Diagnosis of Active Tuberculosis

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Conflicts/Disclosure

• None.
Objectives

• Describe general approach to diagnosing active tuberculosis

• Describe clinical evaluation for diagnosing active tuberculosis

• Describe the microbiological diagnosis of active tuberculosis
Let’s get this out of the way …

- Positive TST or IGRA does not indicate active tuberculosis
- A negative TST or IGRA does not rule out active tuberculosis
Evaluation for Active Tuberculosis

1. Medical History
2. Physical Exam
3. Radiography
4. Microbiologic Testing
Medical Evaluation for TB

1. Medical History

- Symptoms of disease; how long
- History of TB exposure, infection, or disease
- Past TB treatment
- Demographic risk factors for TB
- Medical conditions that increase risk for TB disease
Persons at Risk for Developing TB Disease

Persons at high risk for developing TB disease fall into 2 categories:

- Those who have an increased likelihood of exposure to persons with TB disease
- Those with clinical conditions that increase their risk of progressing from LTBI to TB disease
Increased Likelihood of Exposure to Persons with TB Disease

Persons at risk for exposure to persons with TB disease include:

• Close contacts to person with infectious TB
• Residents and employees of high-risk congregate settings (e.g., correctional facilities, homeless shelters, health care facilities)
• Recent immigrants from TB-endemic regions of the world (within 5 years of arrival to the United States)
Increased Risk for Progression to TB Disease - 1

Persons more likely to progress from LTBI to TB disease include:

• HIV-infected persons

• Those with a history of prior, untreated TB or fibrotic lesions on chest radiograph

• Children $\leq 5$ years with a positive TST
Increased Risk for Progression to TB Disease - 2

Persons more likely to progress from LTBI to TB disease include:

- Underweight or malnourished persons
- Substance abusers (such as smoking, alcohol abusers, or injection drug use)
- Those receiving TNF-α antagonists for treatment of rheumatoid arthritis or Crohn’s disease
Increased Risk for Progression to TB Disease - 3

Persons more likely to progress from LTBI to TB disease include:

• Those with certain medical conditions such as:
  • Silicosis
  • Diabetes mellitus
  • Chronic renal failure or on hemodialysis
  • Solid organ transplantation (e.g., heart, kidney)
  • Carcinoma of head or neck
  • Gastrectomy or jejunoilial bypass
### Symptoms of Tuberculosis

#### Non-specific constitutional symptoms
- Loss of appetite
- Unexplained weight loss
- Night sweats,
- Fever
- Fatigue

#### Respiratory symptoms
- Prolonged cough (3 weeks or longer)
- Shortness of breath
- Hemoptysis
- Chest pain

#### Symptoms of possible extra-pulmonary TB
- Blood in the urine (TB of the kidney)
- Headache/confusion (TB meningitis)
- Back pain (TB of the spine)
- Hoarseness (TB of the larynx)
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Evaluation for Active Tuberculosis

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TB Diagnostic Algorithm

TB Suspect

AFB Microscopy

PCR

Culture

Identification (molecular)

Susceptibility Testing
Stains for Mycobacteria

- Rapid - an hour to perform and report
- Inexpensive indication of whether the specimen contains mycobacteria
- Mycobacteria do not stain with the Gram stain
- “Acid-fast” stains auramine/rhodamine, Ziehl-Neelsen, or Kinyoun stain
- A complex is formed between mycolic acid and dye used in the stain
- This complex is resistant to destaining by mineral acids (thus “acid-fast”)
Acid-fast stains - Issues

• Acid-fast stains are not very specific
  • indicates whether a mycobacterium is present in the specimen
  • does not allow us to know which mycobacteria it is
    • *M. tuberculosis* looks like all the other mycobacterial species on an acid-fast stain

• Acid-fast stains are not very sensitive
  • need 1000-10,000 CFU/ml for a positive AFB smear

• Quality of sputum obtained variable
2-3 AFB Smears are More Sensitive than 1 Smear
Yield of Serial AFB Smears

