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ORIGINAL ARTICLE

Evaluation of Xpert MTB/RIF Ultra performance for pulmonary tuberculosis diagnosis on smear-negative respiratory samples in a French centre

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Abstract

Tuberculosis (TB) is a worldwide public health concern, including in high-resource countries with a low prevalence of TB. Xpert MTB/RIF assay was developed to improve TB and rifampicin (RIF) resistance detection, but sensitivity remains poor on smear-negative sputum. Xpert MTB/RIF Ultra assay was designed to enhance the sensitivity of TB detection in clinical samples. Herein, we evaluated retrospectively the performance of this test on smear-negative respiratory samples. Respiratory specimens with smear-negative and a *Mycobacterium tuberculosis* (MTB) complex-positive culture were retrospectively selected from those taken from patients during routine care, and analysed in the Mycobacteria Laboratory of the Lyon University hospital, France. Specimens were stored at − 20 °C before testing by Xpert MTB/RIF Ultra. For each sample, growth delay and date of anti-TB treatment initiation were recorded. Forty-six samples—29 sputum, 8 bronchial aspirates, 6 broncho-alveolar lavages, and 3 gastric aspirates—were selected. Among samples collected before treatment initiation ($n = 33$), sensitivity was 81.8% (95% CI [64.5; 93.0]) and there was a significant correlation between the quantitative measurements (Ct) of Xpert MTB/RIF Ultra assay and the time to growth detection in culture. Among samples collected after treatment initiation ($n = 12$), sensitivity was 100%, without correlation with time to growth detection due to presence of afterglow DNA in samples. In high-resource settings, the Xpert MTB/RIF Ultra test represents a useful tool for pulmonary TB diagnosis, notably for the paucibacillary forms. Moreover, quantitative measurement of Xpert MTB/RIF Ultra could help to predict time to MTB culture positivity and be used as a quality indicator of MTB culture process.

Keywords Xpert MTB/RIF Ultra · Pulmonary tuberculosis · Diagnosis · High-resource setting · Smear-negative

Background

Tuberculosis (TB) is a worldwide public health concern, including in high-resource countries with a low TB prevalence [1]. Bacteria culture is considered as the gold standard method for *Mycobacterium tuberculosis* (MTB) infection diagnosis and is required to isolate strain for drug sensitivity testing [2]. Nevertheless, the Centers for Disease Control and Prevention recommend using at least one molecular technique per patient for MTB detection [3], notably for smear-negative samples for which nucleic acid amplification (NAA) tests allow a rapid confirmation of MTB infection diagnosis compared to culture. Thus, World Health Organization (WHO) guidelines recommend Xpert MTB/RIF assay use to improve TB and rifampicin (RIF) resistance detection for pulmonary and extra-pulmonary TB diagnosis in adults and children [4]. Nevertheless, the sensitivity remains poor when bacilli load is very low, for example, on smear-negative sputum (reported to range from 28% [5] to 67% [6]), in HIV-infected patients (61%) [6], in children (63%) [7], or for TB meningitis (55.1%) [8]. The Xpert MTB/RIF Ultra assay was designed to enhance the sensitivity of TB detection in clinical samples [9]. Recently, the WHO recommendations include the Xpert MTB/RIF Ultra assay use for the initial diagnosis of

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pulmonary and extra-pulmonary TB [10]. To date, several studies reporting improvement of pulmonary and extra-pulmonary TB diagnosis using Xpert MTB/RIF Ultra assay compared to smear staining, culture, and Xpert MTB/RIF assay were conducted in countries with a high TB prevalence [9, 11–13] or a low TB prevalence [14, 15]. Herein, we evaluated retrospectively the performance of Xpert MTB/RIF Ultra assay on smear-negative respiratory samples compared to culture, in a high-resource setting with a low TB prevalence.

Methods

This study was in accordance with the ethics committee of the Lyon University hospital, France (declared sample collection: DC-2011-1306).

Specimens for the present study were retrospectively selected from those taken from patients during routine care, and analysed in the Mycobacteria Laboratory of the Lyon University hospital, France. Respiratory specimens, including sputum, bronchial aspirates, broncho-alveolar lavages, and gastric aspirates, were collected between March and October 2017 and were selected with negative smear (one smear observed) and *Mycobacterium tuberculosis* complex-positive culture with different time to growth (from 7 to 30 days).

After treatment with the modified Kubica's digestion-decontamination method (*N*-acetyl-L-cysteine–2% NaOH (sodium hydroxide)) [16], sample pellets were re-suspended in a final volume of 2 mL. Smear staining was performed using the acridine orange method [17] (with 50 μL of samples) and MTB cultures were performed by inoculation of 500 μL of samples in mycobacteria growth indicator tubes (MGIT) and using the BACTEC 960® instrument, (Becton Dickinson, Sparks, MD, USA). The remaining samples (around 1 mL) were stored at − 20 °C before testing by the Xpert MTB/RIF Ultra assay (Cepheid, Sunnyvale, CA, USA), according to the manufacturer's recommendations. Semi-quantitative measurements "High", "Medium", Low", "Very Low", and "Trace" of the Xpert MTB/RIF Ultra assay and the Ct (threshold cycle) for the target IS1081-IS6110 were recorded.

