TBM Diagnostics: Xpert Ultra

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Diagnostics History

• Ziehl–Neelsen stain, 1882-1883

• Culture
  – 1882 Koch => Solid Media
  – 1980 BD Bactec instrument
  – 1998 MGIT 960 instrument

• PCR
  – 1990’s Gen-Probe & Roche
  – Dec 2010 Xpert MTB/RIF
  – 2017 Xpert MTB/RIF Ultra
Analytical Sensitivity

- Ziehl–Neelsen stain, ~5000 CFUs
- Culture ~ 10 CFUs

- Xpert MTB/RIF ~100 CFUs
Xpert: Analytical Sensitivity

~100 CFU for a positive Xpert MTB/RIF (Range: 80-130)
Analytical Sensitivity

- Ziehl–Neelsen stain  ~ 5000 CFUs
- Culture  ~ 10 CFUs
- Xpert MTB/RIF  ~ 100 CFUs
- Xpert MTB/RIF Ultra  ~ 10 CFUs
Xpert MTB/RIF

• More volume = More sensitivity
  – Patel et al.
    – 1mL raw = 47% sensitivity vs. Culture
    – 3mL centrifuged = 82% sensitivity vs. Culture

• Nhu et al.
  – centrifuged all CSF, Sensitivity 59% vs. Case Definition
  – Xpert CSF tested: 17% <.8mL; 61% .8-2mL; 22% >2mL

• Bahr et al.
  – 27% sensitivity with 2mL CSF; 72% sensitivity centrifuged
  – Culture 71% sensitivity
## Uganda TBM Results 2015

<table>
<thead>
<tr>
<th>CSF Test</th>
<th>N</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xpert 2mL un-centrifuged CSF</td>
<td>107</td>
<td>28% (5/18)</td>
<td>100% (89/89)</td>
<td>100% (5/5)</td>
<td>87% (89/102)</td>
</tr>
<tr>
<td>Xpert centrifuged CSF</td>
<td>95</td>
<td>72% (13/18)</td>
<td>100% (77/77)</td>
<td>100% (13/13)</td>
<td>94% (77/82)</td>
</tr>
<tr>
<td>Culture</td>
<td>80</td>
<td>71% (12/17)</td>
<td>100% (63/63)</td>
<td>100% (12/12)</td>
<td>93% (63/68)</td>
</tr>
<tr>
<td>AFB Smear by Microscopy</td>
<td>107</td>
<td>22% (4/18)</td>
<td>100% (89/89)</td>
<td>100% (4/4)</td>
<td>86% (89/103)</td>
</tr>
</tbody>
</table>

**Xpert Protocols:**
P=0.008 by McNemar’s test

Xpert MTB/Rif Centrifuged CSF

Xpert MTB/Rif 2mL raw CSF

0 AFB 3
Smear

1

1

3

3

4

1

5

MGIT Culture

Xpert MTB/RIF Ultra
What’s new?

• **Xpert® MTB/RIF Ultra**
  – Larger PCR reaction chamber
  – Improved PCR Probes
    • *Mtb* Targets: IS6110, IS1081, rpoB
  – Sensitivity of ~10 CFU/mL
  
  – Pulmonary clinical trial (FIND & TB-CDRC)
Xpert Ultra in TBM

- Prospectively 129 recruited subjects in Mbarara, Uganda.
- April 2015 - Dec 2016
- Received Xpert Ultra in Nov 2016
- Tested in Dec 2016
- Manuscript written Jan 2016

Supported via R01NS086312; UK MRC/Wellcome Trust: MR/M007413/1
Xpert Ultra Methods

Reference standard:
1) Any positive TB test
2) Probable / Definite TBM per Uniform Case Definition – excluding Xpert Ultra results

Median of 8mL CSF collected, centrifuged 2mL pellet divided for testing.
## Participant Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>TB Meningitis N=22</th>
<th>Other Meningitis N=107</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>32 (30-34)</td>
<td>34 (29-43)</td>
<td>0.24</td>
</tr>
<tr>
<td>Men, percent</td>
<td>59% (13/22)</td>
<td>54% (58/107)</td>
<td>0.82</td>
</tr>
<tr>
<td>Headache duration, days</td>
<td>7 (6.5-14)</td>
<td>4.5 (7-14)</td>
<td>0.83</td>
</tr>
<tr>
<td>CD4 T cell count, cells/µL</td>
<td>72 (43-124)</td>
<td>88 (15-226)</td>
<td>0.83</td>
</tr>
<tr>
<td>Serum C-reactive protein, mg/L</td>
<td>49 (16-74)</td>
<td>52 (11-108)</td>
<td>0.48</td>
</tr>
<tr>
<td>CSF white cells/µL</td>
<td>12 (&lt;4-130)</td>
<td>5 (&lt;4-38)</td>
<td>0.24</td>
</tr>
<tr>
<td>CSF lymphocytes, %</td>
<td>70% (65-83%)</td>
<td>75% (59-80%)</td>
<td>0.89</td>
</tr>
<tr>
<td>CSF total protein, mg/dL</td>
<td>255 (90-510)</td>
<td>170 (65-365)</td>
<td>0.023</td>
</tr>
<tr>
<td>CSF glucose, mg/dL</td>
<td>49 (32-71)</td>
<td>70 (49-95)</td>
<td>0.002</td>
</tr>
<tr>
<td>Antecedent/Prior TB diagnosis</td>
<td>18% (4/22)</td>
<td>3.3% (3/91)</td>
<td>0.026</td>
</tr>
<tr>
<td>Physician TB meningitis diagnosis*</td>
<td>59% (13/22)</td>
<td>43% (44/102)</td>
<td>0.24</td>
</tr>
<tr>
<td>Alive at discharge / last contact</td>
<td>50% (11/22)</td>
<td>70% (64/91)</td>
<td>0.082</td>
</tr>
</tbody>
</table>
## CSF Diagnostic Performance

