Xpert MTB/RIF for Rapid Diagnosis of Tuberculous Lymphadenitis from Fine-Needle-Aspiration Biopsy Specimens[▽]

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This study demonstrates the excellent diagnostic accuracy of the Xpert MTB/RIF test in patients with tuberculous lymphadenitis. The test sensitivity and specificity were 96.7% (95% confidence interval [CI], 86.6 to 100%) and 88.9% (95% CI, 69.6 to 100%), respectively, and it correctly identified 6/6 (100%) of the cytology smear-negative/culture-positive cases and 1 of 2 (50%) rifampin-resistant cases.

Tuberculous lymphadenitis is the most common extrapulmonary manifestation of tuberculosis (TB) (4, 8), and the majority of cases have no active lung involvement. Fine-needle aspiration biopsy (FNAB) offers a feasible and safe option for specimen collection (11, 15). The use of cytology together with the confirmation of acid fastness by Ziehl-Neelsen (ZN) staining and Papanicolaou stain-induced fluorescence microscopy as well as mycobacterial detection by culture offers excellent yields (13, 14) but remains limited by the absence of species confirmation, slow turnaround times, and/or lack of drug resistance guidance. Conventional microbiological culture and drug susceptibility testing are not always available and in rare instances may take 6 weeks or longer (10).

The World Health Organization (WHO)-endorsed Xpert MTB/RIF combines sample processing and real-time PCR in a fully automated platform and detects *Mycobacterium tuberculosis* complex and rifampin resistance in less than 2 h (2, 3, 9). Xpert MTB/RIF has been used successfully on various extrapulmonary specimens, including urine and stool (6), but has not been rigorously evaluated with the use of tissue specimens, such as FNAB specimens.

To determine the diagnostic utility of the Xpert MTB/RIF, FNAB specimens were collected from 50 consenting patients by aspirating two passes of a 23- or 25-gauge needle attached to a 10-ml syringe. Two smears were prepared from each aspirate, one fixed with commercial cytology fixative for Papanicolaou staining and evaluation by fluorescence microscopy and the other air dried for Giemsa and subsequent ZN staining.

Smears were evaluated for adequacy and for a morphological diagnosis, and cases were excluded from the analysis if either one or both passes had inadequate cellular material on smears. Both ZN and Papanicolaou stain-induced fluorescence microscopy evaluations were performed for direct detection of mycobacteria on all specimens. Residual material from one of the aspirates was rinsed in a mycobacterial growth indicator tube (MGIT 960; Becton Dickinson) by aspirating a small volume of fluid into the syringe and expressing it back into the MGIT 960 tube, followed by incubation in a MGIT 960 instrument for mycobacterial culture. Positive cultures were identified as belonging to the *Mycobacterium tuberculosis* complex, and genotypic drug susceptibility testing was done using the genotype MTBDR_{plus} assay (Hain Lifescience, Germany) (1).

The residual material from the remaining aspirate was rinsed as described above into 0.7 ml sterile phosphate-buffered saline (PBS) in a 10-ml headspace glass vial sealed with a TFE/Sil Septa and aluminum open-top seal. Sample preparation buffer was then added to the vial in a 2:1 ratio, incubated at room temperature, and subsequently processed for Xpert MTB/RIF testing as previously described (2).

Performance calculations, including test sensitivity, specificity, and predictive values, were done using Statistica version 8 to compare the diagnostic performance of the Xpert MTB/RIF test to the reference standard (as positive cytology [cytomorphology consistent with mycobacterial infection and direct visualization of the organism on ZN and/or Papanicolaou stain-induced fluorescence microscopy] and/or culture positive for *M. tuberculosis*) (11, 12).

Of the 50 patients recruited, 48 cases had adequate smears for diagnosis (see Fig. 1 for the patient recruitment flow diagram). In total, cytomorphological features associated with TB were seen in 32 (66.7%) patients, nonspecific reactive nodes were identified in 10 (20.8%), acute bacterial lymphadenitis

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* Reference standard = Cytomorphology suggestive of TB with direct visualization of the organism and/or bacteriological culture
 FNAB = Fine Needle Aspiration Biopsy

FIG. 1. Flow diagram of all patients referred for fine-needle aspiration biopsy with possible mycobacterial lymphadenitis.

was seen in 1 (2.1%), malignancy was seen in 4 (8.3%), and an epithelial inclusion cyst was seen in 1 (2.1%) (Table 1).

