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The value of xpert MTB/RIF-generated CT values for predicting the smear status of patients with pulmonary tuberculosis

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ABSTRACT

Background: Smear microscopy is used to assess the patient's infectiousness at the time of initial diagnosis of pulmonary tuberculosis. However, its limited sensitivity and specificity highlights the need for new diagnostic strategies. The aim of our study was to assess the diagnostic accuracy of GX Ct value as a predictor of smear status and its usefulness to quantify mycobacterial load.

Methods: All GX-positive sputum samples during a seven-year period were included in the study. Correlations among Ct values, smear status and TTD on liquid culture were calculated. An optimal Ct value for ruling in infectious patients was established. Clinical and radiological variables were also analyzed.

Results: Sixty-eight samples from 65 patients were included. Ct value and TTD yielded a positive correlation ($\rho = 0.714$; $p < 0.05$), while Ct and smear grade yielded an inverse correlation ($r = -0.71$). An optimal Ct value for ruling in smear positive patients was established at 21.1 cycles (90.5% sensitivity, 61% specificity, 81% PPV and 78% NPV).

Conclusions: Our study confirms the value of GX Ct levels for quantifying mycobacterial load and demonstrates the added value of Ct as a predictor of positive smear status, especially at Ct values below 21.

1. Introduction

Quantification of mycobacterial load is useful for determining disease severity, assessing transmission risk and predicting treatment failure and relapse in patients with pulmonary tuberculosis (TB) [1,2]. Currently, smear microscopy for acid-fast bacilli (AFB) is the only widely implemented method for quantifying mycobacterial load at the time of diagnosis. In addition, since patients who are sputum smear-positive are more likely to transmit tuberculosis [3,4], smear status is used to evaluate the infectiousness of patients in the context of public health contact management and screening.

The GeneXpert MTB/RIF (GX) is an automated molecular test for the detection of *Mycobacterium tuberculosis* (MTB) that estimates mycobacterial load by measuring the threshold-cycle (Ct) of multiple probes targeting the *rpoB* gene. Several small studies have shown that Ct values correlate well with the bacillary load in respiratory specimens compared with conventional methods, such as colony counts on solid agar growth media or measures of *Time-to-Detection* (TTD) in liquid culture [5–7]. However, there are few data about its diagnostic value for predicting the smear status in patients with pulmonary tuberculosis [8–10].

The aim of the present study was to assess the utility of GX Ct values as a rule-in test for smear positivity status. We also investigated the relationship between GX Ct and the smear grade. For this purpose, we performed a study of measures of mycobacterial load, including smear status, liquid culture TTD and GX MTB/RIF Ct values in patients with pulmonary tuberculosis.

2. Material and methods

2.1. Study design

We retrospectively compared the results of the GX MTB/RIF Ct values, smear status, and liquid culture TTD on sputum samples from adult patients with suspected pulmonary tuberculosis attending Hospital 12 de Octubre (Madrid, Spain) from January 2010 to January 2017.

During the study period, smear microscopy and liquid culture were systematically performed for suspected pulmonary tuberculosis, however, the GX was selectively requested by physicians for patients with a high suspicion of disease on the basis of epidemiologic and clinical data, or for patients with suspected infection with a MDR strain. We

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only included samples for which these three analyses were available.

Demographical, clinical, radiological and microbiological data were recorded when available.

2.2. Study samples

All GX-positive sputum samples from patients with suspected pulmonary TB were included. TTD values in liquid culture and microscopy results were recorded for each sample.

2.3. Bacteriological methods

All samples were digested-decontaminated following Kubica-Krasnow method [11] before being microscopically examined and processed for liquid culture in *Mycobacterial Growth Indicator Tube* system (MGIT). In our setting, GX is performed in respiratory samples from patients with high clinical suspicion of pulmonary TB and negative AFB smears, and in patients with positive AFB smears to assess preliminary results for rifampicin resistance.