The time to MGIT growth detection was recorded for each sample and specimens were categorised as follows: 7 to 13 days, 14 to 21 days, and > 21 days. The time at which anti-TB treatment was initiated was also recorded to categorise specimens as before or after anti-TB treatment initiation. Drug susceptibility testing (DST) was performed using the MGIT AST SIRE system and the BACTEC 960® instrument (Becton Dickinson), according to manufacturer instructions [18].

Sensitivity was calculated using the positive MTB culture samples as gold standard. Fisher's exact test was used to test the independence between the time to growth detection and Xpert MTB/RIF Ultra semi-quantitative assessment, and Spearman's correlation test was performed to explore the correlation between the time to growth and the quantitative measurement of DNA quantity of MTB, given by the Ct of IS1081-IS6110. Statistical analyses were performed using RStudio, version 0.99.893 (RStudio Team (2009–2016), RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, USA).

Results

Forty-six respiratory specimens, corresponding to 33 patients with pulmonary TB, were selected. There were 29 sputum, 8 bronchial aspirates, 6 broncho-alveolar lavages, and 3 gastric aspirates. One specimen gave an "error" result with the Xpert MTB/RIF Ultra assay and was therefore excluded from the study. Among the 45 specimens analysed, 33 (corresponding to 26 patients) were collected before initiation of the anti-TB treatment, with 9 samples with MGIT growth delay from 7 to 13 days, 11 samples from 14 to 20 days, and 13 samples with late growth up to 21 days. Twelve samples were collected after initiation of anti-TB treatment (between 11 and 75 days, median 24.5 days). All isolates were RIF-susceptible.

For evaluation of Xpert MTB/RIF Ultra sensitivity, only specimens collected before anti-TB treatment initiation were included ($n = 33$). Results are presented in Table 1 and the sensitivity was 81.8% (95% CI [64.5; 93.0]).

All sample collected after anti-TB treatment ($n = 12$) displayed a positive result for MTB detection (with 8, 2, 1, 1 samples with a "Medium", "Low", "Very Low", and "Trace" semi-quantitative measurements respectively).

For the samples collected before treatment initiation, both variables, the semi-quantitative measurements of Xpert MTB/RIF Ultra assay and the time to growth detection with the BACTEC MGIT system, were not independent ($p = 0.009$, Fisher's exact test). Moreover, the time to growth was significantly positively correlated with the Ct of IS1081-IS6110 (Spearman's correlation test, $p < 0.05$) (Fig. 1). On the contrary, for the samples collected after treatment initiation, the time to MGIT growth detection and the semi-quantitative assessments of Xpert MTB/RIF Ultra assay were independent ($p > 0.05$, Fisher's exact test) and there was no significant correlation with the Ct of IS1081-IS6110 ($p > 0.05$, Spearman's correlation test).

Data on the RIF susceptibility status as detected by Xpert MTB/RIF Ultra were only available for 62% (24/39) of specimens with positive MTB detection. Among the 15 specimens with undetermined status for RIF susceptibility, 12 yielded a "Trace" semi-quantitative MTB result, and 2 a "Medium" and 1 a "Very Low" semi-quantitative MTB result. When available, the RIF susceptibility status was fully concordant with the DST results.

Table 1 Results of Xpert MTB/RIF Ultra for the 33 specimens collected before anti-TB treatment according to growth delay MGIT

		Xpert MTB/RIF Ultra results						Total
		Positive					Negative	
		High	Medium	Low	Very Low	Trace		
Growth delay MGIT BACTEC 960 (days)	7–13	1	5	0	2	1	0	9
	14–20	0	1	0	3	4	3	11
	> 21	0	0	3	1	6	3	13
	Total	1	6	3	6	11	6	33

Discussion

Detection of MTB DNA in the Xpert MTB/RIF Ultra assay is based on amplification of IS6110 and IS1081 genes that are present as multiple copies in the TB genome, allowing better sensitivity than that in the Xpert MTB/RIF assay that is based on the amplification of the simple copy *rpob* gene [9]. Although our study collected small number of samples, the sensitivity of the Xpert MTB/RIF Ultra found herein was similar to that reported by Dorman et al. (63%, 95% CI [54; 71]) on smear-negative pulmonary samples [12]. Interestingly, we found that the semi-quantitative and quantitative measurements of the sample MTB DNA load by Xpert MTB/RIF Ultra assay were related to the time to MGIT growth detection, indicating that these measurements were correlated with viable MTB load in samples. This correlation may be interesting in several respects. Firstly, monitoring the adequacy between the sample's MTB DNA load (provided by the result of the Xpert MTB/RIF Ultra) and the delay to culture positivity could be used as a quality indicator of the whole MTB culture process in clinical laboratory, including the delicate step of sample decontamination. Secondly, assuming that this correlation is maintained for positive smear sputum as already reported for Xpert MTB/RIF [19], semi-quantitative or quantitative measurement results of Xpert MTB/RIF Ultra could be used to assess the contagiousness of patient. However, further studies are necessary to elucidate the transmission rate of pulmonary TB patients based on Xpert MTB/RIF Ultra Ct categories.

