Extrapulmonary TB (EPTB)
Reference standards for diagnostic studies

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Struggling against EPTB for millennia

• Man & TB intertwined for >2 million years
• Possible Bone TB – diagnosed by anatomical appearance and MTB PCR

Which PCR was used?
How sure are we that it was really TB?

Need to label it “possible TB”

Atlit-Yam, Haifa, Israel, 2008
Sites for EPTB

Almost any part of body!
Common important diagnostic groups:
1) Pleural TB
2) CNS TB
3) Abdominal TB
4) Lymph node TB
5) Bone and Jt TB

Performance of TB Culture

- Variable mostly poor for all forms of EPTB
- Pleural TB – 24-58%
  Trajman A, Pai M & Dheda K ERJ 2008
- TB meningitis – 52-87% (87% from 4 lumbar punctures)
  Kennedy DH, Fallon RJ JAMA 1979
- Abdominal TB – ascitic fluid up to 83% (when 1 litre ascitic fluid tapped and centrifuged)
  and tissue as low as 7%
  Singh MM, Bhargava AN, Jain KPK NEJM 1969
- Lymph node TB (‘Scrofula’) – 62% FNA, 71% biopsies
  Polesky A, Grove W, Bhatia G Medicine (Baltimore) 2005
What are the options?

All use one/combinations of the following:

• Invasive
• Often unavailable
• Expensive
• False negatives in 15-20% cases
• Other diseases causes granulomatous inflammation
• AFBs maybe represent NTMs

1) Tissue biopsy/histopathological diagnosis
2) Case definitions/scoring systems – utilise combinations of clinical, basic laboratory and radiology
3) Response to anti-TB treatment
4) Combinations of biomarkers and diagnostic tests

• Wide variations across studies
• Wide variation in performance
• Influenced by disease prevalence therefore not applicable across settings
• TB treatment effective for bacterial disease
• No response does not always mean no disease
• Some alternative diagnoses respond without Rx e.g. Viral meningitis
• Dependent on non-redundancy of test performance
• Expensive to perform numerous tests
• Inclusion bias if including new test

Diagnostic categories for EPTB

1) **Definite TB** – *Mtb.* Culture positive ± histopathological evidence ± other test (e.g. NAAT for TBM with high specificity)

2) **Probable TB** – Culture negative and fulfills case definitions/ high on scoring systems ± response to anti-TB Rx

3) **Possible TB** – lowering on scoring systems/not fulfilling case definition but responding to TB Rx

4) **Indeterminate** e.g. lost to follow-up, NB report

5) **NON TB** – alternative diagnosis proven, no anti-TB Rx, no TB at follow-up (usually >2 months)
Pleural TB studies

Diagram showing how a pleural effusion is drained © CancerHelp UK

Culture/histopathological reference

Utility of quantitative T-cell responses versus unstimulated interferon-γ for the diagnosis of pleural tuberculosis


The reference standard used for diagnosis of TB was culture positivity for Mycobacterium tuberculosis (using pleural fluid or tissue) and/or histology suggestive of TB (caseous necrosis with acid-fast bacilli, with or without granuloma formation). Patients were thus characterised as 1) definite TB (meeting the reference standard), 2) non-TB (no microbiological or histological evidence for TB, alternative diagnosis made, not treated for TB and did not develop TB over 6-month follow-up), and 3) probable TB (empirical anti-TB treatment but not meeting the criteria for definite TB). The definite and non-TB groups were used for sensitivity and specificity calculations. All assays were performed by an experienced laboratory technician who was blinded to patient and clinical details.

Report reference std
Describe diagnostic categories
Describe groups used for accuracy measures
Scoring systems/comboination references

<table>
<thead>
<tr>
<th>Combinations of markers</th>
<th>Reported accuracy</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt; fever + red blood cells + ADA</td>
<td>Very high sensitivity, high specificity</td>
<td>[91]</td>
</tr>
<tr>
<td>ADA + IFN-γ = NAAT</td>
<td>Increase in sensitivity and specificity compared with each separate method</td>
<td>[92]</td>
</tr>
<tr>
<td>Duration of symptoms + protein + leucocyte count +</td>
<td>High sensitivity and specificity</td>
<td>[93]</td>
</tr>
<tr>
<td>lymphocytes % + ADA</td>
<td>High sensitivity and specificity</td>
<td>[94]</td>
</tr>
<tr>
<td>ADA + lymphocytes/neutrophil ratio</td>
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</tbody>
</table>

ADA: adenosine deaminase; IFN: interferon; NAAT: nucleic acid amplification test.

Specific example

Porcell and Vives Med Sci Monit 2003 (Spain)
- Age <35 years (2 points)
- Temperature ≥ 37.8°C (2 points)
- Pleural fluid rbc count < 5 x 10^9/l (1 point)

5 pts 95% 94%

Villegas MV, Labrada LA & Saravia NG Chest 2000
- ADA, unstimulated IFN-γ; NAAT alone or in combination

Call the statistician!

