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Early View

Research letter

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Effect of history of tuberculosis on specificity of Xpert MTB/RIF

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To the editor

Tuberculosis (TB) is the leading cause of mortality among infectious diseases. In 2018, 1.5 million people died due to TB (1). Early diagnosis of TB is key in achieving WHO End TB targets and molecular diagnostic tests have been developed. However, molecular tests cannot differentiate between viable and non-viable bacteria (2, 3), leading to challenges in the interpretation of positive test results in patients with recent TB.

Xpert MTB/RIF ('Xpert', Cepheid, Sunnyvale, USA), a widely used molecular diagnostic, amplifies sequences of the rpoB gene specific to *Mycobacterium tuberculosis* complex bacteria (4, 5). Xpert has a high specificity estimated as 98% (95%CI 97 to 99) in a meta-analysis (6). Growing evidence from case reports or small studies and one larger study by Theron *et al*. suggests a reduced Xpert specificity among patients with TB history (2, 7-9). The rate of Xpert false positives are higher the more recent the history of TB (10). This poses a clinical dilemma: while patients with recent TB have a high risk of reinfection or relapse, they may also have a high risk of a false-positive result (2, 8, 10).

To inform policy and clinical practice, we assessed the effect of TB history on the specificity of Xpert by time since the previous TB episode in settings with different levels of HIV-co-infection (11) in sixteen studies collated by FIND (Foundation of Innovative New Diagnostics, Geneva, Switzerland). While studies were carried out under different protocols, all used Xpert and a common reference standard with liquid and solid cultures that was standardized across studies.

We included all presumptive patients 18 years or older with Xpert and cultures performed. Patients were recruited in 10 countries (Belarus, Cambodia, Georgia, India, Italy, Moldovia, Peru, South Africa, Vietnam and Zimbabwe) between 9 June 2011 and 6 March 2018. We considered Xpert results for the first sample only. Patients with missing Xpert results were excluded from the analysis.

The reference standard was based on Lowenstein Jensen (LJ) and Mycobacterium Growth Indicator Tube (Bactec MGIT; BD Microbiology Systems, Cockeysville, MD, USA). Chest radiographs were considered compatible with TB, if the attending clinician rated the x-ray at least as likely for TB. We estimated the specificity of Xpert as the proportion of Xpert negative out of the culture negative patients. Time since previous TB treatment was calculated as the difference in dates between enrolment and the previous treatment completion. We estimated the specificity of Xpert using logistic regression with a random effect to account for clustering by study. All analyses were performed using Stata version 15 (stataCorp LLC, Texas, USA).

A total of 11,583 patients had Xpert and culture results. We excluded 1,627 (1,615 missing Xpert results, 12 below 18 years old), leaving 10,053 patients for the analysis. Of these, 59% (5,900/10,053) had a positive culture and 55% were positive on Xpert. Overall 16% (1,630/10,053) of patients had a history of TB. The median time since previous TB treatment was 3 years (Interquartile range (IQR), 0.0-6). Patients with TB history tended to be older (median age 39 [IQR 31-48] vs 35 [IQR 26-49], HIV positive and resistant to rifampicin.

False positive Xpert results were observed in 6.5% (95% CI 2.8 -13.7) of patients with TB history and 2% (95% CI 0.6-5.6) without a history of TB. In patients without previous TB, specificity was estimated as 98.0% (95% CI; 96.0-99.7; 3095/3164) while in those with it was 93.5% (95% CI; 80.4-98.1; 465/501). Among the 803 participants with a TB episode within two years of testing, the specificity of Xpert was 92.2% (95% CI 81-97). The specificity increased with time since previous TB. Between two and five years (373 participants), the specificity was 99.0% (95% CI; 86-100) and above five years (454 participants), 98.6% (95% CI; 85.4-99.8). The rate of false positivity for TB detection was similar in both groups in patients with rifampicin resistance regardless of TB history. Of 247 patients with false positive results, 109 had chest X-ray results of which 75% were compatible with active TB. Patients with a non-compatible X-ray were more likely to have a false positive Xpert result with an odds ratio of 4.9 (95% CI; 2-11) adjusting for TB history, HIV, age and sex.

