Extrapulmonary Tuberculosis Diagnostic Approaches

John W. Wilson, MD
Associate Professor of Medicine
Division of Infectious Diseases
Mayo Clinic, Rochester MN
Mayo Clinic Center for Tuberculosis
Approach to TB Investigation: 4 Steps to Success (around the world)

Defining / characterizing:

1. The Host
2. The Syndrome
3. The Microbiology
4. The Treatment
1 - Defining the Host

- Immunocompetent vs. Immunosuppressed – **Especially HIV status**
  - Higher rates of primary TB disease
  - More atypical pulmonary findings
  - Higher rates of extrapulmonary disease & dissemination

- Other medical comorbidities: Diabetes

- Adult vs. Child

- Living status: community vs., hospital, jail, shelter etc.
  - Other cases of TB reported, pattern of spread?
2 - Define the Syndrome – the “itis”

- Pneumonitis – clinical sx’s or via CXR?
- Lymphadenitis, meningitis / cerebritis, pericarditis, hepatitis, peritonitis, pyelonephritis, etc.

Is the syndrome consistent with TB?
Is this new vs. recurrent TB?
Is drug-resistant TB possible? Prev trx?

Treatment approaches based the syndrome – not all the same
3 - Defining the Microbiology

Questions to consider:

1. Is it Infection vs. Non-infection-driven inflammation?

If infection present:

2. Is the Infection mycobacterial, bacterial, fungal, viral, protozoan, helminthic?

- AFB staining, KOH, Gram staining on sputum smear or tissue?
  - Easily done in most laboratories; rapid results
The ideal TB diagnostic test - criteria:

- Provide immediate accurate diagnosis of active TB
- Function optimally for:
  - Adult and pediatric active TB
  - HIV (+) and HIV (-) pts
  - Pulmonary and extrapulmonary TB
- Distinguish between active and latent TB
- Detect drug resistance to 1st line TB drugs
  - Avoid initial treatment failure (and propagation of further drug resistance).
- Inexpensive and appropriate for us within under-resourced (endemic) TB regions

Commercial TB diagnostics available

- Serology testing
  - Variable sensitive and specificity (not commonly used in US)

- IGRA
  - QuantiFERON-TB Gold (Celestis, Australia)
  - T.Spot.TB (Oxford Immunotec, UK)

- Staining
  - Ziehl-Neelsen / Kinyoun staining (Acid-fast)
  - Fluorochrome-based staining

- Culture platforms

- Nucleic amplification platforms
Nucleic acid amplification testing

• Qualitative TB PCR
  • AFB smear (+) and (-) samples
  • Extrapulmonary samples:
    • CSF, urine, tissue, pleural fluid, joint fluid, etc.
    • Variable sensitive – low organism burden

• GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA)
  • Detects MTB and simultaneous RIF drug resistance
    • rpoB gene
  • Qualitative MTB detection
    • AFB (+) sputum sample: 98-100% sensitivity
    • AFB (-) sputum sample: 57-83% sensitivity
    • Extrapulmonary sites: 53-95%

Boehme et al. Lancet 2011;377:1495-505
Limitations in Rapid Molecular Diagnostics

• Should not supplant phenotypic testing
  • clinicians should understand their limitations.

• When rapid molecular tests are negative but suspicion for MDR TB is high, MDR TB treatment should be continued until phenotypic susceptibility results are available

• DNA sequencing may be best suited for evaluating suspected drug-resistant *M. tuberculosis* isolates with discordant results for phenotypic susceptibility and rapid molecular testing
Lymphatic TB (Scrofula)
Consideration to lymphadenopathy / mass

• Non-infectious process?
  • Lymphoma
  • Head/neck neoplasia
  • Infection

• Infection?
  • Granulomatous causes (following slide)
  • Reactive adenopathy / non-granulomatous – e.g.:
    • EBV – mononucleosis
    • CMV
    • other
Granulomatous Lymphadenitis – causes:

