Testing for TB Infection and Disease

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Tuberculosis Clinical Intensive
Sept. 9-11, 2013

Disclosures
• None

Target of Accurate TB Diagnosis
Other Organisms can Interfere with TB Diagnosis

ATYPICALS
FUNGUS

High Accuracy for Diagnosis of HIV in Contrast to TB...

HIV
TB

HIV ANTIBODY
HIV RNA
AFB SMEAR
CULTURE

Mycobacterial Burden

Incubating $10^{3-4}$
Latent $10^{6-5}$
TB scar $10^6$
Active $10^{9-11}$
LTBI vs. TB Disease

<table>
<thead>
<tr>
<th>Latent TB Infection (LTBI)</th>
<th>TB Disease (in the lungs)</th>
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<tbody>
<tr>
<td>Inactive, contained tubercle bacilli in the body</td>
<td>Active, multiplying tubercle bacilli in the body</td>
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<td>TST or blood test results usually positive</td>
<td>TST or blood test results usually positive</td>
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<tr>
<td>Chest x-ray usually normal</td>
<td>Chest x-ray usually abnormal</td>
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<tr>
<td>Sputum smears and cultures negative</td>
<td>Sputum smears and cultures may be positive</td>
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<tr>
<td>No symptoms</td>
<td>Symptoms such as cough, fever, weight loss</td>
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<tr>
<td>Not infectious</td>
<td>Often infectious before treatment</td>
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<tr>
<td>Not a case of TB</td>
<td>A case of TB</td>
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</tbody>
</table>

LTBI: explaining to the patient

- Latent is a clinical term
- “Dormant” infection
- “Inactive”
- Avoid use of “exposed”
- Emphasize non-contagious
- Movement towards calling this “TBI”

Reading the TST

- Measure reaction in 48 to 72 hours
- Measure induration, not erythema
- Record reaction in millimeters, not “negative” or “positive”
- Ensure trained professional measures and interprets the TST
Alternatives to PPD: Specific Mycobacterial Antigens

Interferon-Gamma Release Assays (IGRAs)

- Whole-blood test used to detect *M. tuberculosis* infection
- Two U.S. Food and Drug Administration (FDA) approved IGRAs are commercially available in the U.S.:
  - QuantiFERON®-TB Gold-in-tube test (QFT-GIT)
  - T.SPOT®.TB test (T-Spot)
TST Versus IGRA


IGRA TESTS MORE SPECIFIC THAN PPD

Medical Evaluation for Active TB

- Medical history
- Physical examination
- Test for TB infection
- Chest radiograph
- Bacteriologic examination
Mycobacterial Examination

Mycobacterial examination has 6 stages:

1. Proper specimen collection
2. Examination of acid-fast bacilli (AFB) smears
3. Direct identification (NAAT-nucleic acid amplification test)
4. Specimen culturing and final identification
5. Drug susceptibility testing
6. TB genotyping

Specimen Sources

- Sputum (primary)
- Pulmonary aspiration (secondary)
- Body fluids (CSF, pleural, peritoneal, etc)
- Tissues
- Blood
- Stool (special request)
- Gastric aspiration (less preferred, must reach the lab within 72 hours, must be neutralized to pH 6.0-8.0)
- Other

Acid-fast Bacilli (AFB) smear

- Least sensitive of all AFB Tests (20-75%)
- Requires 10,000 AFB/ml to be positive
- Positive slide cannot determine AFB viability
- Positive slide does not determine whether TB or atypical mycobacteria (M. avium)
- Reported within 24 hours of receiving the specimen in the laboratory
Fluorescent AFB Smear Using Auramine-O Staining

- Very sensitive, takes minutes to read (read under lower magnification)
- Not all that is fluorescent is AFB (need a careful eye)
- Can be confirmed with Ziehl-Neelson (ZN) smear
- Chemical fluorescence, not an immune stain or Direct Fluorescent Antibody

AFB Smear by ZN

- Usually takes about 15 minutes per slide
- May be used to confirm a positive fluorescent smear