<table>
<thead>
<tr>
<th>Study</th>
<th>% of Total Positives Detected by:</th>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; Smear</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Smear</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; Smear</td>
<td></td>
</tr>
<tr>
<td>Walker et al. (2000), <em>Int J Tuberc Lung Dis</em>, 4:246.</td>
<td>77.1%</td>
<td>15.0%</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td>Ipuge et al. (1996), <em>Trans R Soc Trop Med Hyg</em>, 90:258.</td>
<td>83.4%</td>
<td>12.2%</td>
<td>4.4%</td>
<td></td>
</tr>
<tr>
<td>Mathew et al. (2002) <em>J Clin Microbiol</em>, 40:3482-4 (low prevalence pop.)</td>
<td>89.4%</td>
<td>5.3%</td>
<td>5.3%</td>
<td></td>
</tr>
</tbody>
</table>
Acid-Fast Smears Prepared from Early Morning Sputum Specimens Have Better Sensitivity

<table>
<thead>
<tr>
<th>Study</th>
<th>Spot (Random) Specimen Positive (%)</th>
<th>Early Morning Specimen Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ssengooba et al, 2012, Tuberc Res Treat, 2012: 1-6. (MGIT culture positive for MTB)</td>
<td>12/21 (57%)</td>
<td>21/21 (100%)</td>
</tr>
<tr>
<td>Abraham et al, 2012, Indian J Med Res, 135: 249-51 (smear is positive)</td>
<td>21/49 (43%)</td>
<td>32/49 (65%)</td>
</tr>
</tbody>
</table>
Mycobacteria Cultures

Necessary to obtain an isolate of the mycobacterium for:

- species identification
- antimicrobial susceptibility testing
Culture of *M. tuberculosis* complex

- Sensitivity of culture is much better than smear
  - a positive AF smear requires 1000-10,000 CFU/ml of specimen
  - a positive mycobacteria culture requires only 10-100 CFU/mL of specimen

**Culture**

- 2 types of media used:
  - Solid Medium (Lowenstein-Jensen (LJ) or Middlebrook)
  - Broth (Liquid) Medium (FDA-cleared systems - Bactec MGIT and Trek VersaTREK)
  - In general, mycobacteria grow faster in broth but there are some strains that grow better on solid medium
M. tuberculosis Colony Morphology on Solid Medium

Note the “rough and buff” morphology typical of M. tuberculosis
BACTEC MGIT 960 Culture System

MGIT - **Mycobacterial Growth Indicator Tubes** (Becton Dickinson)

- fluorescent indicator in bottom of tube quenched by $O_2$
- $\uparrow$ mycobacterial growth = $\downarrow$ $O_2$ and $\uparrow$ fluorescence
VersaTREK System

- mycobacterial growth causes changes in bottle headspace pressure which are detected by the instrument; sponges in bottle provide increased surface area for growth

Identification of MTB from Culture Isolates

Hybridization Probes

- Step 1: MTB or an NTM?
- Step 2: Monolayer culture dishes
- Step 3: Incubate for 15 minutes
- Step 4: MTB-specific DNA probe
- rRNA from patient’s isolate

Hologic Gen-Probe AccuProbes® (nucleic acid hybridization probes)

Line Probe Hybridization Assays

DNA Sequencing

Matrix-assisted laser desorption ionization – time of flight (MALDI-TOF) mass spectrometry (MS)
Direct Identification of *M. tuberculosis* complex from patient specimen *without waiting* for growth in culture
Nucleic Acid Amplification-based (NAA) tests for MTB

• CDC recommendation:
  • NAA testing be performed on at least one (preferably the first) respiratory specimen from each patient with suspected pulmonary TB
    • if it would alter case management
    • If it would alter TB control activities
  • NAA testing does not replace the need for culture
NAA Tests for Direct Detection of MTB

- FDA-cleared
  - Hologic/Gen-Probe MTD
  - Cepheid GeneXpert MTB/RIF
- CE-marked/RUO in U.S.
  - Hain LineProbe
- Laboratory Develop Tests (LDTs)
  - Rapid cycle/real-time PCR
Direct Detection of MTB from Patient Specimens

*Mycobacterium tuberculosis* Direct Test (MTD) (Hologic Gen-Probe)

