Performance of Xpert MTB/RIF in the Diagnosis of Tuberculous Mediastinal Lymphadenopathy by Endobronchial Ultrasound

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Abstract

Rationale: The Xpert (GeneXpert) MTB/RIF, an integrated polymerase chain reaction assay, has not been systematically studied in extrapulmonary and in particular mediastinal tuberculosis (TB).

Objectives: To investigate the performance of Xpert MTB/RIF in the diagnosis of intrathoracic nodal TB in a large tertiary urban medical center in the UK.

Methods: We collected clinical, cytological, and microbiological data from two cohorts: 116 consecutive patients referred with mediastinal lymphadenopathy with detailed diagnostic information obtained, and an immediately subsequent second cohort of 52 consecutive patients with microbiologically confirmed mediastinal TB lymphadenopathy. All data were derived between January 2010 and October 2012. All patients underwent endobronchial ultrasound and transbronchial needle aspiration (TBNA). The performance of a single Xpert MTB/RIF assay alongside standard investigations, cytology, and microscopy/culture was evaluated against culture-confirmed TB.

Measurements and Main Results: Microbiologically confirmed TB mediastinal lymphadenopathy was diagnosed in a total of

88 patients from both cohorts. Three culture-negative cases with associated caseating granulomatous inflammation on TBNA were given a probable diagnosis. A single Xpert MTB/RIF assay demonstrated overall sensitivity for culture-positive TB of 72.6% (62.3–81.0%). Xpert specificity from cohort 1 was 96.3% (89.1–99.1%). The positive predictive value was 88.9% (69.7–97.1%), negative predictive value was 86.5% (76.9–92.1%), and odds ratio was 51.3 (24.0–98.0) for correctly identifying culture-positive disease. Xpert captured all microscopy-positive cases (14 of 14) and the majority of microscopy-negative cases (48 of 71, 67.6%). Among the cases that were culture positive by TBNA, Xpert identified two-thirds of the multiple drug-resistant TB cases, leading to immediate regimen change up to 5 weeks ahead of positive cultures. The use of Xpert combined with cytology increased the sensitivity to 96.6%.

Conclusions: Xpert MTB/RIF provides a rapid, useful, and accurate test to diagnose mediastinal nodal TB in intermediate-incidence settings. The additional use of TBNA cytology further enhances the sensitivity of Xpert. This combination can facilitate rapid risk assessment and prompt TB treatment.

Keywords: GeneXpert MTB/RIF; tuberculosis, lymph node; endobronchial ultrasound; tuberculosis diagnostics

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The polymerase chain reaction (PCR)-based Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) is a cartridge-based, automated diagnostic test that can identify *Mycobacterium tuberculosis* (MTB) and resistance to rifampicin (RIF) on biological samples in less than 2 hours. The assay has high sensitivity and specificity for the identification of both MTB and rifampicin resistance in expectorated samples of lower airways secretions. However, relatively few performance data have been published to date on nonrespiratory specimens (1–7). We previously demonstrated the usefulness of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) in the investigation of intrathoracic lymphadenopathy, with improved yield for TB when combined with cytological analysis (8). We therefore investigated how the addition of Xpert might further improve the diagnosis and management of mediastinal lymphadenopathy.

Methods

We studied two patient cohorts: cohort 1, all patients undergoing EBUS for mediastinal lymphadenopathy between January 2010 and August 2011 with data collected on diagnostic modalities to assess the specificity of Xpert; and cohort 2, a more targeted cohort of those in whom culture- and/or microscopy-positive TB mediastinal lymphadenopathy was diagnosed to assess the sensitivity of Xpert in microbiologically proven TB. Cohort 2 was extracted from all patients undergoing EBUS in the period immediately after cohort 1 with the same initial criteria. Exclusion criteria were: inadequate lymph node material obtained, current or recent TB treatment, and transfer of care out of the United Kingdom.

“Definite TB” was defined as positive microscopy (auramine stain of the aspirate) and/or culture (from a disease site including, but not limited to, mediastinal nodes) with consistent clinical features. Demographic and immunological data, including HIV status, tuberculin skin test, and interferon-gamma release assay results, were recorded as part of standard care (9).