Microscopy: Decontaminated samples were stained with auramine-thiazine red technique and examined with a fluorescence microscope by well-trained staff. Smears were graded according to the International Union Against Tuberculosis and Lung Disease (IUATLD) scale [12].

Liquid culture: 0.5 mL of each decontaminated sample was inoculated on a supplemented MGIT liquid culture tube (Becton Dickinson, USA) according to manufacturer's specifications. MGIT tubes were incubated at 37 °C and monitored in a BD Bactec™ MGIT™ 960 instrument. All positive cultures were confirmed to be *MTBc* by detection of antigen MPT64 with a commercial immunochromatographic assay (BD MGIT™ TBc Identification Test). TTD for each positive sample was recorded.

GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA): Automated *Real-Time polimerase chain reaction* assay targeting *rpoB* gene was performed as described by the manufacturer. Briefly, 0.5 mL of each decontaminated sample was inoculated in 1.5 mL of GX reagent and incubated for 15 minutes at room temperature with vigorous shaking at 10 minutes and at 15 minutes. 2 mL of each diluted sample was then transferred to a GX MTB/RIF cartridge and loaded into the GeneXpert IV instrument. Interpretation of the results was performed with GeneXpert software version 4.3. Semiquantitative mycobacterial load results were reported as follows: very low (cycle threshold (Ct) > 28), low (Ct 22–28), medium (Ct 16–22) or high (Ct < 16). Ct values for each of the five probes were recorded. Lowest Ct was selected for statistical analysis as described below.

2.4. Statistical analysis

IBM SPSS statistics software version 20.0 for Windows was used for statistical analysis. Spearman Coefficient was performed including all diagnostic and follow-up respiratory samples to establish the relationship between Ct and TTD and between Ct and smear grade. Samples from patients with pulmonary TB in treatment (i.e. GX positive follow-up samples) were excluded from further statistical analysis. Demographical, clinical and radiological data of the newly diagnosed patients were recorded and analyzed.

The performance of Ct values for the detection of smear-positive patients was evaluated using receiver operating characteristic (ROC) curve analysis.

Youden's index was calculated to establish the optimal Ct cut-off value to confirm/rule in smear positivity [13]. Receiver Operator Characteristic (ROC) curves were performed with Graph-Pad Prism software.

Univariate analysis of known clinical, radiological and microbiological variables associated with smear positivity [8] was carried out to evaluate if any of these variables could improve predictive capability of the Ct values. Chi-square test and Student's *t*-test were used for categorical and continuous variables respectively. P values less than 0.05 were considered statistically significant.

Table 1
Comparison between GX semiquantitative results and graded AFB smear status.

Xpert result[a]	AFB smear status[b]					Total
	Negative	Scanty	1+	2+	3+	
Very low, n (%)	5 (71.4)	1 (14.3)	1(14.3)	0	0	7 (100)
Low, n (%)	8 (72.7)	3 (27.3)	0	0	0	11 (100)
Medium, n (%)	10 (34.5)	3 (10.3)	7 (24.1)	7 (24.1)	2 (6.9)	29 (100)
High, n (%)	7 (21.9)	0	0	16 (50.0)	9 (28.1)	32 (100)
Total	30 (38.0)	7 (8.9)	8 (10.1)	23 (29.1)	11 (13.9)	79 (100)

[a] Semiquantitative mycobacterial load defined by GX software: very low ($28 < Ct \leq 38$), low ($22 < Ct \leq 28$), medium ($16 < Ct \leq 22$) and high ($Ct \leq 16$).

[b] Quantified AFB smear status according to International Union Against Tuberculosis and Lung Disease (IUATLD) scale.

3. Results

A total of 82 sputum samples from 79 patients were included in the study. These were 79 diagnostic samples and 3 follow-up samples. Of the 79 GX-positive patients, 30 were smear-negative and 49 were smear-positive.

