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Association between Xpert MTB/RIF cycle threshold values and sputum smear microscopy in patients with pulmonary tuberculosis

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TO THE EDITOR:

In 2010, the WHO endorsed the use of the Xpert MTB/RIF assay (Cepheid; Sunnyvale, CA, USA) in countries with a high burden of tuberculosis, considering it to be a technology capable of revolutionizing the diagnosis of the disease.[1] Xpert MTB/RIF assay results are automatically generated and are reported as either negative or positive for *Mycobacterium tuberculosis*, and in the latter case, as whether the strain is susceptible or resistant to rifampin. Xpert MTB/RIF assay results are also reported as cycle threshold (C_T) values, which correspond to the number of PCR cycles required to detect *M. tuberculosis*. Each additional cycle represents an approximate 50% decrease in the amount of material present in a sample over the previous cycle, thus providing a semiquantitative measure of bacillary burden, and higher C_T values correspond to lower bacillary burden.[2]

Given the WHO recommendation to replace sputum smear microscopy with Xpert MTB/RIF as an initial diagnostic test for tuberculosis (although smear microscopy is still used in some countries), because culture results take several weeks, Xpert MTB/RIF C_T values may be the only way to assess bacillary burden.[3-6] The objective of the present study was to assess the association between Xpert MTB/RIF C_T values and sputum smear microscopy and to assess the diagnostic performance of Xpert MTB/RIF C_T values.

This was a cross-sectional study of prospectively collected data, conducted at the tuberculosis outpatient clinic of a health care center in the city of Alvorada, Brazil, where the incidence of tuberculosis was 84.4 cases/100,000 population between 2017 and 2019.[7] The study was approved by the Research Ethics Committee of the Porto Alegre *Hospital de Clínicas*, located in the city of Porto Alegre (Protocol no. 160063).

Patients aged 18 years or older who had respiratory symptoms suggestive of pulmonary tuberculosis and were able to produce sputum were invited to participate. Those who could not produce sputum were excluded, as were patients with extrapulmonary tuberculosis. The diagnosis of pulmonary tuberculosis was established in accordance with the Third Brazilian Thoracic Association Guidelines on Tuberculosis.[8]

Data were collected on demographic characteristics, smoking, alcohol abuse, symptoms, and comorbidities. Chest X-rays were classified either as being typical of tuberculosis or as being consistent with tuberculosis.[9] Sputum smears were stained by the Ziehl-Neelsen technique for identification of AFB, and culture was performed using the Ogawa-Kudoh method.[8] The Xpert MTB/RIF assay was performed in accordance with the manufacturer's instructions.[2]

Data analysis was performed with IBM SPSS Statistics, version 18.0 (IBM Corporation, Armonk, NY, USA), and MedCalc, version 16.4.3 (MedCalc Software, Mariakerke, Belgium). On the basis of smear microscopy results (positive or negative), we calculated the sensitivity, specificity, positive predictive value, and negative predictive value of Xpert MTB/RIF C_T values, with the corresponding 95% CIs. We also constructed ROC curves to determine the optimal cutoff. In order to calculate sample size, we considered the fact that the sensitivity of a given Xpert MTB/RIF C_T cutoff was 85% in a previous study.[4] Therefore, using a 95% CI and a power of 80%, the required sample size was estimated to be 100 patients at least.

During the study period, 407 patients underwent Xpert MTB/RIF testing. Of those, 150 had a positive Xpert MTB/RIF result and were included in the study. Table 1 describes the characteristics of the study participants. There was a statistically significant difference in mean C_T between sputum smear-positive and sputum smear-negative patients (17.8 ± 4.8 and 22.3 ± 6.7, respectively; p = 0.002). Sensitivity, specificity, positive predictive value, and negative predictive value of an Xpert C_T cutoff of 22.7 were 83.6% (95% CI: 75.8-89.7), 60.7% (95% CI: 40.6-78.5), 90.3% (95% CI: 85.3-93.7), and 45.9% (95% CI: 34.0-58.3), respectively. The area under the ROC curve for this cutoff was 0.70 (95% CI: 0.62-0.77; p = 0.002).

Few studies[4-6] have assessed C_T cutoffs as a measure of bacillary burden. The C_T cutoffs that have been most widely studied are 28[5,6] and 31.8.[4] Hanrahan et al.[6] demonstrated that a C_T cutoff of 28 had good predictive value for smear positivity, with a sensitivity of 89.9% and a specificity of 67.0%. In another study,[5] the authors showed that lower C_T values were associated with HIV negativity and low BMI and also used a cutoff of 28, reporting a sensitivity of 95% and a specificity of 54.1%. In the present study, a C_T value of 22.7 was found to be the optimal cutoff, which is lower than the

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Table 1. Characteristics of the study participants.

Characteristic	(N = 150)
Demographic characteristics	
Age, years	40.9 ± 15.3
Male gender	108 (72.0)
White ethnicity	102 (68.0)
Active smoking	84 (56.0)
Alcohol abuse	31 (20.7)
Symptoms	
Cough	145 (96.7)
Weight loss	112 (74.7)
Dyspnea	62 (41.3)
Fever	72 (48.0)
Night sweats	97 (64.7)
Hemoptysis	13 (8.7)
HIV positivity	31 (20.7)
Diabetes	18 (12.0)
Radiological features	
Typical of tuberculosis	103 (68.7)
Consistent with tuberculosis	47 (31.3)
Sputum smear positivity	122 (81.3)
Cycle threshold, Xpert MTB/RIF	
	18.7 ± 5.5
Ccpositi(detected)	

*Data presented as mean ± SD or as n (%).

cutoffs used in most studies. However, the decision regarding the optimal C_T cutoff differs according to the context and the objectives of testing. In order to identify as many smear-positive patients as possible, higher C_T values should be chosen. In the context of limited resources, however, patients with lower C_T values should be prioritized for respiratory isolation.[4]

In one meta-analysis,[4] cutoffs of 27.7 and 31.8 were shown to have a sensitivity of 85% and 95%, respectively, as well as a specificity of 67% and 35%, for smear-positive samples. However, the authors concluded that the moderate diagnostic accuracy of C_T values compared with that of sputum smear microscopy, as well as different needs in contexts with varying prevalence of sputum smear positivity, may preclude the use of C_T values as a surrogate for sputum smear microscopy in all contexts.

One of the limitations of the present study was the fact that participants were recruited at a single tuberculosis outpatient clinic. However, we believe that the findings are applicable to similar contexts. In addition, we did not assess whether C_T values can predict infectiousness and transmission, although smear positivity alone has been shown to be an imperfect measure of infectiousness, with evidence of transmission from smear-negative but culture-positive cases.[10] Despite these limitations, to our knowledge, this is the first study in Brazil to assess the accuracy of C_T values as a surrogate for sputum smear microscopy.

In conclusion, Xpert MTB/RIF C_T values are associated with sputum smear results and are lower in smear-positive patients. A C_T value cutoff of 22.7 showed good predictive value for smear positivity.

AUTHOR CONTRIBUTIONS

GCP: study design, methodology, investigation, formal analysis, and writing of the original draft; GRP, KGD, and LRM: methodology, investigation, writing, revision, and editing; and DRS: study design, methodology, investigation, formal analysis, and writing of the original draft.

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