<table>
<thead>
<tr>
<th>1. Noninfectious granulomatous disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Sarcoidosis lymphadenitis</td>
</tr>
<tr>
<td>2) Sarcoid-like lymphadenitis</td>
</tr>
<tr>
<td>3) Berylliosis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. Infectious granulomatous disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Suppurative</td>
</tr>
<tr>
<td>1) Tularemia lymphadenitis</td>
</tr>
<tr>
<td>2) Cat scratch lymphadenitis</td>
</tr>
<tr>
<td>3) Yersinia lymphadenitis</td>
</tr>
<tr>
<td>4) Lymphogranuloma venereum</td>
</tr>
<tr>
<td>5) Fungal infection</td>
</tr>
<tr>
<td>B. Non-suppurative</td>
</tr>
<tr>
<td>1) Tuberculous lymphadenitis</td>
</tr>
<tr>
<td>2) Atypical mycobacterial infection</td>
</tr>
<tr>
<td>3) BCG-lymphadenitis</td>
</tr>
<tr>
<td>4) Toxoplasma lymphadenitis (Piringer-Kuchinka lymphadenopathy)</td>
</tr>
<tr>
<td>5) Lepra</td>
</tr>
<tr>
<td>6) Syphilis</td>
</tr>
<tr>
<td>7) Brucellosis</td>
</tr>
<tr>
<td>8) Fungal infection (Cryptococcus, Histoplasma, Coccidioidomycosis, Pneumocystis)</td>
</tr>
</tbody>
</table>
Diagnostic approach for lymphatic TB - I

- Head/neck/axilla – cervical chain LNs most common
  - **Excisional LN biopsy**
    - Most invasive (surgical procedure)
    - Best diagnostic yield / most tissue histology
      - Esp if non-TB considerations high in DDx
        - Other granulomatous infections
        - Lymphoma and other neoplasms
      - HIV negative patients
        - Lower organism burdens
    - May provide for more rapid symptomatic response

Fontalilla et al. CID 2011;53(6):555-562
Artenstein et al. CID 1995;20:876-82
Diagnostic approach for lymphatic TB - II

- Head/neck/axilla – cervical chain LNs most common
  - **Fine needle aspiration (FNA)**
    - Non-surgical procedure (office procedure)
    - High safety
    - Variable results – can add to delays in dx:
      - ‘Indeterminate results’ may necessitate excisional biopsy
  
  Fontalilla et al. CID 2011;53(6):555-562
  Artenstein et al. CID 1995;20:876-82

- FNA has higher yield in HIV (+) pts (compared to HIV (-) pts
  - Higher LN organism burden – easier detection

  Acta Cytol 1991;35:325-32
## Primary Diagnostic tests in Tuberculosis

Lymphadenitis – some variability

<table>
<thead>
<tr>
<th>Location (Year)</th>
<th>Culture (+)</th>
<th>AFB (+)</th>
<th>GI (+)</th>
<th>Culture + GI (+)</th>
<th>NAAT (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>California (1992) [28]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excisional Biopsy</td>
<td>28/30 (93%)</td>
<td>11/30 (37%)</td>
<td>23/30 (77%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>FNA</td>
<td>18/29 (62%)</td>
<td>10/29 (35%)</td>
<td>16/29 (55%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>France (1999) [9]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excisional Biopsy</td>
<td>12/39 (31%)</td>
<td>2/39 (5%)</td>
<td>32/39 (82%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>FNA</td>
<td>8/26 (31%)</td>
<td>2/26 (8%)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>California (1999) [29]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FNA</td>
<td>44/238 (18%)</td>
<td>58/238 (24%)</td>
<td>84/238 (35%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>India (2000) [30]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excisional Biopsy</td>
<td>4/22 (18%)</td>
<td>5/22 (23%)</td>
<td>13/22 (59%)</td>
<td>17/22 (77%)</td>
<td>15/22 (68%)</td>
</tr>
<tr>
<td>FNA</td>
<td>2/22 (10%)</td>
<td>4/22 (18%)</td>
<td>7/22 (32%)</td>
<td>9/22 (41%)</td>
<td>12/22 (55%)</td>
</tr>
<tr>
<td><strong>California (2005) [5]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excisional Biopsy</td>
<td>24/34 (71%)</td>
<td>15/39 (38%)</td>
<td>36/31 (88%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>FNA</td>
<td>48/77 (62%)</td>
<td>5/19 (26%)</td>
<td>47/76 (62%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>UK (2010) [12]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FNA</td>
<td>65/97 (67%)</td>
<td>22/97 (23%)</td>
<td>77/97 (79%)</td>
<td>88/97 (91%)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**NOTE.** NA, not available; AFB, acid-fast bacilli; GI, granulomatous inflammation; NAAT, nucleic acid amplification test; FNA, fine-needle aspiration.