Sedation for fiberoptic bronchoscopy was achieved with midazolam and fentanyl. TBNA samples were obtained using an echo-bronchoscope (BF-UC160F/180F-OL8; Olympus, Tokyo, Japan) and 22-gauge transbronchial aspiration needles. All nodal passes were from one or several of the following stations: 2R, 2L, 4R, 4L, 7, 10R,

10L, 11R, 11L. Between 4 and 14 passes were taken for each patient, depending on the rapid on-site evaluation of sequential samples. If rapid on-site evaluation was suggestive of TB, this station was subsequently sampled two further times and needle washings sent purely for TB PCR, smear, and culture.

TBNA specimens were concentrated by centrifugation and processed using the Xpert MTB/RIF platform; the residual deposit was decontaminated and processed using conventional microbiological techniques of microscopy with auramine-phenol and Ziehl-Neelsen staining and liquid culture (BACTEC MGIT 960 Systems; Becton Dickinson, Oxford, UK). Cytology with respect to TB was graded as follows: I, epithelioid granulomatous reaction with caseation; II, epithelioid granulomatous reaction without caseation; III, nongranulomatous reaction with necrosis; IV, nonspecific; V, inadequate sample. Positive TB cytology has been defined previously as Grade I–III (8, 10).

The study was approved as service evaluation. Patient consent was not required by the ethics committee. It was undertaken at St Mary's Hospital, Imperial College Healthcare NHS Trust.

Results

Cohort 1 comprised 132 consecutive patients undergoing EBUS between January

2010 and August 2011; 16 were excluded (inadequate material [$n = 5$], recent/current TB treatment [3], incomplete data and/or transfer of care out of center [8]). One hundred sixteen cases were further analyzed with clinical features as shown in Table 1. Follow-up was completed 6 months after recruitment. In cohort 1, 42 patients were given a final diagnosis of TB (36 definite, 6 on clinical grounds), and standard treatment was given.

Seventy-four patients (64% of cohort 1) were given a diagnosis other than TB, including 27 with sarcoidosis (23% of cohort, 34% of all non-TB cases) and 20 with cancer (17% of cohort, 27% of non-TB). Twenty-five patients had no definite disease diagnosed, with lymphadenopathy considered reactive in most cases. Three patients failed empirical anti-TB therapy, and sarcoidosis was later rediagnosed in these patients. TB diagnoses were more common in Black African and South Asian patients, in whom TB was overrepresented relative to their prevalence in the overall cohort (Table 1). Forty-five of 52 (87%) patients with definite TB in cohort 2 were of South Asian origin.

Cohort 2 comprised 57 patients in whom mediastinal TB was clinically diagnosed, from which 52 cases were confirmed microbiologically (51 culture-positive, 1 microscopy-positive only). A total of 88 microbiologically confirmed cases, 36 from cohort 1 and 52 from cohort 2, were taken for further analysis.

Table 1. Cohort 1 demographics

		% of Cohort	TB	% of All TB
Ethnicity, n				
South Asian	39	34	21	54
White UK	35	30	0	0
Black African	23	19	13	57
White Europe	7	6	0	0
Black Caribbean	5	4	1	20
Middle East	4	3	1	25
Other	3	3	0	0
Age, yr				
Range	14–85			
Average	48			
Median	46			
Sex, n				
Male	73			
Female	43			
Ratio	1.7 M:1 F			
HIV, n				
No. of patients	8			
Known diagnosis	7			
New diagnosis	1			

Definition of abbreviations: F = female; M = male; TB = tuberculosis.

Table 2. GeneXpert and cytology sensitivity data from both cohorts

	n	Sensitivity* (%)
GeneXpert	84	72.6 (62.3–81.0)
Cytology	84	92.9 (85.3–96.7)
Cytology or Xpert	88	96.6 (90.5–98.8)

Cytology refers to transbronchial needle aspirate grade 1–3.

*95% Confidence intervals shown in parentheses.

Performance of Xpert MTB/RIF

To assess sensitivity, 88 cases of definite tuberculosis were pooled from cohort 1 (36) and cohort 2 (52). Xpert demonstrated an overall sensitivity for culture-positive TB of 72.6% (95% confidence interval, 62.3–81.0) (Table 2). Within cohort 1, a single Xpert assay had a high specificity 96.3% (89.1–99.1%), high positive predictive value (PPV) and negative predictive value (NPV), and an odds ratio of 51.3 (24.0–98.0) for correctly identifying culture-positive disease (Table 3). The inclusion of three further cases of probable TB (culture-negative TB but grade 1 cytology and consistent clinical features) did not significantly alter these values.