Latent class analysis

- Statistical method to compensate for imperfect “reference” tests
- A probabilistic model is assumed for the relationship between the new diagnostic test, one or more imperfect “reference” tests, and the unobserved, or latent, disease status
- Minimum of 3 “conditionally independent” tests
- Call the statistician!
TB meningitis studies and attempts to unify case definitions for diagnostic studies

Multiple case definitions for TBM - 14 different ones referenced!

- All involve combinations of:
  1) Clinical
  2) CSF findings ±
  3) Radiology ±
  4) TB Rx response

- Each definition includes definite and presumptive tuberculous meningitis cases.
- Clinical features include headache, meningeal signs, and either clinical presentations of meningitis lasting for more than 3 weeks.
- CSF: typical tuberculous meningitis (cellular pleocytosis, PMNs ≤ 10%, lymphocytes ≥ 60%, protein ≥ 1.9 g/L, and glucose < 0.4 g/L).
- Supportive criteria:
  - CSF chemistry and protein levels within normal limits
  - CSF pleocytosis with predominance of lymphocytes
  - CSF culture or MTB PCR positive

Marais S et al. Lancet 2010

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Adults only

- TB meningitis (TBM)
  - Patients: HIV seropositive, ≤ 15 years of age
  - Case definition includes definite and probable tuberculosis meningitis cases.
  - Definite tuberculosis meningitis: CSF positive for MTB and/or positive culture for MTB (≥ 8 colonies).
  - Probable tuberculosis meningitis: clinically suspected infection demonstrated by positive CSF or meningitis on one or more of the following four criteria:
    - CSF cell count with granulomas and/or caseating granulomas
    - CSF protein > 0.95 g/L
    - CSF glucose < 0.4 g/L
    - CSF PCR positive
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- Marais S et al. Lancet 2010
A uniformed case-definition

• Absence of standardisation in TBM diagnostic categorisation and clinical case definition makes comparison of studies difficult
• May 2009 – Cape Town - TBM workshop
• Develop consensus case-definition for future studies and evaluation of new tests

Panel 2: Consensus tuberculous meningitis diagnosis

Clinical entry criteria
- Symptoms and signs of meningitis including one or more of the following: headache, irritability, vomiting, fever, neck stiffness, convulsions, focal neurological deficits, altered consciousness, or lethargy.

Tuberculous meningitis classification

Definite tuberculous meningitis
- Patients should fulfill criterion A or B:
  A) Clinical entry criteria plus one or more of the following: acid-fast bacilli seen in the CSF; Mycobacterium tuberculosis cultured from the CSF; or a CSF positive commercial nucleic acid amplification test.
  B) Acid-fast bacilli seen in the context of histological changes consistent with tuberculous in the brain or spinal cord with suggestive symptoms or signs and CSF changes, or visible meningitis (on autopsy).

Probable tuberculous meningitis
- Clinical entry criteria plus a total diagnostic score of 10 or more points (when cerebral imaging is not available) or 12 or more points (when cerebral imaging is available) plus exclusion of alternative diagnoses. At least 2 points should either come from CSF or cerebral imaging criteria.

Possible tuberculous meningitis
- Clinical entry criteria plus a total diagnostic score of 6-9 points (when cerebral imaging is not available) or 8-11 points (when cerebral imaging is available) plus exclusion of alternative diagnoses. Possible tuberculous cannot be diagnosed or excluded without doing a lumbar puncture or cerebral imaging.

Not tuberculous meningitis
- Alternative diagnosis established, without a definitive diagnosis of tuberculous meningitis or other convincing signs of dual disease.

Clinical entry criteria – patients must have symptoms of meningitis

Definite TB:
1) CSF smear or culture positive or CSF PCR.
2) AFB in context of histology with CSF changes

Probable TB and possible TB:
1) Clinical entry criteria + diagnostic score

Not TB:
1) Alternative diagnosis established

Removed TB Rx

Marais S et al Lancet 2010
**Case definition**

Using weighted scoring:

1) **Probable TB**
   - ≥10 (no radiology)
   - ≥12 (radiology)

1) **Possible TB**
   - 6-9 (no radiology)
   - 6-12 (radiology)

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**TB Lymphadenitis**

- Reflect similar issues as two previous examples
- Studies use culture positive or combinations of tests (e.g. AFB positive or suggestive or PCR positive)
- Complexities of response to treatment
- Clinical scoring systems variable and complex

TB-Lymphadenitis on TB treatment:
- 7% persistence of enlarged nodes
- 7% transient enlargement
- 14% developed new nodes during therapy
- 21% had residual adenopathy after therapy was complete

Daley P, Thomas S & Pai M *I/TLD 2007*
Conclusions

• No good reference standards for EPTB
• Various combination ‘reference’ standards used
• Reporting diagnostic categories and groups used for analysis imperative
• Consensus case definitions/disease categorisation will allow better comparison across studies