protein gene, *TRC4* (conserved repetitive element), *GCRS* (guanine-cytosine-rich repetitive sequence), *hupB*, *dnaJ*, MTP-40 protein gene and PPE gene have been employed in these PCR assays [12, 13].

The self-contained and automated GeneXpert MTB/RIF nucleic acid amplification test (NAAT) represents a significant advancement for the laboratory diagnosis of tuberculosis and for the detection of rifampicin resistance [14, 15]. Recent studies have evaluated the performance of Xpert assay in the diagnosis of PTB and EPTB even in countries with medium or low burden, using a culture-based reference standard to develop shared guidelines on use of Xpert [16-29].

The aim of this study was to investigate the accuracy of GeneXpert in diagnosing extrapulmonary tuberculosis.

■ MATERIAL AND METHODS

This retrospective study was carried out into two Italian Microbiology laboratories: at Niguarda Ca' Granda, Milan hospital and Papa Giovanni XXIII, Bergamo hospital. Both are accredited by the Italian Ministry of Health for laboratory diagnosis. Xpert system was in use from 4 and 2 years respectively.

We analysed all consecutive results on respiratory and non-respiratory samples from January 2010 to June 2014. The pulmonary samples were sputa, bronchial lavages and tracheal-bronchial aspirates. Many specimen representative of each form of EPTB were considered, such as biopsies, gastric aspirates and urines. Body fluids other than cerebrospinal fluid (CSF) were classified as total cavitory fluids (*i.e.* pleural fluid, peritoneal fluid, ascitic fluid, pericardic fluid, sinovial fluid and drainage fluid).

Respiratory samples and non-sterile non-respiratory samples were routinely decontaminated with NALC NaOH 3% standard protocol and concentrated before the test. The sterile specimens were homogenized (if needed) before resuspension and concentration. The protocol for the Xpert[®]MTB/RIF PCR suggested by Cepheid (Sunnyvale, CA, USA) was strictly followed for the all specimens: 0.5mL of concentrated sample was added to 1.5mL Xpert[®]MTB/RIF sample reagent,

after 15 minutes it was put into the cartridge for the analysis on GenExpert[®] system.

For all samples we performed acid-fast microscopy with auramine staining, liquid culture with MGIT 960 (Becton Dickinson Biosciences, Sparks, MD, USA) and solid culture: Lowenstein-Jensen (Becton Dickinson Biosciences, Sparks, MD, USA) at Niguarda Ca'Granda hospital and International Union Tuberculosis Medium – IUTM (Liofilchem s.r.l, Italy) at Papa Giovanni XXIII hospital. The isolated mycobacteria were identified using the probe assay GenoType[®] Mycobacterium CM (HAIN lifescience GmbH).

We compared retrospectively the results of Xpert[®]MTB/RIF obtained on pulmonary (12725) and extrapulmonary (2856) clinical specimen with culture results and clinical data. The samples, whose culture was contaminated by not alcohol-acid resistant bacteria, were 499 (38 extrapulmonary and 461 pulmonary), 3.2 %, were eliminated from the analysis, whereas for samples, which were PCR-positive and culture negative, we evaluated medical records and epidemiologic register for clinical diagnosis of TB. In our region there is an epidemiologic register for infectious diseases, where every patient is classified as suspected or confirmed case for TB following the European guidelines 28/IV/2008, which are based both on laboratory results and on clinical data. We considered a patient positive for TB, when is registered as a confirmed case. For 7 patients, clinical data were not available, thus they were eliminated from the analysis.

The statistical analysis evaluated sensitivity, specificity, negative and positive likelihood ratios (LR) and negative and positive predictive values.

■ RESULTS

From January 2010 and June 2014, we investigated with Xpert MTB/RIF 12257 (81,7%) respiratory and 2818 (18.3%) extrapulmonary specimens. Respiratory samples were 12257 for 8780 patients; whereas extrapulmonary specimens were 2818 for 2219 patients. Globally we performed 15075 tests for 10477 patients and 522 of them were examined both on respiratory and extrapulmonary samples. We compared retrospectively the Xpert results with culture results and medical records for clinical diagnosis of TB.

Among 12257 respiratory samples, 844 (6.9%) scored positive for DNA amplification. As a matter of fact, 717 samples were also culture positive for MTC, whereas 123 were followed by a negative culture results and were clinically confirmed TB cases. Finally, 4 samples were culture negative but were not clinically confirmed for tuberculosis, therefore they were considered false positive results for Xpert MTB/RIF (Table 1).