<table>
<thead>
<tr>
<th>CSF Test</th>
<th>Volume Tested</th>
<th>N</th>
<th>Sensitivity vs. composite end point</th>
<th>Sensitivity vs. case definition</th>
<th>Assay Error Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xpert Ultra</td>
<td>0.5mL</td>
<td>129</td>
<td>95% (21/22)</td>
<td>70% (16/23)</td>
<td>2.3% (3/129)</td>
</tr>
<tr>
<td>Xpert MTB/Rif</td>
<td>1.0mL</td>
<td>129</td>
<td>45% (10/22)</td>
<td>43% (10/23)</td>
<td>4.7% (6/129)</td>
</tr>
<tr>
<td>MGIT Culture</td>
<td>0.5mL</td>
<td>129</td>
<td>45% (10/22)</td>
<td>43% (10/23)</td>
<td>1.6% (2/129)</td>
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Composite end-point included any positive CSF Xpert Ultra, Xpert, or Bactec Mycobacterial growth indicator tube (MGIT) culture. Sensitivity vs. uniform clinical case definition for definite (n=14) or probable (n=9) TB meningitis excluded Xpert Ultra results in defining case status. Error in culture reflects contamination with non-tuberculous mycobacterium growth.
Of 8 participants positive by Xpert Ultra only, 6 had recently initiated HIV therapy, and all 8 were negative by testing with:

- Cryptococcal Antigen
- Gram’s stain,
- culture, and
- multiplex PCR for 14 common meningitis pathogens (Biofire).
Next Gen Sequencing for Confirmation

Sequencing from the residual volume left over in the Xpert Ultra cartridges

<table>
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<tr>
<th>ID</th>
<th>Xpert Ultra Category</th>
<th>MGIT Culture</th>
<th>Xpert MTB/RIF</th>
<th>Next Generation Sequencing of M. tuberculosis Genes</th>
</tr>
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<tr>
<td>5001</td>
<td>Trace</td>
<td>Negative</td>
<td>Negative</td>
<td>IS6110 detected</td>
</tr>
<tr>
<td>5004</td>
<td>Very Low</td>
<td>Positive</td>
<td>Positive</td>
<td>IS6110, IS1081, rpoB (WT) detected</td>
</tr>
<tr>
<td>5005</td>
<td>Trace</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative*</td>
</tr>
<tr>
<td>5103</td>
<td>Trace</td>
<td>Positive</td>
<td>Negative</td>
<td>IS6110 detected</td>
</tr>
<tr>
<td>5194</td>
<td>Very Low</td>
<td>Positive</td>
<td>Positive</td>
<td>IS6110, IS1081, rpoB (WT) detected</td>
</tr>
<tr>
<td>5116</td>
<td>Trace</td>
<td>Negative</td>
<td>Negative</td>
<td>IS6110 detected</td>
</tr>
<tr>
<td>5285</td>
<td>Very Low</td>
<td>Negative</td>
<td>Positive</td>
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Acknowledgements

Nathan Bahr
Fiona Cresswell
Graeme Meintjes

Radha Rajasingh
David Meya
Conrad Muzoora
Josh Rhein
Darlisha Williams
Reuben Kiggundu
Mahsa Abassi
Kathy Huppler Hullsiek

Financial support for this research was provided by the National Institute of Neurologic Diseases and Stroke; National Institute of Allergy and Infectious Diseases; UK MRC/Wellcome Trust Global Clinical Trials

Supported via R01NS086312;
UK MRC/Wellcome Trust: MR/M007413/1
Pathogen-Response Framework

Modified from:
Diagnostic Framework: TB Meningitis

Organism Burden

Upward movement on the y-axis represents an increase in organism burden. The y-axis is labeled with different diagnostic methods:

- AFB Smear: ~5000 CFU/mL
- GeneXpert: ~100 CFU
- Culture: 10 CFU/mL

These methods are used to detect the presence of Mycobacterium tuberculosis in the patient's samples.

The x-axis represents the strength of the immune response:

- Weak
- Strong

Effective Th1 Immune Response

The diagram illustrates the relationship between organism burden and immune response. When the immune response is strong, subclinical (latent) infection can be detected with AFB Smear at ~5000 CFU/mL. As the immune response weakens, the organism burden increases, leading to clinical disease and eventually death.