A positive correlation was found between the Ct value and the TTD in liquid culture, with Spearman ρ coefficient of 0.714 ($p < 0.05$).

The distribution of smear grades within each semiquantitative category of GX is shown in Table 1. An inverse relationship between increasing smear grade and GX Ct ($r = -0.71$) was observed.

ROC curve for GX Ct as a test for detection of smear-positive patients is shown in Fig. 1. Youden's index yielded a Ct cut-off value of 21.1. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for Ct 21.1 were 90.5% (95% CI, 77.4%–87.3%), 61% (95% CI, 38.5%–80.3%), 81% (95% CI, 66.3%–90.4%), and 78% (95% CI, 52%–92.6%), respectively.

Univariate analysis of known smear-positive associated variables is shown in Table 2. Samples from smear-negative patients had a significantly higher mean Ct value than those from smear-positive patients (20.9 (± 5.8) vs. 16.9 (± 4.9), p value = 0.001). Lower mean TTD values were associated with smear positivity (6.3 (± 3.1) vs. 10.1 (± 5.0), $p < 0.001$). Regarding clinical and radiological features, no significant associations between these variables and smear-positive status were observed.

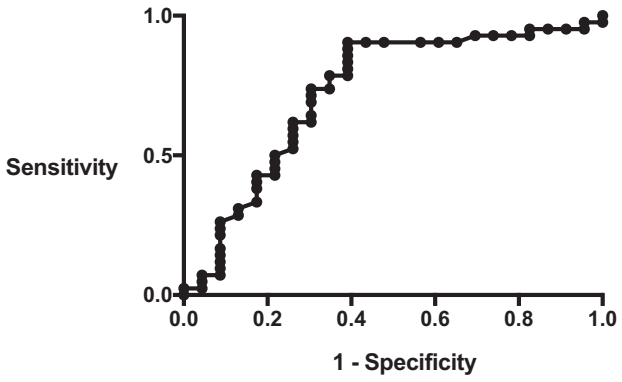

Fig. 1. Ct cutoff for smear-positive samples.

Table 2
Univariate analysis of variables associated with smear positivity.

	Total (N = 79)	Smear positive (N = 49)	Smear negative (N = 30)	*P value*[*]
Demographical and epidemiological data				
Age, mean (± SD)	40.9 (± 20.2)	43.5 (± 20.2)	36.6 (± 19.9)	0.147
Male sex, n(%)	47(59.5)	29 (59.2)	18 (60.0)	0.943
Non-European country of origin, n(%)	37 (46.8)	22 (44.9)	15 (50.0)	1.000
HIV-coinfected, n(%)	2 (2.5)	2 (4.2)	0 (0.0)	0.257
Previous TB, n(%)	15(19.0)	12 (24.5)	3 (10)	0.111
Smoker or ex-smoker, n (%)	30 (38.0)	22 (48.9)	8 (32.0)	0.171
Clinical and radiological data				
Fever (>38 °C), n(%)	37 (46.8)	24 (51.1)	13 (44.8)	0.597
Cough, n(%)	67 (84.8)	40 (85.1)	27 (90.0)	0.533
Hemoptysis, n(%)	23 (29.1)	15 (31.9)	8 (26.7)	0.624
Dysnea, n(%)	26 (32.9)	15 (31.9)	11 (37.9)	0.591
Weight loss, n(%)	38 (48.1)	23 (48.9)	15 (51.7)	0.813
Presence of cavities on chest X-ray, n(%)	38 (48.1)	26 (53.1)	12 (42.9)	0,389
Microbiological data				
TTD, mean (± SD)	7.7 (± 4.3)	6.3 (± 3.1)	10.1 (± 5.0)	**<0.001**
Ct, mean (± SD)	18.2 (± 5.6)	16.6 (± 4.9)	20.9 (± 5.8)	**0.001**

[*] *P values* < 0.05 were considered statistically significant.