In respiratory samples we observed a sensitivity and a specificity of 87,1% and 99,9%, respectively, with a negative likelihood ratio of 0,13 (Table 2).

The extrapulmonary specimens were 2818: gastric aspirates 375 (13.3%), biopsies 744 (26.4%), CSF 160 (5.7%), total cavitory fluids 968 (34.4%), urines 235 (8.3%) and pus 336 (11.9%): 323 samples were positive for DNA amplification. Among them, 250 were followed by a positive culture for MTC, whereas 72 samples were culture negative; TB was clinically confirmed in all 71 of them and for 1 sample it was excluded. We identified also 68 culture positive for Nontuberculous Mycobacteria (MNT), which were all PCR negative as expected (Table 1).

There were many differences in the results of the extrapulmonary specimens. The sensitivity of urine, pus and CSF was 88.2%, 95.6% and 100% respectively, the negative likelihood ratios (LR-) were 0.12, 0.04 and 0 respectively (Table 2). There-

fore, the performance of Xpert MTB/RIF on this specimens is comparable to the one obtained on respiratory samples.

On the other hand, the sensitivity of gastric aspirates and biopsies was 81,8% and 83,6% and LR- was 0.18 and 0.16 respectively. On the contrary, the total cavitory fluids results were significantly different from the other specimens, i.e. the performance of Xpert MTB/RIF was worst than expected. As a matter of fact, the sensitivity was 53.7% and the LR- was 0.46, in particular during the analysis we noticed that the results of pleural fluids alone were 38.1% and 0.62 respectively, whereas the ones for the other cavitory fluids (*i.e.* the remaining fluids: peritoneal, ascitic, pericardic, sinovial and drainage fluid) were 70% and 0.3 (Table 2).

Because of the very low prevalence in our country, in all specimens the negative predictive values scored more than 96%. Moreover, the results of positive predicted values were even better scoring always more than 98%.

The time needed to perform a PCR Xpert MTB/RIF testing is fundamental also for the detection of multidrug-resistant TB cases as WHO recommends. We detected 15 respiratory and 6 extrapulmonary samples carrying an *rpoB* mutation thanks to this system, that were confirmed by antibiotic susceptibility testing and by genotypic

Table 1 - Sample distribution for materials, PCR results and clinical diagnosis.

Sample type	Respiratory specimen	Extrapulmonary specimen	Gastric aspirate	Total cavitory fluid	LCR	Biopsy	Urine	Pus
GeneXpert positive and MTC positive culture	717 5.8%	250 8.9%	39 10.4%	18 1.9%	7 4.4%	107 14.4%	15 6.4%	64 19%
GeneXpert negative and MTC positive culture	124 1.0%	63 2.2%	12 3.2%	19 2%	0	26 3.5%	2 0.8%	4 1.2%
GeneXpert positive, MTC negative culture and TB diagnosis	123 1.0%	72 2.5%	15 4%	4 0.4%	3 1.9%	26 3.5%	0	24 7.1%
GeneXpert positive, MTC negative culture and no TB diagnosis	4 0.1%	1 0.1%	1 0.3%	0	0	0	0	0
GeneXpert negative, NTM positive culture	458 3.7%	68 2.4%	19 5.1%	3 0.3%	0	39 5.2%	0	7 2.1%
GeneXpert negative and culture negative	10831 88.4%	2364 83.9%	289 77.0%	924 95.4%	150 93.7%	546 73.4%	218 92.8%	237 70.6%
Total	12257	2818	375	968	160	744	235	336

Table 2 - Sample distribution for materials and statistical features.

Sample type (n°)	Respiratory specimen (12257)	Extrapulmonary specimen (2818)	Gastric aspirate (375)	CSF (160)	Biopsy (744)	Urine (235)	Pus (336)	Total cavitory fluid (968)	Only pleural fluid (714)	Other cavitory fluid (254)
Sensitivity %	87.1	83.6	81.8	100	83.6	88.2	95.6	53.6	38	70
Specificity %	99.9	99.9	99.7	100	100	100	100	100	100	100
Positive LR	2460.1	2035.1	252.8	∞	∞	∞	∞	∞	∞	∞
Negative LR	0.13	0.16	0.18	0	0.16	0.12	0.04	0.46	0.62	0.3
PPV %	99.5	99.7	98.2	100	100	100	100	100	100	100
NPV %	98.9	97.5	96.2	100	95.7	99.1	98.4	98	98.1	97.5

mutations. In addition there were no samples, in those Xpert did not identify a mutation on *rpoB* gene, which were resistant to rifampicine during the susceptibility testing.

