Xpert® MTB/RIF for the 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 was 96.7% (95%CI, 86.6-100) and 88.9% (95%CI, 69.6-100) respectively, and 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 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 staining and Papanicolaou stain-induced fluorescent microscopy as well as mycobacterial detection by culture offers excellent yields (13,14), but remains limited by the absence of species confirmation, slow turn-around 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 polymerase chain reaction (PCR) in a fully automated platform and detects *Mycobacterium tuberculosis* complex and rifampin resistance in less than 2 hours (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.

To determine the diagnostic utility of the Xpert® MTB/RIF, FNAB were collected from 50 consenting patients by aspirating two passes of a 23- or 25-g needle attached to a 10ml syringe (IRB 05/03/043). Two smears were prepared from each aspirate, one fixed with commercial cytology fixative for Papanicolaou staining and evaluation by fluorescent 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 fluorescent 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, USA) 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 *Mycobacterium tuberculosis*
complex and genotypic drug susceptibility testing was done using Genotype MTBDRplus
assay (Hain Lifesciences, Germany) (1).

The residual material from the remaining aspirate was rinsed as above into 0.7 ml sterile
phosphate buffered saline (PBS) in a 10ml 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 fluorescent microscopy) and/or culture positive for *M. tuberculosis* (11,12)).
Of the 50 patients recruited, 48 cases had adequate smears for diagnosis (see Figure 1 for patient recruitment flow diagram). In total, cytomorphological features associated with TB were seen in 32 (66.7%) patients, non-specific reactive nodes identified in 10 (20.8%), acute bacterial lymphadenitis in 1 (2.1%), malignancy in 4 (8.3%) epithelial inclusion cyst 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, 86.6-100) (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, 69.6-100). 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 one 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 the 1 of the 2 rifampin resistant cases. The average time to result for
microbiological culture was 18.5 days (range 9-55 days), while the Xpert® MTB/RIF test result was available within 2 hours 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% was reported, respectively. Our study is the first to evaluate the performance of Xpert® MTB/RIF in diagnosing tuberculous lymphadenitis through the use of 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 TB/HIV endemic areas. It is unlikely that our patient cohort had exacerbated disease as compared to patients presenting at primary health-care clinics as these patients are routinely referred from the primary health-care clinic to the referral centre 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
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appropriate diagnosis and treatment for patients with tuberculous lymphadenitis.

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129
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181 needle aspiration biopsy: cytomorphology, ZN staining and autofluorescence --

184 Mycobacterial autofluorescence in Papanicolaou-stained lymph node aspirates: a

187 biopsy: an undervalued diagnostic modality in paediatric mycobacterial disease.
Table 1: Demographics and diagnostic outcome of patients referred for fine needle aspiration biopsy with possible mycobacterial lymphadenitis.

<table>
<thead>
<tr>
<th>Age</th>
<th>Number (N=48)</th>
<th>Percent</th>
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</thead>
<tbody>
<tr>
<td>&lt;5 years</td>
<td>2</td>
<td>4.2</td>
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<tr>
<td>5-20 years</td>
<td>6</td>
<td>12.5</td>
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<tr>
<td>&gt;20 years</td>
<td>40</td>
<td>83.3</td>
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<table>
<thead>
<tr>
<th>Gender</th>
<th>Number (N=48)</th>
<th>Percent</th>
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</thead>
<tbody>
<tr>
<td>Male</td>
<td>20</td>
<td>41.7</td>
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<tr>
<td>Female</td>
<td>28</td>
<td>58.3</td>
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<table>
<thead>
<tr>
<th>Patients on TB treatment</th>
<th>Number (N=48)</th>
<th>Percent</th>
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<tr>
<td></td>
<td>2</td>
<td>4.2</td>
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</table>

<table>
<thead>
<tr>
<th>HIV infection status</th>
<th>Number (N=48)</th>
<th>Percent</th>
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<tbody>
<tr>
<td>Positive</td>
<td>9</td>
<td>18.8</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>6.6</td>
</tr>
<tr>
<td>Unknown</td>
<td>36</td>
<td>75</td>
</tr>
<tr>
<td>Culture +/-HIV +</td>
<td>4/9</td>
<td>44.4</td>
</tr>
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<table>
<thead>
<tr>
<th>Cytological features</th>
<th>Number (N=48)</th>
<th>Percent</th>
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<tbody>
<tr>
<td>Reactive lymph node</td>
<td>10</td>
<td>20.8</td>
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<tr>
<td>Features consistent with TB</td>
<td>32</td>
<td>66.7</td>
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<tr>
<td>Acute bacterial infection</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>Malignant or suggestive of malignancy</td>
<td>4</td>
<td>8.3</td>
</tr>
<tr>
<td>Epithelial inclusion cyst</td>
<td>1</td>
<td>2.1</td>
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</table>
Cases with cytomorphology suggestive of TB

<table>
<thead>
<tr>
<th>Smear, Culture, GeneXpert</th>
<th>Count</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>+, +, +</td>
<td>22</td>
<td>45.8</td>
</tr>
<tr>
<td>+, +, −</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>−, +, +</td>
<td>6</td>
<td>12.5</td>
</tr>
<tr>
<td>+, −, +</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>−, −, +</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>−, −, −</td>
<td>1</td>
<td>2.1</td>
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</tbody>
</table>

*a Smear + = Cytomorphology suggestive of TB with direct visualization of organism.

+ positive, − negative
Table 2: Diagnostic accuracy of the Xpert® MTB/RIF test versus various reference standards.

<table>
<thead>
<tr>
<th>Reference standard</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Predictive values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/N (%)</td>
<td>95% CI</td>
<td>n/N (%)</td>
</tr>
<tr>
<td>Xpert® vs. Reference Standard*</td>
<td>29/30 (96.6)</td>
<td>86.6-100</td>
<td>16/18 (88.9)</td>
</tr>
<tr>
<td>Xpert® vs. Culture</td>
<td>28/29 (96.6)</td>
<td>86.1-100</td>
<td>16/19 (84.2)</td>
</tr>
<tr>
<td>Xpert® vs. smear−Culture+</td>
<td>6/6 (100)</td>
<td>100</td>
<td>(100)</td>
</tr>
<tr>
<td>Autofluorescence vs. Culture</td>
<td>22/29 (75.9)</td>
<td>60.3-91.4</td>
<td>18/19 (94.7)</td>
</tr>
<tr>
<td>ZN vs. Culture</td>
<td>12/29 (41.4)</td>
<td>23.5-59.3</td>
<td>19/19</td>
</tr>
</tbody>
</table>

*Reference standard 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.
LEGENDS

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

Initial study group n=50

Cytological screening

Inadequate FNAB for diagnosis n=2

Cytomorphology consistent with an inflammatory process in a lymph node

Yes n=43

Xpert®MTB/RIF n=43

Positive n=30

Reference standard
Yes n=29

No n=1

Negative n=13

Reference standard
Yes n=1

No n=12

No n=1

Positve n=1

Reference standard
Yes n=0

No n=1

Negative n=4

Reference standard
Yes n=0

No n=4

Yes n=1

No n=1

*Reference standard = Cytomorphology suggestive of TB with direct visualization of the organism and/or bacteriological culture

FNAB = Fine Needle Aspiration Biopsy