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Diagnosis of extrapulmonary tuberculosis using the Xpert® MTB/RIF assay

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Abstract

The Xpert® MTB/RIF assay has been CE-marked for rapid molecular diagnosis of TB in Europe and has been endorsed by the WHO as a replacement for sputum smear microscopy for diagnosis of pulmonary TB in low- and middle-income countries. However, few data are available to inform recommendations for use of the assay for testing nonsputum clinical samples when investigating suspected extrapulmonary TB (EPTB). We review and discuss the findings of Tortoli and colleagues, who evaluated the assay used for this purpose in a large study of adults and children in Italy. They provide a per-sample analysis of 268 diagnoses of EPTB at a range of anatomic sites (sensitivity: 81.3%; 95% CI: 76.2–85.8) and data for 1206 samples in which EPTB was excluded (specificity: 99.8%; 95% CI: 99.4–100). We discuss how this paper forms an important addition to the growing body of literature demonstrating the utility of Xpert MTB/RIF for EPTB diagnosis when applied to diverse types of clinical samples.

Keywords

diagnosis; extrapulmonary; nucleic acid amplification test; tuberculosis; Xpert®

TB remains a key challenge to global public health and our ability to tackle this disease has been severely hampered by inadequate diagnostic assays [1]. Diagnosis of extrapulmonary TB (EPTB) remains especially challenging since the number of *Mycobacterium tuberculosis* (MTB) bacilli present in tissues at sites of disease is often low and clinical specimens from deep-seated organs may be difficult to obtain. Histology is time-consuming to undertake and establishing a diagnosis of TB with high specificity remains difficult. Tissue microscopy after special staining is often negative and when mycobacteria are seen, it is impossible to distinguish MTB from nontuberculous mycobacterial disease. Reliance on culture, the

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mainstay of diagnosis, often leads to considerable delays, compromising patient care and outcomes.

Nucleic acid amplification tests for rapid TB diagnosis are increasingly being used. The US CDC recommends that nucleic acid amplification tests be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB [2]. However, no recommendation exists for their use in the investigation of patients suspected of having EPTB as the evidence base is limited.

The Xpert® MTB/RIF assay (Cepheid Inc., CA, USA) marks an important development in the field of rapid molecular TB diagnostics [3,4]. This multifunctional diagnostic platform is an automated, closed system that performs real-time PCR and can be used by operators with minimal technical expertise, enabling diagnosis of TB and simultaneous assessment of rifampicin resistance to be completed within 2 h. Sputum samples can be analyzed with very minimal processing, yielding positive diagnoses in 99–100% of patients with smear-positive pulmonary TB and 57–83% of patients with smear-negative pulmonary TB in clinical evaluation studies [3]. The Xpert MTB/RIF assay was rapidly endorsed by the WHO in December 2010 as a replacement for sputum smear microscopy, particularly in settings with high rates of HIV-associated TB and multidrug-resistant TB [101].

Since Xpert MTB/RIF was specifically developed and optimized for testing sputum samples and initial large-scale evaluations were in patients with pulmonary TB, WHO endorsement specifically applied to the investigation of pulmonary TB. More recently, however, evaluations of the assay have extended to a variety of nonrespiratory clinical samples from patients with EPTB. The evidence base for use in the investigation of EPTB remains comparatively weak, however, and many more studies assessing a variety of clinical samples other than sputum are therefore needed. However, compared with pulmonary disease, investigation for use in EPTB is far more complex because of the diversity of clinical sample types, difficulties in obtaining adequate tissue for analyses and in extraction of MTB DNA from samples, the challenge of providing a rigorous gold standard for comparison, and the range of potential ways of processing samples prior to analysis. Here we review the article by Tortoli and colleagues [5], discuss the findings of their large study of EPTB patients in Italy and compare these findings with those of other recently published studies utilizing samples other than sputum.

Methods

This was a primarily laboratory-based study, with data provided by eight nationally accredited laboratories in Italy. A total of 1493 consecutive extrapulmonary clinical samples undergoing investigation for possible EPTB were included. These samples were obtained from 1068 patients; 494 (33.5%) samples were from patients aged ≤18 years.

Sample processing differed according to specimen type. Nonsterile samples were subjected to standard *N*-acetylcysteine sodium hydroxide decontamination and concentration by centrifugation, whereas sterile samples only underwent simple mechanical homogenization (if required), concentration and resuspension in saline. All samples underwent fluorescence microscopy for acid-fast bacilli and culture on solid (Lowenstein–Jensen) and liquid (MGIT, Becton Dickinson Biosciences, MD, USA) media. Samples were also tested with the Xpert MTB/RIF assay with adherence to the manufacturer's protocol.

