Performance of Xpert MTB/RIF on pleural tissue for the diagnosis of pleural tuberculosis

To the Editor:

Tuberculosis (TB) remains the second leading cause of death from an infectious disease in adults. Extrapulmonary TB (EPTB) accounts for about 25% of all cases of active TB. Pleural TB is the second most common manifestation of EPTB.

Existing tests for the diagnosis of pleural TB have major limitations in terms of accuracy, time to diagnosis and drug resistance testing, and require special expertise for sample acquisition and interpretation of the results. Biopsy of the pleural tissue for combined histological examination and culture is considered the diagnostic gold standard, albeit imperfect [1, 2].

The Xpert MTB/RIF assay (Xpert; Cepheid, Sunnyvale, CA, USA) is a rapid, World Health Organization (WHO) endorsed, automated PCR test optimised for respiratory specimens that can detect both *Mycobacterium tuberculosis* (MTB) and rifampicin resistance [3, 4]. Given the limitations of available tests for the diagnosis of pleural TB, several studies have evaluated the performance of Xpert using pleural fluid as a sample type. Overall, these studies show limited accuracy with sensitivity averaging around 44% [5–7]. However, the preferred specimen for the diagnosis of pleural TB is pleural tissue. To date, the evaluation of Xpert performed on pleural tissue has been limited to isolated samples within larger studies [4, 6, 7].

We enrolled consecutive adult patients that were evaluated for pleural TB in the pulmonary clinic and inpatient ward at the Christian Medical College, Vellore, India. Pleural TB was suspected based on clinical symptoms and radiographic evidence of a pleural effusion. Information on demographics, comorbidities, presenting symptoms and results of diagnostic evaluation were collected prospectively. The institutional review boards of the Christian Medical College and McGill University, Montreal, Canada, approved the study.

All recruited patients underwent thoracentesis for evaluation of pleural fluid. One specimen was processed with routine diagnostics including fluorescence smear microscopy, adenosine deaminase (ADA; Diazyme Laboratories, San Diego, CA, USA), liquid cultures (Mycobacterium Growth Indicator Tube; Becton Dickinson, Sparks, MD, USA) and solid cultures (Löwenstein–Jensen medium). The second sample was used for Xpert testing. A pleural biopsy was performed, when clinically indicated and safely feasible. Pleural tissue was evaluated with histopathology, smear microscopy and culture.

TABLE 1 Xpert results for pleural fluid and pleural tissue

Diagnostic test results contributing to the diagnosis	Subjects n	Xpert positive in pleural fluid	Xpert positive in pleural tissue
Tuberculosis	33	4[#]	0[¶]
Positive pleural tissue culture	8	2	0
Positive pleural fluid culture	0	0	0
Positive pleural fluid smear	1	1	0
MTB identified at other site	3	0	0
Histopathology with granulomas	24	2	0
Lymphocytic exudate with elevated ADA	18	1	0
Alternative diagnosis	63	0[#]	1[¶]
Definite malignancy by pathology or cytology	36	0	1
Pleural tissue culture and histopathology negative for tuberculosis	65	0	1

MTB: *Mycobacterium tuberculosis*; ADA: adenosine deaminase. [#]: sensitivity 13.3% (four out of 30; 95% CI 4–31%) and specificity 100% (61 out of 61; 95% CI 94–100%); [¶]: sensitivity 0% (none out of 17; 95% CI 0–20%) and specificity 97.4% (37 out of 38; 95% CI 86–100%).

Pleural fluid was centrifuged (1370 ×g for 15 min) and the concentrated sediment, resuspended in 1 mL of the original supernatant, was used for Xpert [5]. Pleural tissue was finely ground and re-suspended in 1 mL of sterile saline [8]. The Xpert "sample reagent" was added (2:1 ratio for both pleural fluid and pleural tissue samples) and, after incubation, 2 mL were transferred into a G4 cartridge.

We defined two composite reference standards (CRS) for the diagnosis of TB. The first CRS (CRS-1) identified confirmed TB if either acid-fast bacilli were identified on microscopic evaluation of pleural tissue or fluid, culture from pleural tissue or fluid was positive for MTB, histopathology of pleural tissue identified granulomas or MTB was identified in any other sample (*e.g.* sputum) from the same patient.