Clin Inf Dis 2011;53(6):555-62
Diagnostic approach to peripheral LN

Progressive lymphadenopathy & no comorbid explanation, or clinical history consistent with TB, or PPD (+)

HIV (-)
- High prevalence of MTB or underdeveloped area
  - FNA
  - MTB culture (+) or smear (+)
    - Treatment
    - Excisional biopsy
  - Culture (-); histopathology not consistent with MTB
    - Treatment
    - Excisional biopsy
  - Histopathology consistent with MTB; culture (-)
    - Treatment trial; consider excisional biopsy

HIV (+)
- Developed country
  - FNA
  - MTB culture (+)
    - Treatment
    - Smear (+), or histopathology consistent with MTB, and culture (-)
    - Empirical anti-TB treatment; excisional biopsy
  - Culture (-); histopathology not consistent with MTB
    - Expectant management

Results not consistent with MTB
- Rule out other infections or neoplasms
US based Lymphatic TB study – 106 patients
- a quality pathology lab

**TABLE 4. Interpretation of Initial Biopsies**

<table>
<thead>
<tr>
<th>Biopsy Type</th>
<th>Positive ZN Pathology</th>
<th>Positive Fluorochrome</th>
<th>Positive M. TB Culture Microbiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>No./No. Performed (%)</td>
<td>No./No. Performed (%)</td>
<td>No./No. Performed (%)</td>
<td>No./No. Performed (%)</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>20/58 (34)*</td>
<td>25/111 (23)*</td>
</tr>
<tr>
<td>Surgical Biopsy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>15/39 (38)</td>
<td>9/34 (26)</td>
<td>24/34 (71)</td>
</tr>
<tr>
<td>Granulomas with necrosis†</td>
<td>29/41 (71)</td>
<td>13/29 (45)</td>
<td>7/25 (28)</td>
</tr>
<tr>
<td>Granulomas without necrosis‡</td>
<td>7/41 (17)</td>
<td>1/7 (14)</td>
<td>2/6 (33)</td>
</tr>
<tr>
<td>Necrosis and/or inflammation‡</td>
<td>4/41 (10)</td>
<td>1/3 (33)</td>
<td>0/3 (0)</td>
</tr>
<tr>
<td>Other§</td>
<td>1/41 (2)</td>
<td>0/0 (0)</td>
<td>0/0 (0)</td>
</tr>
<tr>
<td>Fine Needle Aspirate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>79</td>
<td>5/19 (26)</td>
<td>16/77 (21)</td>
<td>48/77 (62)</td>
</tr>
<tr>
<td>Cytology performed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granulomas with necrosis†</td>
<td>21/76 (28)</td>
<td>4/8 (50)</td>
<td>1/20 (5)</td>
</tr>
<tr>
<td>Granulomas without necrosis‡</td>
<td>26/76 (34)</td>
<td>1/7 (14)</td>
<td>4/26 (15)</td>
</tr>
<tr>
<td>Necrosis and/or inflammation‡</td>
<td>21/76 (28)</td>
<td>0/3 (0)</td>
<td>10/21 (48)</td>
</tr>
<tr>
<td>Other§</td>
<td>8/76 (10)</td>
<td>0/1 (0)</td>
<td>0/7 (0)</td>
</tr>
<tr>
<td>No cytology performed</td>
<td>3</td>
<td></td>
<td>1/3 (33)</td>
</tr>
</tbody>
</table>

Abbreviations: ZN = Ziehl-Neelsen; M. TB = Mycobacterium tuberculosis.
*Not all specimens had a ZN stain performed in pathology, and 9 specimens were not sent for culture.
†Includes necrotizing granulomas or granulomas with necrosis in the biopsy.
‡Combined due to the large degree of overlap.
§Nasopharyngeal carcinoma (surgical specimen), reactive hyperplasia and atypia, inadequate material, benign lymphocytes, fat necrosis and fibrosis.

PCR – TB detection via DNA/RNA amplification

• Variety of PCR platforms
  • MTB detection
  • MTB Drug resistance

• Very helpful when positive
  • When performed by quality lab (minimize false positivity)

• Does NOT exclude TB diagnosis if test result negative
Diagnostic approach for lymphatic TB - III

• Hilar and mediastinal adenopathy
  • Often associated with primary pulmonary tuberculosis
    • E.g. Pediatric TB and in HIV (+) adults
  • Bronchoscopy with transbronchial biopsies
    • Small sample sizes
  • In HIV (-) / immunocompetent adults: important to exclude more common etiologies:
    • Sarcoidosis
    • Malignancies: lymphoma, lung and metastatic neoplasms
    • Histoplasma, Tularemia, toxoplasmosis, respiratory anthrax, etc.
Genitourinary TB
Genitourinary TB – Diagnostic options:

- Radiologic imaging:
  - Intravenous urography – often show:
    - Ureteral strictures
    - Renal pelvis distortions/hydronephrosis
    - Bladder fibrosis