Compared to the reference standard, Xpert MTB/RIF correctly identified 29 out of 30 TB cases (sensitivity, 96.7%; 95% CI, 86.6 to 100) (Table 2). The possible false-negative result had a prolonged transit interval of 9 days before Xpert MTB/RIF testing, which may have affected the result. Xpert MTB/RIF was positive in two cases, with negative cytomorphology and culture (specificity, 88.9%; 95% CI, 69.6 to 100). The cytomorphology from one of the false-positive results showed a necrotizing suppurative lymphadenitis, which is consistent with TB. However, no organisms could be identified on microscopy or culture. The cytomorphology of the other false-positive result showed an epithelial inclusion cyst, and the reason for this false-positive result remains unknown. One case had a negative culture result with positive cytology (including mycobacterial identification) and a positive Xpert MTB/RIF

test. This patient had been on TB treatment for 1 month at the time of specimen collection, which provides the likely explanation for this discrepant result.

The Xpert MTB/RIF test was positive in all 6 smear-negative culture-positive cases and correctly identified 1 of the 2 rifampin-resistant cases. The average time to results for microbiological culture was 18.5 days (range, 9 to 55 days), while the Xpert MTB/RIF test result was available within 2 h of commencing the test. This represents a substantial reduction in diagnostic delay, thereby permitting real-time decision making and planning of treatment (5).

A recent study by Hillemann et al. demonstrated the effectiveness of Xpert MTB/RIF on extrapulmonary tissue (6). In that study, the combined sensitivity and specificity of 77.3% and 98.2% were reported, respectively. Our study is the first to evaluate the performance of Xpert MTB/RIF in diagnosing tuberculous lymphadenitis through the use of

TABLE 1. Demographics and diagnostic outcomes of patients referred for fine-needle aspiration biopsy with possible mycobacterial lymphadenitis

Characteristic	No. of patients (<i>n</i> = 48)	% of patients
Age		
<5 yrs	2	4.2
5–20 yrs	6	12.5
>20 yrs	40	83.3
Gender		
Male	20	41.7
Female	28	58.3
On TB treatment	2	4.2
HIV infection status		
Positive	9	18.8
Negative	3	6.6
Unknown	36	75
Culture positive/HIV positive	4/9	44.4
Cytological features		
Reactive lymph node	10	20.8
Features consistent with TB	32	66.7
Acute bacterial infection	1	2.1
Malignancy or suggestive of malignancy	4	8.3
Epithelial inclusion cyst	1	2.1
Cases with cytomorphology suggestive of TB ^a		
Smear +, culture +, GeneXpert +	22	45.8
Smear +, culture +, GeneXpert –	1	2.1
Smear –, culture +, GeneXpert +	6	12.5
Smear +, culture –, GeneXpert +	1	2.1
Smear –, culture –, GeneXpert +	1	2.1
Smear –, culture –, GeneXpert –	1	2.1

^a Smear +, cytomorphology suggestive of TB with direct visualization of the organism. +, positive; –, negative.

FNAB specimens. Study limitations include the small number of rifampin-resistant cases identified and the fact that the research was conducted in a referral center, as ideally the technique is suited to use in peripheral laboratories to be effective in controlling the disease. A positive aspect of the study is that the patient population is representative of patients presenting with peripheral lymphadenopathy in most areas where TB/HIV are endemic. It is unlikely that our patient cohort had exacerbated disease compared to patients presenting at primary health care clinics, as these patients are routinely referred from the primary health care clinic to the referral center for FNAB.

In conclusion, FNAB is a simple procedure which can be performed in an outpatient setting by clinicians or nursing staff after a short training period (7, 15). It is ideal for use in resource-limited settings, including more remote and rural areas (15). Specimen collection is simple and safe. With the use of a transport vial, virtually no sample preparation is required and there is minimal risk of contamination. Furthermore, the transmission risk to the operator may also be reduced. Combining FNAB and rapid genotypic diagnosis using automated systems should greatly improve access to

TABLE 2. Diagnostic accuracy of the Xpert MTB/RIF test versus various reference standards^a

Reference standard	Sensitivity		Specificity		Predictive value (%)	
	<i>n/N</i> (%)	95% CI	<i>n/N</i> (%)	95% CI	Positive	Negative
Xpert vs reference standard	29/30 (96.6)	86.6–100	16/18 (88.9)	69.6–100	93.5	94.1
Xpert vs culture	28/29 (96.6)	86.1–100	16/19 (84.2)	63.6–100	90.3	94.1
Xpert vs smear +, culture +	6/6 (100)	100	100	100	100	100
Autofluorescence vs culture	22/29 (75.9)	60.3–91.4	18/19 (94.7)	79.3–100	95.7	72
ZN vs culture	12/29 (41.4)	23.5–59.3	19/19	100	100	52.8

^a Reference standard is as defined in the text (positive cytology [cytomorphology consistent with mycobacterial infection with direct visualization of the organism] and/or mycobacterial culture). ZN, Ziehl-Neelsen stain; *n*, index group; *N*, control group; –, negative; +, positive.

appropriate diagnosis and treatment for patients with tuberculous lymphadenitis.

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