The second CRS (CRS-2) included all of the above and, in addition, included patients classified as TB cases if the pleural fluid was found to be a lymphocytic exudate with ADA levels >40 $U \cdot L^{-1}$, in the absence of any other diagnosis to explain the pleural effusion. Pleural TB was ruled out if either histopathology or cytology was diagnostic for malignancy, or both pleural tissue culture and histopathology showed no evidence of TB, and TB was not identified from any other sample.

We calculated sensitivity and specificity of Xpert as performed on pleural fluid and pleural tissue using the two reference standards. The analysis and reporting followed the Standards for the Reporting Diagnostic Accuracy (STARD) [9].

We enrolled 96 patients between August 2012 and May 2013. The median (interquartile) age of patients was 46 (33–57) years and 20% were female. All but two patients presented with symptoms suggestive of TB (*i.e.* fever, cough, weight loss, night sweats or shortness of breath). 29% of patients had an immunocompromising illness that would put them at higher risk for TB (diabetes, end-stage renal disease, *etc.*) and 18% reported a prior history of active TB.

Based on CRS-1, we diagnosed 28 patients with active TB, of whom eight (29%) were tissue culture-confirmed. The majority (57%) of diagnoses were based solely on histopathology. Culture from pleural fluid did not yield any positive result (table 1). MTB was identified from another site (*i.e.* sputum or transbronchial biopsy) in three patients, suggesting that their pleural effusion was TB-related. Based on CRS-2, we diagnosed five additional cases of possible TB.

Xpert on pleural fluid detected four out of 25 patients with confirmed TB as defined by CRS-1 (Xpert not performed on three TB patients), resulting in a sensitivity of 16.0% (95% CI 5–36%). The specificity was 100% (66 out of 66; 95% CI 95–100%). Using the CRS-2, Xpert sensitivity was 13.3% (four out of 30; 95% CI 4–31%) and specificity 100% (61 out of 61; 95% CI 94–100%). Xpert on pleural fluid detected two out of eight cases that were tissue culture positive (table 1) and the one case that was pleural fluid smear positive (and also had an Xpert positive on a lymph node biopsy). Only one out of the four pleural fluid Xpert positive cases would have been detected based on biochemical findings (*i.e.* lymphocytic exudate with elevated ADA).

Xpert on pleural tissue in 55 patients was negative in all of the 14 confirmed TB cases as defined by CRS-1 (sensitivity 0%, 95% CI 0–23%). Three additional cases identified by CRS-2 were also Xpert negative. One false-positive result was obtained in a case with a histopathological diagnosis of malignancy, without any evidence of a concomitant TB infection (specificity 97.6%, 95% CI 87–100%) (table 1).

We observed no invalid results for Xpert testing of pleural fluid. In contrast, two invalid results were obtained when testing pleural tissue (one in a TB patient). For patients with positive Xpert result, the time to detection of TB was reduced to a few hours compared to 4 days on average for a diagnosis based on histopathology and 2–3 weeks for a diagnosis based on liquid culture.

To our knowledge this is the largest study to date evaluating the performance of Xpert using pleural tissue for the diagnosis of pleural TB. The study highlights the limited sensitivity of Xpert. Xpert performed on pleural tissue did not detect any of the identified TB cases. Other studies on Xpert have described good performance when testing tissue from various sites (*e.g.* lymph node), suggesting that it could be an alternative to culture in tissue specimens [7]. Most of these studies have used only a culture reference standard [10]. Culture, however, is limited in its ability to detect EPTB and the comparison to only culture-confirmed cases is likely to overestimate the sensitivity of Xpert. Nonetheless, in our study Xpert missed all cases with positive cultures on tissue.

The low sensitivity in pleural fluid observed in this study has been described in previous studies [5–7, 10]. Explanations for the limited sensitivity of Xpert in pleural fluid and tissue samples could relate to PCR inhibitors or insufficient sample volume in this paucibacillary disease. Further research on the optimisation of sample processing should be considered to enhance the sensitivity of the test [4]. In summary, our findings suggest that Xpert is of limited use in the diagnosis of pleural TB.