AFB Smear (shown in red) are tubercle bacilli
Interpretation of the AFB Smear

<table>
<thead>
<tr>
<th>AFB Seen (1000X)</th>
<th>Report</th>
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</thead>
<tbody>
<tr>
<td>• 0</td>
<td>• No AFB seen</td>
</tr>
<tr>
<td>• 1-2/300 fields</td>
<td>• Doubtful, repeat</td>
</tr>
<tr>
<td>• 1-9/100 fields</td>
<td>• Rare 1+ AFB</td>
</tr>
<tr>
<td>• 1-9/10 fields</td>
<td>• Few 2+ AFB</td>
</tr>
<tr>
<td>• 1-9/field</td>
<td>• Moderate 3+</td>
</tr>
<tr>
<td>• &gt;9/field</td>
<td>• Numerous 4+</td>
</tr>
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New CDC Guidelines of Use of NAA

MMWR January 16, 2009

• “NAA testing should be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities.”

Nucleic Acid Amplification (NAA) Test

*Amplified MTD* by Genprobe is the only FDA approved method

MTD [Mycobacterium Tuberculosis Direct]
Detects mRNA
NAA: Molecular Detection of *M. tb*

- Rapid Test - 24-48 hrs from smear positive / negative slide
- AFB smear +: 97% sensitive/100% specific
- AFB smear -: 72% sensitive/99% specific
- Non-Bloody Pulmonary Specimens only

- *M. celatum* and *M. terrae*-like organisms may cross-react. However, these organisms are rare clinical isolates.

AFB Culture Test

- More sensitive than AFB smear
- 10 AFB/ml can produce a positive result
- Culture may be AFB positive even if smear was reported negative for AFB
- Rapid broth testing – normally positive within 1-2 weeks. Requires 6 weeks to report culture as negative

AFB Tests Performed on Growth in Mycobacteria Culture

- Genetic DNA Probe Identification (not amplified)
- HPLC (high performance liquid chromatography) Identification
- Biochemical Identification Confirmation
- Drug Susceptibility
- DNA Genotype
- MDDR (molecular detection of drug resistance) (CDC)
Susceptibility Testing of *M. tuberculosis*

**When to test**
- All new (initial) *M. tb* isolates
- Suspected new drug resistance
- Repeat after 90 days if specimens continue to produce *M. tb*
- Relapse or failed therapy

Primary Anti-TB drugs
- Isoniazid
- Rifampin
- Ethambutol
- Pyrazinamide
- (Streptomycin)
Secondary Anti-TB Drugs

- Fluoroquinolone (ciprofloxacin, levofloxacin or moxifloxacin)
- Ethionamide
- Cycloserine
- Capreomycin
- Amikacin
- Kanamycin
- Streptomycin
- PAS

CDC – Molecular Detection of TB Drug Resistance (MDDR)

- Rapid testing for DNA sequences associated with 1st and 2nd line drug resistance
- NAAT (+) sputum specimens or culture isolates
- 3-4 day turn-around-time
- Must meet the following criteria:
  - Known Rifampin resistance
  - Known MDR
  - High risk of Rifampin resistance or MDR-TB (e.g. previous TB, MDR-TB contact, foreign born)
  - High profile patient (e.g. daycare worker, nurse)
  - Mixed or non-viable culture
  - Adverse reaction (e.g. RIF allergy)

CDC MDDR

- **First-line** MDDR to detect MDR-TB
  - rpoB (Rifampin)
  - inhA and katG (Isoniazid)
- **Second-line** MDDR to detect XDR-TB
  - gyrA (Fluoroquinolones)
  - rrs (Kanamycin, Amikacin, Capreomycin)
  - eis (Kanamycin)
  - blyA (Capreomycin)
  - pncA (Pyrazinamide)
  - embB (Ethambutol)

- Used alone, MDDR and DST are imperfect, used together, the accuracy of the detection of drug resistance can be improved
TB DNA Genotyping
 Universally Offered by CDC