Diagnostic accuracy was first assessed by simple comparison with mycobacterial culture results. Analysis was then made against a TB diagnostic gold standard that incorporated all culture-positive diagnoses plus any additional diagnoses in patients with radiological and/or histopathological evidence of TB that improved during the course of TB treatment.

Results

Results of testing with Xpert MTB/RIF were indeterminate for 17 (1.1%) samples and so complete results were available for 1476 samples. Clinical follow-up data were also missing for two patients in whom ascertainment of outcomes was needed. Although 425 samples were from patients who provided more than one sample, all analyses were presented on a per-sample basis and not on a per-patient basis.

M. tuberculosis was cultured from 238 samples and an additional 30 samples that tested culture-negative were judged to be from patients who had TB according to the composite gold standard (Table 1). Thus, a total of 268 samples were obtained from sites of EPTB disease. Patients who provided the remaining 1206 samples had no evidence of EPTB. The clinical samples for which there was a positive TB diagnosis (n = 268) were tissue biopsies or fine-needle aspirates (35%), gastric aspirates (23%), pus (21%), urine (6%), cerebrospinal fluid (5%) and other body fluids (peritoneal, synovial and pericardial: 4%) (Table 1).

Compared with culture results, the sensitivity and specificity of Xpert MTB/RIF were 79.0% and 97.3%, respectively. Compared with the composite diagnostic gold standard, the sensitivity of Xpert MTB/RIF was 81.3% (95% CI: 76.2–85.8) and the specificity was 99.8% (95% CI: 99.4–100). Of the two false-positive Xpert results in the latter analysis, one was from a patient with bladder cancer who had received therapeutic BCG intravesical instillation, which is a very plausible explanation.

The sensitivity of Xpert MTB/RIF was much higher for smear-positive disease (99.0%) compared with smear-negative disease (70.3%). Analyses stratified by sample type and by patient age (adult vs child) were also presented. Using the composite gold standard, the sensitivity in samples from children (86.9%) tended to be higher than that in samples from adults (77.6%). This may be a reflection of the specific sample types, which differed in proportion by age. Sensitivity (all ages) exceeded 75% for tissue biopsies and fine-needle aspirates (88.3%; 95% CI: 82–95), gastric aspirates (78.7%; 95% CI: 68–89), pus samples (87.3%; 95% CI: 67–100), cerebrospinal fluid (85.7%; 95% CI: 67–100) and urine (87.5%; 95% CI: 71–100). Lower sensitivity was observed for pleural fluid samples (44.4%; 95% CI: 21–67) and other body fluids including pericardial, peritoneal and synovial fluids (50%; 95% CI: 19–81).

Seven patients had confirmed rifampicin-resistant disease and all were correctly identified by MTB/RIF and no false-positive results were reported, as has been the case in other studies [3].

Discussion

This paper is an important addition to the literature. It represents the largest number of nonrespiratory samples tested with Xpert MTB/RIF of any study published to date and provides a much larger number of EPTB diagnoses compared with most other studies, with the exception of the study from India by Vadwai and colleagues (Table 1) [6–12]. Comparisons with culture only as well as the composite gold standard that included histological and radiological data and response to TB treatment were entirely appropriate, acknowledging the fact that not all disease can be culture-confirmed. Since the study was retrospective and primarily laboratory-based, concerns could be raised about the nonmicrobiological component of the gold standard. However, these comprised only 11% of diagnoses and their inclusion had only a minor impact on the calculated sensitivity of Xpert MTB/RIF but improved specificity a little, reaching 99.8%, which is entirely consistent with most other studies (Table 1).

The observed sensitivity of Xpert MTB/RIF of 81.3% for EPTB is also entirely consistent with seven other published studies (Table 1) in which reported sensitivities ranged from 25.0 to 95.1% and exceeded 50.0% in all but one small study of patients with pleural effusions (Table 1). The heterogeneity between studies may reflect differences between patient populations, patient selection, type of EPTB, the quality of the samples, differences in sample processing and the diagnostic gold standard used. A limitation of the data presented by Tortoli and colleagues is that no per patient analysis was performed and these are the relevant data from a clinical perspective. The per-patient diagnostic accuracy might differ from the per-sample analysis if certain patients provided multiple specimens, although this difference is unlikely to be substantial.