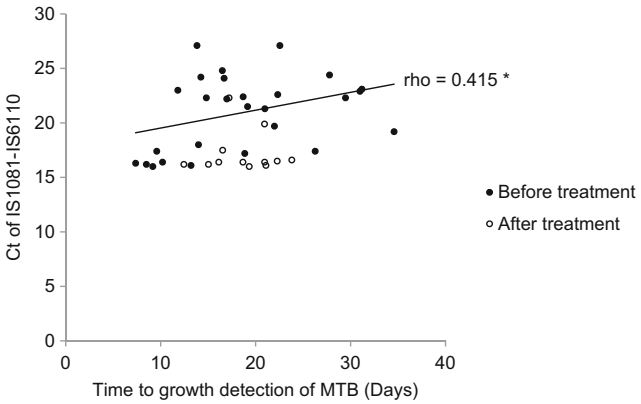

Fig. 1 Representation of the time to MTB growth detection according to the quantitative measurement of DNA in specimens. Filled circles indicate specimens collected before antibiotic treatment ($n = 33$); empty circles indicate specimens collected after antibiotic treatment ($n = 12$). Spearman's correlation test, $*p < 0.05$

In our study, specificity of Xpert MTB/RIF Ultra was not directly evaluated, since only samples with MTB-positive cultures were tested. Several authors report poorer specificity when Xpert MTB/RIF Ultra is used to test subjects with a recent history of TB due to the presence of persistent dead bacilli DNA because this assay is capable of detecting very low amounts of MTB DNA [9, 12, 20]. This induces a potential risk to overtreatment, notably in lower prevalence settings [21]. In accordance with a poor specificity due to the presence of persistent dead bacilli DNA, we found herein that the time to MGIT growth detection was not correlated with the semi-quantitative or quantitative measurement of Xpert MTB/RIF Ultra, because of the detection of additional DNA released by killed bacilli leading to overestimation of the bacteria load.

It is difficult to discuss the performance of the Xpert MTB/RIF Ultra test for RIF resistance status detection as only susceptible isolates were identified; the only comment possible is that there were no false-positive RIF resistance results. The proportion of interpretable RIF susceptibility results was, however, far below that reported by Chakravorty et al. (93.5%; 187/200) [9] and Dorman et al. (86%; 588/684) [12], probably explained by the choice of specimens (only smear-negative specimens in the present study). The majority of uninterpretable RIF susceptibility results were categorised as "Trace", (i.e. there were IS1081-IS6110 genes, but no *rpob* amplification [9], including the targets rpoB1, rpoB2, rpoB3, and rpoB4), corresponding to paucibacillary specimens, and precluding susceptibility status determination. Moreover, 3 specimens ("Medium" or "Very Low") also gave an undetermined status for RIF susceptibility. For 1 of the "Medium" specimens, the culture yielded a mixture of MTB and 1 nontuberculous mycobacteria (NTM; *Mycobacterium avium*) which could have interfered with an accurate *rpob* molecular

beacon hybridization, though Chakravorty et al. indicate that NTM does not significantly interfere with RIF susceptibility status [9]. For the second specimen detected "Medium", undetermined status for RIF susceptibility could be due to the anti-TB treatment, which might have altered *rpob* gene integrity. Finally, for the last specimen categorised "Very Low", only part of *rpob* gene (target rpoB4) did not amplify because of an insufficient amount of DNA.

Conclusions

Taken together, the results indicate that the new Xpert MTB/RIF Ultra test constitutes a useful tool for pulmonary TB diagnosis in high-resource settings with a low TB prevalence, notably for paucibacillary smear-negative sputum. This test allows a significant time saving in pulmonary TB diagnosis, since it detects low-burden MTB samples otherwise yielding late positive cultures (up to 3 weeks). Moreover, monitoring of the correlation between the MTB DNA load measured by Xpert MTB/RIF Ultra and the time to MGIT culture positivity could serve as an additional quality indicator of the whole MTB culture process.

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Author's contribution EH, CG, IF, JPR, GL, and OD designed the experiments. EH, AM, LC, and CB performed the experiments. EH, GL, and OD wrote the manuscript.

Compliance with ethical standards

This study was in accordance with the ethics committee of the Lyon University hospital, France (declared sample collection: DC-2011-1306). In accordance with French legislation, written informed patient consent was not required to compare technical performance of assays with clinical specimens collected following clinical recommendation.

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