Improved diagnosis of extrapulmonary tuberculosis by antigen detection using immunochemistry-based assay

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Overview

• Introduction: extrapulmonary tuberculosis (TB) & diagnostic challenges

• New diagnostic method
  – Biopsies
  – Fluids

• Further research plans
Burden & distribution of extrapulmonary TB

Source: United States, CDC, 2008
Burden of extrapulmonary TB (2)

• HIV-TB co-infection - > 50% extrapulmonary

• Pediatric TB – higher proportion extrapulmonary
Is extrapulmonary TB infectious?

- EPTB / sputum neg TB accounts for 13% of TB transmission (Tostmann, 2008)

- Transmission from genitourinary TB (D’ Agata, 2001)
Diagnostic challenges EPTB

- Clinical criteria without lab: over-diagnosis (~25%)
- Acid-fast stain/microscopy: detection limit 10000 bacilli/ml
- Culture: detection limit 100 bacilli/ml
- Histology/cytology
  - differentiation from other granulomatous diseases
  - atypical histological with HIV coinfection
- PCR based methods: better sensitivity, expensive, PCR machine, sensitive to contamination
- Serology: not recommended
Immunohistochemistry & immunocytochemistry

- Immunochemistry - more sensitive than acid fast staining; intact bacillary cell-wall is not a prerequisite.

- Potential to distinguish between different mycobacterial species.

- Limited studies for use as a diagnostic test probably due to non-availability of specific antibodies for *M. tuberculosis* antigens
MPT64 antigen

- In-house rabbit polyclonal antibody for detection of MPT64 antigen: 26-kDa secreted mycobacterial protein
  - specific for the *M. tuberculosis* complex
  - Distinguish pathogenic from atypical mycobacteria.
Material

- Extrapulmonary TB
  - Lymph nodes
  - Pleura
  - Abdomen
  - CNS
- Tissue biopsies - pleura, lymph nodes, abdominal TB
- Cell smears - pleural fluid, ascitic fluid, CSF & lymph node aspirates
Diagnostic procedures

– Acid-fast staining
– Culture (LJ medium)
– Immunohistochemistry/immunocytochemistry
– PCR for IS6110 (specific for *M. tuberculosis* complex)
### Positive results of diagnostic procedure in TB and non-TB biopsies

<table>
<thead>
<tr>
<th>Diagnostic Procedure</th>
<th>Cervical lymph node biopsies n(%)</th>
<th>Abdominal biopsies n(%)</th>
<th>Pleural biopsies (HIV coinfection) n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TB* (n=120)</td>
<td>TB* (n=33)</td>
<td>TB* (n=25)</td>
</tr>
<tr>
<td></td>
<td>Non-TB (n=32)</td>
<td>Non-TB (n=18)</td>
<td>Non-TB (n=11)</td>
</tr>
<tr>
<td>AFB stain</td>
<td>14(12)</td>
<td>0</td>
<td>2 (8)</td>
</tr>
<tr>
<td>LJ Culture**</td>
<td>27(22)</td>
<td>4 (12)</td>
<td>3(12)</td>
</tr>
<tr>
<td>Immunochemistry-MPT64</td>
<td>96(80)</td>
<td>25(76)</td>
<td>20 (80)</td>
</tr>
<tr>
<td>Nested-PCR</td>
<td>104(87)</td>
<td>28(85)</td>
<td>16 (64)</td>
</tr>
</tbody>
</table>
Validity of MPT64-IHC as diagnostic test

- PCR for IS6110 (specific for \textit{M. tuberculosis} complex) was used as “gold standard”