This study represents the largest dataset to date to assess the effect of TB history on the specificity of Xpert for TB. The specificity of Xpert was lower in those with TB history compared to those without it and increased as time since previous TB increased. In patients with TB history within two years, specificity was reduced. False positive was associated with negative chest X-rays even when adjusted for TB history and HIV, consistent with previous findings (12). This suggests that a negative chest X-ray could aid in deciding whether to start a patient with a positive Xpert result and recent TB history on therapy. This might be a viable strategy in patients without HIV. In HIV patients, the risk of not treating a patient who may truly have TB might outweigh the risk of overtreatment. These results only provide an association and not causation. However, the decreased specificity in patients with recent TB history is coupled with increased sensitivity, as shown with Xpert MTB/RIF Ultra (10), which supports the causal link. A causal link is further supported by an association between false positive results and decreasing bacterial load in those with TB history. High Cycle threshold values (Ct) predict false Xpert positivity however, discriminatory power of Ct values in predicting false positive remain minimal (9). A study showed patients with false positive

Xpert remained healthy and asymptomatic after 17 months of follow up (12). However, PET-CT showed positivity consistent with active disease at the end of therapy (2, 8), suggesting Xpert false positivity might not stem only from non-viable bacteria alone but could also be related to viable bacteria still present after a patient is clinically cured.

A limitation of our study was that Ct values for Xpert were not reported and that clinical outcome data were not available. Study strengths include the size of the dataset with a common reference standard. Data from different settings suggest likely generalizability. The overall specificity of Xpert in our study of 98% is identical with the pooled specificity reported in a recent Cochrane review including 95 studies (6).

Our results may have implications for the design of diagnostic algorithms in patients with TB history, particularly if the prior episode was within two years. Consideration of other conditions and careful clinical decision before treatment initiation in this group is important. The impact of false positive results on patient outcomes remain a relevant question for future studies and modelling studies, such as the one by Kendall *et al* for Xpert Ultra (13), should weigh the impact of likely overtreatment among patients with previous TB history in different epidemiological settings. Importantly, while our findings pertain directly to Xpert, similar results should be expected with other molecular TB assays. In conclusion, Xpert specificity is reduced among patients with previous TB history, and this reduction is greater among those with TB in the last two years.

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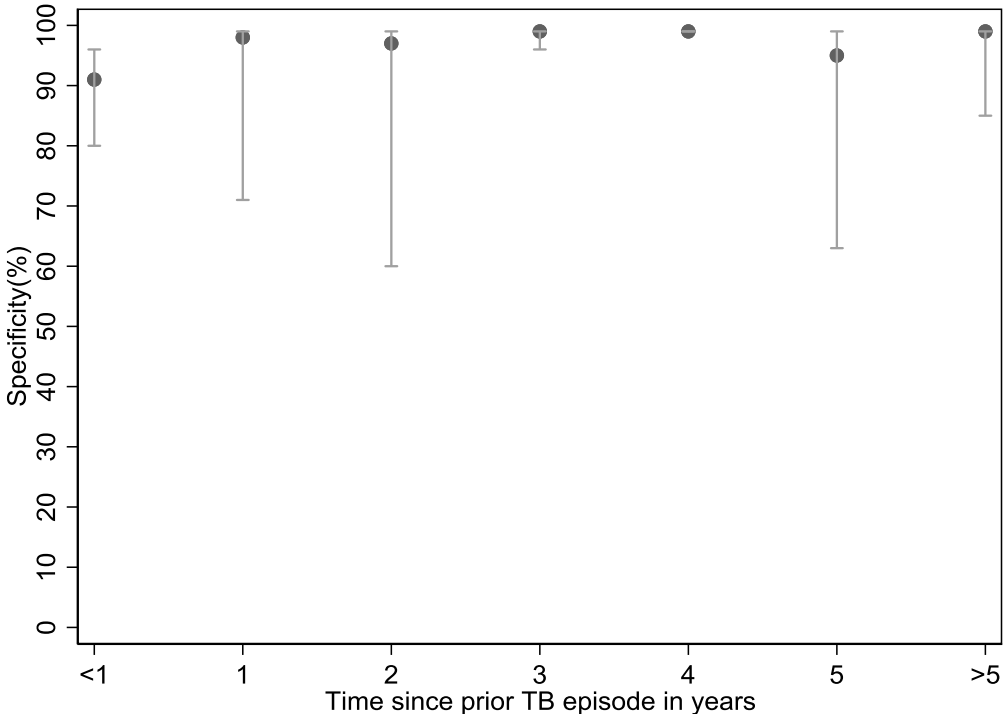
Figure 1: Xpert MTB/RIF specificity by time since previous tuberculosis episode



Black circles: Specificity estimated using logistic regression with a random effect to account for clustering by study
Error bars: 95% confidence intervals
X-axis: Time period (in years)