Eighty-five of 88 (96.6%) definite TB cases originated from TBNAs (84 culture-positive, 1 microscopy-positive only); the remaining 3 cases were from extramediastinal nodal sites. Of the 85 TBNA samples, 14 were microscopy-positive, cultured at 6 to 19 days (mean, 13.3 d), and 14 of 14 (100%) of these were identified by Xpert. Seventy-one were microscopy-negative, cultured at 10 to 39 days (average, 16.5 d), and 48 of 71 (67.6%) were identified by Xpert (Figure 1). The three patients in whom culture confirmation came from an extra-TBNA source all had positive Xpert TBNAs. Xpert

identified significantly more of the earlier than the later positive cultures: 42 of 47 (89.4%) of those culture positive by Day 15 versus 19 of 37 (51.4%) culture positive at 16 to 39 days ($P < 0.0001$). The median time from EBUS to initiation of TB treatment in those with a positive Xpert in cohort 1 was 3.0 days; the median in those with negative Xpert was 8.0 days.

In addition to the definite cases of microbiologically proven TB, there were nine Xpert-positive/culture-negative cases, and six of these were judged to have TB on clinical features and given treatment.

TBNA Cytology and Combined Cytology/Xpert Performance

Positive TBNA cytology (grade I–III) showed overall diagnostic sensitivity from both cohorts of 92.9% (85.3–96.7%) for culture-positive TB (Table 2). In cohort 1, sensitivity for cytology remained high at 94.6% (82.3–98.5%), with a high NPV at 95.7% (85.8–98.8%); however, specificity and PPV were low at 55.6% (44.7–65.9%) and 49.3% (38.0–60.7%), respectively, and so the odds ratio for having TB in the event of a positive result remained low (Table 3). This reflects the potential finding of granulomatous inflammation and/or necrosis in a wide variety of diseases. Where a composite of “either cytology- or single Xpert-positive” was used, the sensitivity increased to 96.6% (90.5–98.8%), with only 3 of 85 definite cases not identified by either cytology or Xpert. It is notable that 20 of 85 (23.5%) patients with culture-positive TB had grade II TBNA cytology.

Multidrug-resistant TB

Xpert correctly identified two samples (one smear positive, one smear negative) for rifampicin resistance within 24 hours of processing and up to 5 weeks ahead of

drug-susceptibility test results, enabling appropriate treatment at the outset. A third smear-negative case was not captured by Xpert despite the fact that known mutations D516A and L533P included within the Xpert MTB/RIF system were ultimately found by the local reference laboratory line probe assay. There were no false-positive results.

HIV-TB

Of the nine HIV-positive cases, TB was diagnosed and treated in six (four definite, two clinical diagnoses—grade II cytology, immunodiagnostics positive, response to treatment), one pulmonary *Mycobacterium avium-intracellulare complex* (MAC) infection, one sarcoidosis, and one indeterminate. Five of six (83.3%) TBNAs from those receiving diagnoses and given TB treatment were positive by Xpert, whereas only one of six was microscopy positive. All three non-TB cases were Xpert negative, including the microscopy-positive MAC case.

Non-TB Diagnoses

Non-TB diagnoses were available in detail for cohort 1. From this cohort, sarcoidosis was diagnosed in 27 cases (23.3%) based on cytology, negative cultures, and clinical features: 22 of 25 (88%) were immunodiagnosis negative; cytology grade II in 24 of 27 (89%) cases, grade IV in 3 of 27 (11%); all were Xpert negative. No patient with grade I appearances had sarcoidosis. Cancer was diagnosed in 20 cases (17%); this includes one potentially false-positive Xpert result: an 82-year-old white woman from London found to have small cell lung cancer was Xpert positive but microscopy/culture negative and had no features specific for TB. Imaging showed mediastinal node calcification suggesting past and latent tuberculous infection.

Table 3. GeneXpert and cytology performance data from cohort 1 only

	n	Sensitivity, (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR–	OR
GeneXpert	36	66.7 (47.4–79.3)	96.3 (89.1–99.1)	88.9 (69.7–97.1)	86.5 (76.9–92.1)	17.8 (5.8–55.9)	0.35 (0.2–0.6)	51.3 (24.0–98.0)
Cytology	36	94.6 (82.3–98.5)	55.6 (44.7–65.9)	49.3 (38.0–60.7)	95.7 (85.8–98.8)	2.1 (1.6–2.8)	0.1 (0.03–0.4)	21.9 (7.2–55.0)

Definition of abbreviations: LR– = negative likelihood ratio; LR+ = positive likelihood ratio; NPV = negative predictive value; OR = odds ratio; PPV = positive predictive value.