4. Discussion

Smear microscopy is the first performed test in patients with a suspicion of pulmonary TB. A positive smear result reinforces the suspicion of TB and is associated with a high transmission risk [10,14–16]. However, it has a limited sensitivity and is unable to distinguish between non-tuberculosis mycobacteria and MTB. To address these limitations, the World Health Organization has recommended the GX as the initial test for pulmonary TB diagnosis [17]. Since infection control programs and contact tracing guidelines rely upon the smear status, further studies are needed to clarify how patients who undergo only GX testing should be managed. Thus, the present study was performed to assess the utility of GX Ct values as a rule-in test for smear positivity status. We also investigated the relationship between GX Ct and the smear grade.

It is difficult to select a single Ct value that is both a sensitive and specific for predicting smear-positive status. Based on ROC analysis, we found that a GX Ct value at Youden's index had moderate good rule-in value for smear positivity (cut-off Ct = 21.1; VVP: 81%) and optimal clinical utility (sensitivity 90.5%). Bellow a Ct cut-off point of 21.1, 81% of patients are correctly classified as smear positive cases. Other Ct cut-off points have been proposed by other authors. In a previous study, Theron et al. [8] reported that GX Ct values had poor clinical utility as a rule-in test for smear positivity, but they suggested that a Ct cut-off value of 31.8 provided moderately good rule-out value for smear positivity. This cut-point showed high values of sensitivity and NPV (95.8 and 80%) but moderate clinical utility. Blakemore et al. [9] reported that a Ct of 27.7 was 98% sensitive and 48% specific for smear-positive status. However, it should be noted that in this study samples were stained with Ziehl-Neelsen smear which is known to be less sensitive than the fluorescent auramine-thiazine red staining used in our study.

The smear quantitative result is used to assess the patient's infectiousness at the time of initial diagnosis. Here, we compared the semiquantitative result of GX with those of smear microscopy. We found that the GX Ct results correlate well with smear grades ($r = -0.71$). However, when we studied the distribution of smear grades for all samples within each semiquantitative category, we observed that GX quantitative categories do not consistently separate samples into the same categories as smear microscopy grading. It is difficult to know what proportion of the variation is caused by variation in smear classification and what is attributable to variation in the reproducibility of the Ct. Since smear grading is operator dependent and has variable operational sensitivity; we think that GX could provide more robust results than smear grading.

We also observed a significant number of sputum samples that were classified as smear-negative had relatively high mycobacterial load according to semiquantitative result of GX. This observation suggests that a low Ct value can identify samples with high amount of MTB bacilli, even if that sample is smear-negative. Since smear-negative patients are a substantial possible source of TB transmission [4], we think that GX quantitation could be used to identify the subset of potentially infectious smear-negative patients.

Finally, our results demonstrate a relatively strong correlation between GX Ct and TTD in liquid culture. Stronger correlations have been reported between TTD and cfu per milliliter in previous studies [18,19]. As suggested by Blakemore et al. [9], this observation may reflect the fact that both tests quantify the same fraction of metabolically cultivable mycobacterial cells that remain after sample processing. On the contrary, GX measures MTB DNA that is independent of bacterial metabolic status or sample processing protocol. Therefore, initial mycobacterial load may be more accurately estimated by using GX.

Although the conclusiveness of our results is limited by the small sample size, our data suggest that in those patients with suspected pulmonary TB who are tested by GX instead of smear microscopy at the time of initial diagnosis, GX could discriminate between infectious and non-infectious patients with varying values of sensitivity and specificity depending on the chosen cut-off. A Ct cut-off of approximately 21 best predicted smear-positive status.

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Transparency declaration

This study does not present any conflicts of interest for the authors.

Ethics approval statement

This study was reviewed and approved by local ethics committee of Hospital Universitario 12 de Octubre. Informed consent was not required, as routinely collected data from clinical practice were anonymized and used as our data source.

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