- CT Abdomen / CT urogram:
  - Calcification present in >50% renal TB cases
  - Distal ureteral most common site
  - Multiple strictures
  - Bladder wall thickening

Nat Rev Urol 2011; 8:678-688
Genitourinary TB – Diagnostic options:

- Urine AFB smear and culture
  - 3 early morning samples – ideal
  - Low – variable sensitivity (based on disease severity)
    → 11 – 80%
    Clin Inf Dis 1994; 18;557-561

- Nucleic acid amplification:
  - Sensitivity: 87- 99%
  - Specificity 92 - 98%

  J Urol 2000; 164;584-588
  J Cell Mol Biol 2003; 2;2032-41
Tuberculosis Osteomyelitis

Pott’s Disease

- Backache is primary symptom
  - Nonspecific symptoms
- Slow progression
- Diagnosis often delayed
Tuberculosis Osteomyelitis – Imaging

• Spinal x-ray
  - Can be normal early in disease
  - Later stages: disc degeneration; osteolytic destruction

• CT spine
  - Good for axial bone changes

• MRI spine
  - Excellent for disc and soft tissue changes
  - Best for spinal cord impingement
  - Most sensitive test for early disease

• Unlike bacterial/pyogenic vertebral osteomyelitis, vertebral TB disease may *spare* the disc space in up to 50% cases (with adjacent vertebral body disease)

Clev Clin J of Med. 2004; 71(7)537-549
J Craniovertr Junct Spin. 2012; 1(2)74-85
Tuberculosis Osteomyelitis – laboratory testing

• CT guided bone biopsy
  • Small tissue sample

• Surgically obtained tissue
  • AFB staining – variable: can be (-) in > 50% cases
  • Mycobacterial culture
  • 1 UK series of Spinal TB:
    • Ziehl-Nielson stain was positive in 15 of 20 (75%) specimens
    • culture was positive for *M tuberculosis* in 16 (76%)
      Postgrad Med J. 2006; 82(963): 46–51

• Nucleic acid amplification / PCR
  J Craniovertr Junct Spin. 2012; 1(2)74-85
Pericardial TB
Pericardial TB – diagnostic approach

- **CXR:** enlarged cardiac silhouette in almost all cases
  - Pleural effusions 40-60% cases
- **ECG:** low voltage; nonspecific T wave changes
- **ECHO:** pericardial thickening; pericardial effusions
  - Can develop constrictive physiology
- **CT/MRI:** Pericardial thickening, effusions
  - Matted mediastinal LNs (lymphatic drainage of pericardium)

Pericardial TB - Diagnosis

- Pericardiocentesis
  - Bloodstained pericardial fluid > 80% cases
  - Exudative fluid
  - Leukocyte pleocytosis – predominantly lymphocytes and monocytes
  - Adenosine deaminase (ADA), lysozyme assay

- Microbial examination of pericardial fluid:
  - Low yield by AFB staining (0-42%)
  - Mycobacteria cultures (53-75%)

- Pericardial biopsy
  - Histopathology examination
  - 10-60% sensitivity for TB diagnosis

- PCR testing (of fluid and/or tissue)
Basic approach to Pericardial TB diagnosis:

- **Non-invasive:**
  - Obtain samples from other sources: sputum, urine, etc.
    - AFB stain, PCR, culture
    - CXR, Echo, CT – supporting findings

- **Invasive:**
  1. Pericardiocentesis
  2. Pericardial biopsy

- Empiric therapy based upon supporting findings above (even from other anatomic locations)
CNS Tuberculosis
CNS TB imaging

- MRI – most sensitive to detect meningeal enhancement
  - Basal meninges most commonly affected
  - Tuberculomas

- Meningeal biopsy – when ddx is broad and TB very unclear

Eur J Radiol 2012; 81(5):974-8
CNS Tuberculosis

- CSF evaluation – low TB bacillary burden
  - WBC count up to 1500/mm³
    - lymphocytic predominance is usual
    - ¼ cases can have PMN pleocytosis, usually early
  - Protein is elevated
  - glucose is characteristically low
  - AFB smear: low yield, 5-30% from single examination
    - Higher yield with repeated LPs
  - Culture yield – 45-90%
    - Higher in HIV (+) pts (up to 88%)
  - PCR – low by itself - 50-60%
    - PCR should NOT replace AFB examination and culture

Acta Neurol Scand 2010; 122:75-90
Jour of Inf 2009. 59;167-87
CNS Tuberculosis

- Adenosine deaminase – can be very helpful
  - Correlates with lymphocyte proliferation
  - Sensitivity 99-100% - for CNS TB
  - Specificity lower – depends upon cut-off value:
    - Elevated ADA levels also seen in:
      - Lymphoma with meningeal involvement
      - CNS malaria
      - CNS brucellosis
      - CMV meningeal disease (HIV + pts)
      - Cryptococcal meningitis (HIV+ pts)

Jour of Inf 2009. 59;167-87
Cerebrosp Fluid Resear 2006, 3:5
## Adenosine deaminase – in CNS TB

**Table 1:** The mean ADA activity (with range) in the CSF of TBM patients (n = 117), non-TBM infectious meningitis patients (n = 60) and control patients with non-infectious neurological disorders (n = 104). The data are expressed as mean ± SD.