- Transcription-mediated amplification of *M. tuberculosis* complex rRNA directly from respiratory specimens
- Clinical specificity: 99-100%
- Clinical sensitivity:
  - smear positive: 91-95%
  - smear negative: 83-100%
- Technically challenging
  - inhibition from specimen components a concern
  - open PCR system so false positives due to cross-contamination of specimens are possible.
  - cross-reactions occur with some rare mycobacteria: *M. celatum*, *M. terrae*-like organisms, *M. holsiatricum*
Direct Detection of MTB from Patient Specimens

Cepheid Xpert® MTB/RIF Test

- WHO-endorsed
- Runs on the Cepheid GeneXpert platform
- FDA-approved for respiratory specimens
- Detects *M. tuberculosis* complex and provides information about RIF resistance
- Results in about 2 hrs; minimal hands-on needed

Source: www.finddiagnostics.org
Xpert MTB/RIF accuracy for detection of *Mtb* complex

- Limit of Detection is 131 CFU/ml (package insert)

- Chang et al, 2012, J Infect 64:580-8:
  - Meta-analysis of 18 studies with 10,224 patients total
  - Pulmonary TB:
    - Sensitivity, Smear positive disease – 98.7%
    - Sensitivity, Smear negative disease – 75.0%
    - Specificity - 98.2%
  - Extrapulmonary TB:
    - Sensitivity - smear positive, 95.2%; smear negative 70.7%
    - Specificity – 82.6%

- **Time to diagnosis comparison:**
  - Smear microscopy = same day (but non-specific)
  - Broth culture took an average of 16 days
  - Solid media plate cultures took an average of 20 days
  - Xpert MTB/RIF– same day diagnosis
Direct Detection of MTB from Patient Specimens
Line Probe Assays
(Hain Lifesciences)

M. tuberculosis complex
direct detection

Not approved for diagnostic use in the U.S.

Source: http://www.hain-lifescience.de
Direct Detection of MTB from Patient Specimens
Laboratory-developed PCR Tests (LDTs)
Example of Real-time PCR Workflow in our Laboratory

Approximate turn-around time = 4h
Direct comparison of Mayo LDT PCR assay with the GenProbe MTD test

<table>
<thead>
<tr>
<th>Assay</th>
<th>MTD</th>
<th>Agreement (%)</th>
<th>kappa coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>49</td>
<td>538/542 (99.3%)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>LightCycler</td>
<td></td>
<td></td>
<td>0.96</td>
</tr>
<tr>
<td>PCR</td>
<td>3</td>
<td>489</td>
<td></td>
</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>
Limitations of NAA tests for Direct Detection of *M. tuberculosis*

- Inhibition from specimen components a concern for falsely negative results
  - Inhibition control needed unless system lab has checked for inhibitors in all specimen types
- PCR detects presence of nucleic acid but doesn’t indicate if the organism is still viable
  - Patient could be being treated successfully but may still have a positive PCR result
- Culture is more sensitive so always perform culture too
  - Negative PCR result does not rule out *M. tuberculosis* infection
  - Culture isolate is needed for drug susceptibility testing
Comparison of TB Diagnostic Modalities

Proportion of TB Cases Detected by Each Method

Drug Resistance Testing of *M. tuberculosis* complex
**M. tuberculosis** complex Drug Resistance Testing

- Agar proportion is the current gold standard for all drugs except pyrazinamide
  - not rapid (14-21 days)
  - labor-intensive, technically complex
  - no FDA-cleared, commercially-available kit

Organism is resistant to drug A in the upper right compartment (>1% of inoculum shown by upper left control quadrant is growing in presence of drug). Organism is susceptible to drugs B & C in the lower compartments. Control quadrant in upper left contains no drugs.
Rapid Broth Susceptibility Testing for MTB
FDA-cleared, semi-automated with MGIT or VersaTREK systems

Compare growth rates in bottles/tubes +/- critical concentrations of drug

CDC goal is results for first-line drugs reported within 15-30 days after receipt of the specimen
**M. tuberculosis** complex resistant isolates