@ERSpublications
Xpert MTB/RIF, an approved molecular test for pulmonary TB, is of limited use in the diagnosis of pleural TB http://ow.ly/nSnoD

Devasahayam J. Christopher[1], Samuel G. Schumacher[2,3], Joy S. Michael[4], Robert Luo[5], Thangakunam Balamugesh[1], Paramasivan Duraikannan[1], Nira R. Pollock[6,7], Madhukar Pai[2,3] and Claudia M. Denkinger[2,3,7]
[1]Dept of Pulmonary Medicine, Christian Medical College, Vellore, and [4]Dept of Microbiology, Christian Medical College, Vellore, India. [2]McGill International TB Centre, McGill University, Montreal, and [3]Dept of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, Canada. [5]Dept of Pathology, Stanford University, Stanford, CA, [6]Dept of Laboratory Medicine, Boston Children's Hospital, Boston, MA, and [7]Division of Infectious Disease, Beth Israel Deaconess Medical Centre, Boston, MA, USA.

Correspondence: C.M. Denkinger, Dept of Epidemiology and Biostatistics, McGill University, 3650 Saint-Urbain, K-3.09, Montreal, QC, H2X 2P4, Canada. E-mail: claudia.denkinger@mail.mcgill.ca

Received: June 18 2013 | Accepted after revision: July 05 2013 | First published online: July 30 2013

Support statement: This work was supported by the Canadian Institutes of Health Research (grants MOP-89918) and a Grand Challenges Canada award (0026-01-04-01-01). M. Pai is supported by the European and Developing Countries Clinical Trials Partnership (EDCTP-TBNEAT grant) and the Fonds de recherche du Québec-Santé. C.M. Denkinger is supported by a Richard Tomlinson Fellowship at McGill University (Montreal, Canada) and a fellowship of the Burroughs–Wellcome Fund from the American Society of Tropical Medicine and Hygiene. S.G. Schumacher is supported by the Quebec Respiratory Health Training Program. The funders had no role in the analysis of data and decision to publish.

Conflict of interest: Disclosures can be found alongside the online version of this article at www.erj.ersjournals.com

Acknowledgements: We are grateful to the study coordinators (Deepa Shankar, Shabana Gulam, Priya Samon and Amala Arumugam, all Department of Pulmonary Medicine, Christian Medical College, Vellore, India), and to the physicians of the Department of Pulmonary Medicine at the Christian Medical College for their support and contributions to this study.

References

1 Dinnes J, Deeks J, Kunst H, *et al.* A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. *Health Technol Assess* 2007; 11: 1–314.
2 Porcel JM. Tuberculous pleural effusion. *Lung* 2009; 187: 263–270.
3 Steingart KR, Sohn H, Schiller I, *et al.* Xpert(R) MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database Syst Rev* 2013; 1: CD009593.
4 Weyer K, Mirzayev F, Migliori G, *et al.* Rapid molecular TB diagnosis: evidence, policy-making and global implementation of Xpert MTB/RIF. *Eur Respir J* 2013; 42: 252–271.
5 Friedrich SO, von Groote-Bidlingmaier F, Diacon AH. Xpert MTB/RIF assay for diagnosis of pleural tuberculosis. *J Clin Microbiol* 2011; 49: 4341–4342.
6 Moure R, Martin R, Alcaide F. Effectiveness of an integrated real-time PCR method for detection of the *Mycobacterium tuberculosis* complex in smear-negative extrapulmonary samples in an area of low tuberculosis prevalence. *J Clin Microbiol* 2012; 50: 513–515.
7 Tortoli E, Russo C, Piersimoni C, *et al.* Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. *Eur Respir J* 2012; 40: 442–447.
8 Vadwai V, Boehme C, Nabeta P, *et al.* Xpert MTB/RIF: a new pillar in diagnosis of extrapulmonary tuberculosis? *J Clin Microbiol* 2011; 49: 2540–2545.
9 Bossuyt PM, Reitsma JB, Bruns DE, *et al.* The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. *Ann Intern Med* 2003; 138: W1–W12.
10 Causse M, Ruiz P, Gutierrez-Aroca JB, *et al.* Comparison of two molecular methods for rapid diagnosis of extrapulmonary tuberculosis. *J Clin Microbiol* 2011; 49: 3065–3067.