Only a single published study from South Africa has previously assessed the use of Xpert for diagnosis of TB in children, but only respiratory samples were tested [13]. For sputum culture-positive disease, Xpert MTB/RIF performed on two induced sputum samples detected 75.9% of TB cases compared with 37.9% using smear microscopy. This compares to a sensitivity of 86.9% (95% CI: 80–93) using Xpert MTB/RIF for EPTB in children in the study by Tortoli and colleagues [5]. Unfortunately, failure to report the absolute number of children included, the number of samples tested per child and the actual numbers of each sample type from children precludes full appreciation of the data. Nevertheless, these data, together with those from Nicol and colleagues [13], provide some important progress in the arena of pediatric TB diagnosis, which remains a huge challenge.

Five-year view

Five of the eight studies presented in Table 1 are studies of ‘convenience’, being laboratory-based evaluations of routine samples, whereas just three studies prospectively recruited patients for diagnostic evaluation [10,12]. Further prospective studies that incorporate much larger numbers of patients with suspected TB at the most common extrapulmonary anatomic sites, including pleural TB and TB meningitis, are needed. Sample preparation algorithms need to be optimized for different sample types, the volumes of body fluids that need to be concentrated by centrifugation prior to testing need to be defined and guidance on how the assay might be used for some sample types at the point-of-care are needed. Other studies are needed to address the role of Xpert MTB/RIF in the diagnosis of EPTB in specific clinical settings. For example, fine-needle aspiration biopsy of lymph nodes [10] might provide an important adjunct to testing respiratory samples when screening HIV-infected patients for TB prior to antiretroviral therapy in sub-Saharan Africa [14,15]. Studies must also assess the impact of the use of Xpert MTB/RIF on time to TB diagnosis and clinical outcomes, thereby permitting cost–effectiveness analyses to be performed. As the body of evidence grows, these emerging data need to be reviewed and synthesized so that national and international recommendations for the use of Xpert MTB/RIF in the diagnosis of EPTB can be established. Very encouraging data generated thus far suggest that this assay could come to play an important routine role in EPTB diagnosis.

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References

1. Lawn SD, Zumla AI. Tuberculosis. Lancet. 2011; 378:57–72. [PubMed: 21420161]

2. Centers for Disease Control and Prevention. Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. MMWR Morb. Mortal. Wkly Rep. 2009; 58:7–10. [PubMed: 19145221]
3. Lawn SD, Nicol MP. Xpert(R) MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Future Microbiol. 2011; 6:1067–1082. [PubMed: 21958145]
4. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. N. Engl. J. Med. 2010; 363:1005–1015. [PubMed: 20825313]
5. Tortoli E, Russo C, Piersimoni C, et al. Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. Eur. Respir. J. 2012 doi:10.1183/09031936.00176311. (Epub ahead of print).
6. Armand S, Vanhuls P, Delcroix G, Courcol R, Lemaitre N. Comparison of the Xpert MTB/RIF test with an IS6110-TaqMan real-time PCR assay for direct detection of Mycobacterium tuberculosis in respiratory and nonrespiratory specimens. J. Clin. Microbiol. 2011; 49:1772–1776. [PubMed: 21411592]
7. Causse M, Ruiz P, Juan Bautista GA, Casal M. Comparison of two molecular methods for the rapid diagnosis of extrapulmonary tuberculosis. J. Clin. Microbiol. 2011; 49:3065–3067. [PubMed: 21653775]
8. Friedrich SO, von Groote-Bidlingmaier F, Diacon AH. Xpert MTB/RIF assay for diagnosis of pleural tuberculosis. J. Clin. Microbiol. 2011; 49:4341–4342. [PubMed: 21998430]
9. Hillemann D, Rusch-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF System. J. Clin. Microbiol. 2011; 49:1202–1205. [PubMed: 21270230]
10. Ligthelm LJ, Nicol MP, Hoek KG, et al. Xpert MTB/RIF for rapid diagnosis of tuberculous lymphadenitis from fine-needle-aspiration biopsy specimens. J. Clin. Microbiol. 2011; 49:3967–3970. [PubMed: 21880965]
11. Moure R, Munoz L, Torres M, Santin M, Martin R, Alcaide F. Rapid detection of *Mycobacterium tuberculosis* complex and rifampin resistance in smear-negative clinical samples by use of an integrated real-time PCR method. J. Clin. Microbiol. 2011; 49:1137–1139. [PubMed: 21191053]
12. Vadwai V, Boehme C, Nabeta P, Shetty A, Alland D, Rodrigues C. Xpert MTB/RIF, a new pillar in the diagnosis of extrapulmonary tuberculosis? J. Clin. Microbiol. 2011; 49:2540–2545. [PubMed: 21593262]
13. Nicol MP, Workman L, Isaacs W, et al. Accuracy of the Xpert MTB/RIF test for the diagnosis of pulmonary tuberculosis in children admitted to hospital in Cape Town, South Africa: a descriptive study. Lancet Infect. Dis. 2011; 11:819–824. [PubMed: 21764384]
14. Lawn SD, Brooks SV, Kranzer K, et al. Screening for HIV-associated tuberculosis and rifampicin resistance before antiretroviral therapy using the Xpert MTB/RIF assay: a prospective study. PLoS Med. 2011; 8:E1001067. [PubMed: 21818180]
15. Lawn SD, Wood R. Tuberculosis in antiretroviral treatment services in resource-limited settings: addressing the challenges of screening and diagnosis. J. Infect. Dis. 2011; 204(Suppl. 4):S1159–S1167. [PubMed: 21996698]