■ DISCUSSION

TB remains a serious public health problem. In children as in adults, TB continues to be considered a challenge for the rapid and accurate diagnosis of pulmonary and extrapulmonary presentations. Ineffective TB detection and transmission of drug-resistant TB strains endanger TB control activities [6].

Currently, the access to molecular tests for sensitivity to anti-tuberculosis drugs is limited because of high costs and insufficient resources. In recent years, new tests were commercialized, able to improve the diagnosis of TB and also more quickly to detect the drug resistances [15].

Several papers show the performance and the utility of PCR for early diagnosis of EPTB and also a significant difference in PCR results due to several target genes, such as the gold standard utilized in different laboratories [23, 24].

In many cases suspect of EPTB, when the traditional microbiological tests fail, the PCR results together with the clinical presentation and/or histopathology can be extremely useful to set the anti-tuberculosis therapy [13].

Some of these assays have been approved by the WHO and one of these, Xpert MTB/RIF assay was approved by the Food and Drug Administration (FDA) in the United States in June 2013 [8].

Without doubt, the most significant advantage of GeneXpert tests is the speed with which the results become available. The automated process

of the GeneXpert employs only about 90 minutes to complete the reaction. Also, compared with other PCR testing methods, the automated, cartridge-based test obviates the need for extensive laboratory support. The GeneXpert is considered a test which can be used also in smaller hospitals and clinics [10].

One of the disadvantages of PCR testing, also in GeneXpert, is that, in contrast to TB culture, it will give a positive result even if the pathogens are not viable. In our patients, active TB has to be confirmed clinically and by means of various imaging modalities. Another disadvantage is that the GeneXpert notices the drug resistance only for rifampicin, therefore a mono-resistance for isoniazid can be not detected if not by other PCR testing methods, such as GenoType MTBDRplus [13].

In our paper, we have carefully evaluated the literature on the diagnostic accuracy of the GeneXpert MTB/RIF assay when used to test non-respiratory samples, specially Maynard-Smith systematic review [23].

Our data show a very high specificity as in most studies, confirming its performance and usefulness in diagnosing TB so to inform timely and accurately clinical colleagues when the samples for the detection of *Mycobacterium tuberculosis* complex are positive. In contrast, sensitivity is extremely heterogeneous, with much higher values, typically for CSF samples, pus, urine and lymph node biopsies compared to results of cavitory and pleural fluids.

The paucity of mycobacteria in samples and not so much to inhibitory factors encountered in the PCR can explain the reduced sensitivity of the MTB/RIF assay also if soluble PCR inhibitors such as heme, bilirubin, and bile salts in tissue and stool are well known. These PCR inhibitors

interfere with the DNA polymerase and decrease the sensitivity of PCR-based NAATs. It is conceivable that some inhibitors get into the reaction vial although the Xpert system removes many substances during the wash and filtration steps [30]. These findings strongly support the recently released WHO recommendations for the use of GeneXpert MTB/RIF for TB diagnosis by testing CSF and tissue samples [15].

A good sensitivity was also observed when testing gastric aspirate, highlighting a possible new opportunity in the diagnosis of sputum-scarce pulmonary TB, which is particularly useful in children. Other potential sources of heterogeneity in sensitivity include small samples sizes, patient age and sample processing methodology.

These data suggest that Xpert MTB/RIF assay is an accurate, sensitive, and specific test for the rapid detection of pulmonary end extra-pulmonary TB. The closed cartridge GeneXpert is considered for its performance and simplicity a valid diagnostic test for routine in reference TB laboratories also in countries with low or intermediate incidence, and may absolutely support in limiting the diffusion of TB in such settings [7, 20]. As a matter of fact, GeneXpert represents a significant advancement for the laboratory diagnosis of TB especially for its low turn around time. Therefore, it's possible to give clinicians results in less than 24 hours. Even if it is more costly relative to other products, GeneXpert also gives information on rpoB mutation in the same analysis.

Further studies are required to support our data and what has already been published in medical literature for the use of Xpert MTB/RIF in the diagnosis of EPTB from extrapulmonary samples in a large range of TB patients.

In conclusion, the authors suggest the opportunity to perform the Xpert MTB/RIF also on non-respiratory samples in EPTB patients with adding a note on the medical report, which indicates the values of PPV and NPV of molecular method in use.

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