- If the isolate is resistant to any agent
  - preliminary report issued
  - consider confirming resistance by 2\textsuperscript{nd} method or 2\textsuperscript{nd} lab
  - consider initiating testing of secondary agents to avoid delays

- If the isolate is resistant to only PZA consider
  - speciation
    - *M. bovis* is mono-PZA-resistant
    - most isolates of *M. tuberculosis* are PZA-susceptible
Newest Method for Mtb DST LDT (Not FDA-cleared) MIC Plate

- broth microdilution method
- multi-center studies supporting FDA-submission completed
- rapid (14 days)
- contains INH, RIF, EMB and 9 second-line drugs
- test 1st and 2nd line drugs simultaneously with same inoculum
- provides MIC endpoint – helpful for isolates with MIC near critical concentration (CC) breakpoint that give fluctuating results w/CC method

Molecular detection of *Mtb* drug resistance markers

Why?

Rapid determination of potential drug resistance compared with phenotypic methods

Limited availability at this time except for the CDC MDR TB program
Molecular Detection of *M. tuberculosis* Drug Resistance at the CDC

- Offered for *M. tuberculosis* complex isolates and nucleic-acid amplification-positive (NAAT+) sputum sediments
- Provides rapid identification of mutations associated with resistance to many TB drugs
- Limitations include
  - Insufficient data to definitively associate all mutations detected with resistance;
  - Not all mechanisms of resistance are known
  - Not all resistance loci are sequenced
- Use in conjunction with conventional DST results
Molecular resistance testing for MTB at the CDC

<table>
<thead>
<tr>
<th>Drug</th>
<th>Locus/Loci examined</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>rifampin</td>
<td><em>rpoB</em></td>
<td>97.1</td>
<td>97.4</td>
</tr>
<tr>
<td>isoniazid</td>
<td><em>inhA</em> &amp; <em>katG</em></td>
<td>86.0</td>
<td>99.1</td>
</tr>
<tr>
<td>fluoroquinolones</td>
<td><em>gyrA</em></td>
<td>79.0</td>
<td>99.6</td>
</tr>
<tr>
<td>kanamycin</td>
<td><em>rrs</em> &amp; <em>eis</em></td>
<td>86.7</td>
<td>99.6</td>
</tr>
<tr>
<td>amikacin</td>
<td><em>rrs</em></td>
<td>90.0</td>
<td>98.4</td>
</tr>
<tr>
<td>capreomycin</td>
<td><em>rrs</em> &amp; <em>tlyA</em></td>
<td>55.2</td>
<td>91.0</td>
</tr>
<tr>
<td>ethambutol</td>
<td><em>embB</em></td>
<td>78.8</td>
<td>94.3</td>
</tr>
<tr>
<td>pyrazinamide</td>
<td><em>pncA</em></td>
<td>86.0</td>
<td>95.9</td>
</tr>
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Xpert MTB/RIF and Rifampin resistance

• Target is rpoB: gene encoding beta subunit of bacterial RNA polymerase

• Mutations in an 81bp region of the rpoB gene are responsible for ~96% of RIF resistance in Mtb;

• also predicts MDR TB since the majority of RIF-resistant isolates will also be INH-resistant

• Some false positive RIF resistance with Xpert
  • PPV is lower in low prevalence settings
  • CDC recommends reporting Xpert RIF-R as a preliminary result pending confirmation with sequencing; growth-base DST is still required
Line Probe Assays

*M. tuberculosis* complex
direct detection and INH/RIF resistance

Not approved for diagnostic use in the U.S.

Source: [http://www.hain-lifescience.de](http://www.hain-lifescience.de)
Diagnosis of TB: Summary

• Medical evaluation is critical
  • Identify risk of exposure and risk of reactivation
  • Clinical symptoms can be suggestive but often nonspecific

• AFB stains are rapid but insensitive and nonspecific

• Molecular tests are available for rapid identification of MTB from culture as well as from initial specimens

• Mycobacterial culture should always be ordered

• Drug susceptibility testing should be performed on all positive cultures

• Molecular tests are available for rapid identification of MTB from culture as well as from initial specimens