• “Fingerprint” of each isolate

• Michigan Department of Community Health – Eastern States

• California Department of Public Health – Western States

Genotyping Advantages

Used with traditional investigations, genotyping has
• Identified outbreaks not previously recognized
• Confirmed/detected transmission
• Identified risk factors for recent infection
• Demonstrated re-infection with different strains
• Identified weaknesses in conventional contact investigations
• Documented lab cross-contamination
Epidemic Curve of Investigation of a Multistate TB Outbreak

3 Examples of TB suspects

- Each presents on October 1, 2012
- EASY #1 case, with smear + pulmonary TB
- MEDIUM #2 case, with smear negative pulmonary TB
- DIFFICULT #3 case, with suspected MDR-TB

SAMPLE CASES....
Case #1  Easy

From: Current Approaches to Tuberculosis in the United States

Figure Legend:
Admission chest radiograph showing bilateral lung infiltrates with prominence in the right upper lobe and lingula of the left lung.

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| 11        | PPD or IGRA | 2nd smear positive |
| 12        | 2nd smear positive | 3rd smear positive |
| 13        | NAAT on 1st + smear | 4th smear positive |
| 14        | IGRA result positive |
| 15        | INH | RIFAMPICIN | PZA | ETHAMBUTOL |
| 16        | AFB in liquid culture | DIRECT PROBE + M.Tb |
| 17        | 1st line drug susceptibility | DNA genotype |
| 18        | 1st line drug susceptibility | DNA genotype |
| 19        | 1st line drug susceptibility | DNA genotype |
| 20        | 1st line drug susceptibility | DNA genotype |
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| 27        | 1st line drug susceptibility | DNA genotype |
| 28        | 1st line drug susceptibility | DNA genotype |
| 29        | 1st line drug susceptibility | DNA genotype |
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| 31        | 1st line drug susceptibility | DNA genotype |
**#1 case**

**OCTOBER 2012**

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- **SUSPECTED PPD or IGRA**
- **SPUTUM COLLECTED**
- **1ST SMEAR POSITIVE**
- **PPD 15mm**
- **2ND SMEAR POSITIVE**
- **IGRA RESULT**
- **INH**
- **RIFAMIPIN**
- **PZA**
- **ETHAMBUTOL**
- **AFB IN LIQUID CULTURE**
- **DIRECT PROBE**
- **M.Tb**

**1ST LINE DRUG SUSCEPTIBILITY DNA GENOTYPE**

**AFB SMEAR FEW**

**CXR IMPROVED**

#1 case

2 month milestone for negative culture...

**DECEMBER 2012**

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**#2 case**

**Case #2 Medium Difficulty**

57 yr male

- Routine cultures negative
- No improvement
- Bronchoscopy AFB smear negative
- HIV +
- CD4 478 cells/mm³
#2 case

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<tbody>
<tr>
<td>HIV+ TB SUSPECT</td>
<td>HIV+ or HIV ELISA</td>
<td>PPD or IGRA</td>
<td>SMEAR NEGATIVE</td>
<td>IGRA</td>
<td>SMEAR POSITIVE</td>
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**#2 case**

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<td>AIDS PT TB SUSPECT</td>
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<td>IGRA</td>
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<td>IGRA RESULT POSITIVE</td>
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HIV can Interfere with TB Diagnosis

- ATYPICALS
- FONDS
Case #3
Difficult

- African male
- Treated 3 times for TB in Africa
- Flies to USA for “help”
- HIV negative

#3 case MDR suspect

MDR-TB SUSPECT
Call Lab!
MDDR POSITIVE
PPD or IGRA
SPUTUM COLLECTED
1ST SMEAR POSITIVE
PPD READ 15 mm
2ND SMEAR POSITIVE
NAAT on 1ST + SMEAR POSITIVE

#3 case MDR suspect

OCTOBER 2012
MDC Lab Confirmation of 2nd Line Drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Sensitivity</th>
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<tbody>
<tr>
<td>INH</td>
<td>R</td>
</tr>
<tr>
<td>Rifampin</td>
<td>R</td>
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<td>PZA</td>
<td>R</td>
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<tr>
<td>Ethambutol</td>
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<td>Cycloserine</td>
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Mycobacterial Examination

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3-Way Interactions to Provide Modern TB Care…

Questions & Discussion