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Sensitivity</th>
<th>Specificity</th>
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</thead>
<tbody>
<tr>
<td><strong>LYMPH GLAND</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norway (n=32)</td>
<td>95</td>
<td>62</td>
</tr>
<tr>
<td>Tanzania (n=35)</td>
<td>88</td>
<td>90</td>
</tr>
<tr>
<td>India (n=152)</td>
<td>93</td>
<td>98</td>
</tr>
<tr>
<td><strong>ABDOMINAL TB</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>India (n=51)</td>
<td>89</td>
<td>95</td>
</tr>
<tr>
<td><strong>Pleura</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(HIV-coinfection)</td>
<td>72 (80)</td>
<td>61 (100)</td>
</tr>
<tr>
<td>South Africa (n=36)</td>
<td></td>
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</tr>
</tbody>
</table>
M.tuberculosis specific protein MPT64 in TB lymph nodes
M. tuberculosis specific protein MPT64 in TB lymph nodes
*M. tuberculosis* specific protein MPT64 antigen in abdominal TB lesions

Intestinal wall  Peritoneum
Mycobacterial MPT64 antigen in HIV-TB coinfected pleural TB lesions

Granulomatous inflammation, n=14

MPT64
Mycobacterial MPT64 antigen in HIV-TB coinfected pleural TB lesions - atypical histology

H&E stain

Acid-fast stain

IHC-MPT64
## Positive results of various diagnostic procedures on fluids & aspirate

<table>
<thead>
<tr>
<th>Diagnostic Procedure</th>
<th>Lymph node aspirate n (%)</th>
<th>Cerebrospinal fluid n (%)</th>
<th>Pleural fluid n (%)</th>
<th>Ascitic fluid n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TB (n=120)</td>
<td>TB (n=16)</td>
<td>TB (n=21)</td>
<td>TB (n=33)</td>
</tr>
<tr>
<td></td>
<td>Non-TB (n=30)</td>
<td>Non-TB (n=11)</td>
<td>Non-TB (n=20)</td>
<td>Non-TB (n=19)</td>
</tr>
<tr>
<td>AFB stain</td>
<td>15(12)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LJ** Culture</td>
<td>24(20)</td>
<td>1(6)</td>
<td>4(19)</td>
<td>3(9)</td>
</tr>
<tr>
<td>Immunochemistry-MPT64</td>
<td>84(70)</td>
<td>10 (62)</td>
<td>16(76)</td>
<td>18(54)</td>
</tr>
<tr>
<td>Nested-PCR</td>
<td>88(74)</td>
<td>10 (62)</td>
<td>15(74)</td>
<td>16(48)</td>
</tr>
</tbody>
</table>

*Note: LJ** Culture is likely a typo and should be corrected to LJ Culture.*
Immunocytochemical staining of mycobacterial antigen MPT64

CSF

LN Aspirate

Pleural fluid

Ascitic fluid

B

D
Validation of **Immunocytochemistry**

Nested-PCR used as gold standard

- Sensitivity = 96%
- Specificity = 96%
Conclusion

Immunochemistry with a-MPT64 is:

- rapid, sensitive and specific method for establishing etiological diagnosis of TB
- unlike PCR, not sensitive to contamination, does not require sophisticated equipment, can be established in a pathology/cytology lab.
Further Research

- To assess the feasibility of implementation and validity of the assay in the routine diagnostic settings.
- To assess improvement in case detection comparing the intervention and control hospitals.
- To improve the diagnostic capacity and sustain the quality at the TB diagnostic laboratories in low-income settings by training and academic building of the staff.
- Cost-effectiveness analysis of the assay after implementation at selected hospitals for future scale-up.
A hospital implementing WHO endorsed DOTS strategy and a pathology laboratory will be selected from each of the 5 sites; Tanzania, Zanzibar, India, Pakistan, and Norway.

1-3 control from each site (except Norway)
Hospital out patient

TB guidelines

Pulmonary TB suspect
- Radiology
- sputum
- Clinical data
  - HUS-antigen detection by immunochemistry (hypothesis 2)
  - AFB microscopy & Culture
  - Histopathology/cytology

Extrapulmonary TB suspect
- Information for economic evaluation
- Samples
  - Radiology
  - Biochemical tests
- PCR/HIV
- Immunochemistry based assay (Hypothesis 1)
- HUS- new assay (hypothesis 3)

Define TB case, Treatment (DOTS), Follow-up, data registration

Response to treatment
- TB case

No response to treatment
- Reassessment