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>ADA activity (U/L/min) Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Tuberculous Meningitis (n = 117)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture positive (n = 27)</td>
<td>14.31 ± 3.87</td>
<td>2.99–26.94</td>
</tr>
<tr>
<td>Clinically suspected (n = 90)</td>
<td>17.67 ± 4.18</td>
<td>9.01–26.94</td>
</tr>
<tr>
<td>2. Non TBM infectious meningitis (n = 60)</td>
<td>13.29 ± 3.16</td>
<td>2.99–21.02</td>
</tr>
<tr>
<td>Pyogenic meningitis (n = 41)</td>
<td>9.25 ± 2.14</td>
<td>4.99–13.96</td>
</tr>
<tr>
<td>Viral meningitis (n = 19)</td>
<td>10.11 ± 1.99</td>
<td>5.11–13.96</td>
</tr>
<tr>
<td>3. Non-infectious neurological disorders (n = 104)</td>
<td>7.39 ± 0.93</td>
<td>4.99–9.00</td>
</tr>
<tr>
<td>Headache (n = 32)</td>
<td>2.71 ± 1.96</td>
<td>0.00–7.68</td>
</tr>
<tr>
<td>Stroke (n = 29)</td>
<td>0.98 ± 0.19</td>
<td>0.11–1.20</td>
</tr>
<tr>
<td>Venous sinus thrombosis (n = 13)</td>
<td>4.18 ± 1.19</td>
<td>1.92–5.83</td>
</tr>
<tr>
<td>Guillain-Barré syndrome (n = 12)</td>
<td>1.82–4.12</td>
<td></td>
</tr>
<tr>
<td>Epilepsy (n = 6)</td>
<td>5.38 ± 2.16</td>
<td>2.63–7.68</td>
</tr>
<tr>
<td>Other neurological disorders (n = 12)</td>
<td>2.36 ± 0.79</td>
<td>1.01–3.18</td>
</tr>
<tr>
<td></td>
<td>1.18 ± 0.47</td>
<td>0.5–1.87</td>
</tr>
</tbody>
</table>

Cerebrosp Fluid Resear 2006, 3:5

**Figure 1:** Box plots of CSF ADA activity in TBM (CP-culture positive; CS-clinically suspected), non-TBM infectious meningitis (PM-pyogenic meningitis; VM-viral meningitis) and control group of non-infectious neurological disorders (OTH). The plots show the 90th percentile (bars), 75th and 25th percentile (box) and median (bar in box). N = numbers of individuals in each group. Dashed line represents the calculated ADA cut off value.
GI/Intestinal Tuberculosis
GI/Intestinal Tuberculosis

- Intestinal TB can mimic Crohn’s disease
  - Ileocecal location most common

- Note infection with M. bovis in global regions where unpasteurized milk consumption common
  - Brucellosis also in ddx globally

- Primary diagnostic approaches:
  - Tissue histology
  - Microbial staining
  - Culture or PCR
Demographics of TB and Crohn’s disease

Global TB incidence

Global Crohn’s disease incidence

Am J Gastroenterol 2009; 104:1003–1012
Intestinal TB – radiology findings

- CT, PET or MRI:
  - Enlarged para-aortic lymph nodes
  - Asymmetric bowel wall thickening
  - Ascites
  - Inflammatory mass – bowel wall/lymph nodes/omentum
  - Narrowing of terminal ileum; thickening of ileocecal value

- Ultrasound
  - Documenting and aspirating ascites
Intestinal TB – laboratory testing

• Direct microscopy / AFB staining
  • 10-30% sensitivity of culture (+) samples

• Adenosine deaminase (ADA)
  • Proportional to T-cell activation

• Culture methodologies
  • Higher yield than AFB staining
  • Stool cultures for mycobacteria

• PCR/DNA amplification – variable results
  • LN sampling – comparable to other lymphatic forms of TB
  • Fecal PCR – variable results
THE END
Questions?