Cytology refers to transbronchial needle aspirate grade 1–3; 95% confidence intervals shown in parentheses.

Figure 1. Breakdown of culture-positive transbronchial nodal aspirates; microscopy/culture vs. GeneXpert. NEG = negative; PCR = polymerase chain reaction; POS = positive; TB = tuberculosis.

Discussion

We have examined the role of the Cepheid GeneXpert MTB/RIF PCR platform in the largest study of mediastinal lymphadenopathy reported to date. A recent study evaluated high pretest probability cases investigated with EBUS and included data on nucleic acid amplification (non-Xpert assay) with positive results in 7 of 24 patients (11). Tortoli and colleagues described a study of Xpert in extrapulmonary TB but encompassing samples from a range of disease sites (6). Our first cohort consisted of all patients consecutively referred to the service without preselection. We have shown high sensitivity and very high specificity when Xpert is compared with the gold standard of positive culture. Overall Xpert sensitivity for microbiologically proven TB in our study of 72.6% is consistent with reported sensitivities of 61 to 98% in respiratory (5, 7, 12–15) and nonrespiratory samples (3–7). In the only comparable study of Xpert on lymph node material performed in South Africa, the 97% sensitivity (29 of 30 cases correctly identified) was in cervical nodes in a high HIV-prevalence setting (4). Reported sensitivity is consistently very high in microscopy-positive cases, and all 14 such cases in our cohort were correctly identified, whereas 48 of 71 (68.6%) microscopy-negative but culture-proven cases correctly identified also compares favorably with previous literature.

Xpert specificity for definite TB at 96.3% was consistent with specificities reported in the literature of 97 to 100% in

respiratory and nonrespiratory samples (3–7, 12–15). However, the gold standard of culture is likely to underestimate actual TB, and, notably, there were nine Xpert-positive/culture-negative cases, six of whom were given clinical diagnoses as judged by clinical features and given treatment.

The PPV of Xpert alone was high at 88.9%, similar to the 94% from the South African study (4). The NPV was 86.5% and reflects the lower sensitivity of the assay in our cohort. Xpert in isolation gave a high positive likelihood ratio of nearly 18 and a high odds ratio of 51 and therefore would support the use of a positive result to start empirical treatment pending microbiological data or justify it in its absence. However, its modest negative likelihood ratio does not strongly refute a diagnosis where other factors support the diagnosis.

Our finding that sensitivity of Xpert is greater in TBNA achieving a faster positive culture is consistent with other findings in respiratory specimens and likely relates to bacillary biomass (16, 17). Mediastinal nodal aspirates, traditionally believed to be paucibacillary, can still be captured by Xpert. The small subgroup of HIV-positive individuals in this study does not allow strong conclusions, but the higher sensitivity of Xpert (80% positive) is consistent with the hypothesis that they have a greater bacillary burden.

One limitation of this study is the absence of a universally accepted gold standard other than culture and therefore disagreement on how to attribute a case to TB in its absence. It is possible that the gold standard should include both those

culture positive and Xpert positive. Xpert may be generating an increased yield through improved sensitivity compared with culture, with six culture-negative cases clinically consistent with TB.

This study is broadly representative of the ethnic presentation of TB in London, which currently has an intermediate incidence for tuberculosis of 42 of 100,000 (Public Health England). Although no formal preselection took place in enrollment, we acknowledge that the group referred for EBUS is already selected from the background community with respect to mediastinal nodal disease and may impart inclusion bias when considering Xpert performance in a given TB incidence setting.

In summary, EBUS-TBNA is increasingly used as a tool for microbiological diagnosis in mediastinal disease. We have demonstrated that a single GeneXpert assay in this setting and in isolation can provide improved sensitivity when compared with microscopy alone and has very high specificity. Taken together with other supporting features that include cytology, Xpert further increased sensitivity and specificity, improved predictive values, and facilitated more rapid assessment of suspected TB. Although rapid detection of rifampicin resistance enabled immediate treatment changes several weeks ahead of culture, the inability of the Xpert system to identify all *rpoB* mutations highlights the need for alternative sequence-based detection methods. The evidence here provides support for the use of GeneXpert testing in low-intermediate incidence settings where mediastinal tuberculosis is suspected. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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