Website

101. WHO. Tuberculosis diagnostics automated DNA test. www.who.int/tb/features_archive/xpert_factsheet.pdf

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Key issues

- Diagnosis of extrapulmonary TB (EPTB) is a major challenge.
- This large study found that when testing a range of nonrespiratory sample types from both adults and children suspected of having EPTB, Xpert® MTB/RIF had a sensitivity of 81.3% and specificity of 99.8%.
- The data from this study add to a rapidly growing body of literature that collectively show that Xpert MTB/RIF provides a rapid EPTB diagnosis in approximately 50–80% of cases in a majority of studies. Xpert MTB/RIF is likely to play an important role in providing rapid molecular diagnostic assessment of suspected EPTB.
- Further studies are required to evaluate the usefulness and cost–effectiveness of the Xpert MTB/RIF assay for nonsputum samples in high TB-endemic regions.

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Table 1

Summary of studies (n = 8) published before 7 March 2012 in which the diagnostic accuracy of Xpert® MTB/RIF for extrapulmonary TB was assessed.

Study (year)	Country	TB gold standard diagnoses (n)	TB not diagnosed (n)	Main sample types testing positive for TB (n)	Gold standard for TB diagnosis	Xpert sensitivity, % (95% CI)	Xpert specificity, % (95% CI)	Ref.
Index study								
Tortoli *et al.* (2012)	Italy	268	1206	Tissue biopsies/fine-needle aspirates (94); pleural fluid (18); gastric aspirates (61); pus (55); CSF (14); urine (16); peritoneal/ synovial/pericardial fluid (10)	Culture (solid and liquid) or suggestive radiology/ histology with documented positive response to TB treatment	81.3 (76.2–85.8)	99.8 (99.4–100)	[5]
Other studies								
Armand *et al.* (2011)	France	32	NA	LNs (16); pleural (7); bone (5)	Culture (solid and liquid media)	53.1 (34.7–70.9)	NA	[6]
Causse *et al.* (2011)	Spain	41	299	Tissue biopsies (18); CSF (6); gastric aspirates (8); pleural fluid (4); purulent exudates (5)	Culture (solid and liquid media)	95.1 (83.5–99.4)	100 (98.8–100)	[7]
Friedrich *et al.* (2011)	South Africa	20	5	Pleural fluid (25)	Culture (liquid media)	25.0 (8.7–49.1)	100 (47.8–100)	[8]
Hillemann *et al.* (2011)	Germany	45	476	Tissue (30); gastric aspirate (8); urine (5)	Culture (solid and liquid media)	77.3 (60.5–87.1)	98.2 (96.0–98.9)	[9]
Ligthelm *et al.* (2011)	South Africa	30	18	Fine-needle aspiration LN biopsy	Composite standard: positive cytology + AFB and/or culture of MTB	96.6 (86.6–100)	88.9 (69.6–100) (note: only 18 samples)	[10]
Moure *et al.* (2011)	Spain	108	41	All smear-negative. Pleural fluid (26); LNs (34); abscess aspirates (17); tissues (12)	Culture (solid and liquid media)	58.3 (48.5–67.8)	100 (91.4–100)	[11]
Vadwai *et al.* (2011)	India	283	250	Tissue biopsies (105); pus (98); body fluids (24)	Composite of smear, culture, clinical, radiology and histology	80.6 (75.5–85.0)	99.6 (97.8–100)	[12]

Only studies with at least 20 gold standard diagnoses of extrapulmonary TB were included.

AFB: Acid-fast bacilli; CSF: Cerebrospinal fluid; LN: Lymph node; MTB: *Mycobacterium tuberculosis*; NA: Not available.