Mayo Clinic Center for Tuberculosis

Diagnosis of Latent & Active Tuberculosis

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Disclosures: None
Objectives

• Describe Screening for LTBI: TST and IGRA

• Describe laboratory methods used for the detection, identification, new DNA based methods which include susceptibility testing for *M tuberculosis*

• Review typical TB related chest x-ray findings
Epidemiology of Tuberculosis
TB in Foreign-Born Immigrants to US

- Proportion of TB cases foreign-born increased from 29% to 66% (1993-2014)
- Mexico 21%, Philippines 12%, India 8%, Vietnam 8%
- US-born TB cases decreased by ~80% (1993-2012)
- ~90% MDRTB (97 in 2014) occur among Foreign-born
  - Anticipate XDR TB (2 cases 2014); TDR TB
- Concentrated in NY, CA, FL, TX (50% of US cases)
- Active cases most often arise from prior infection
- ~55% occur within 5 yrs of immigration
  - ≤ 2 yrs in US 75/100,000
  - > 2 yrs in US 16/100,000

CDC; Cain et al: JAMA 2008
Refugee & Immigrant TB Screening

• Within Country of Origin
  – Adults: Evaluated for Active TB only
  – Children (<15 yrs) & TB contacts screened (TST) in some countries but no LTBI Rx

• Arrival within US
  – TB Suspects are expected to f/u w/ local health dept (not mandated)
  – Applicants for adjustment of status evaluated for LTBI (Rx not mandated)

• Not evaluated…Estimates ~30%
  – Visitors, Temp Workers, Undocumented
  – Student visa

Immigration process doesn’t deal with LTBI for you…
“Tuberculosis is a social disease with medical implications”

–Sir William Osler
Focus of TB Control in the US: Targeted Testing & Rx for LTBI

- Few cases due to transmission from other active cases (↓ HIV related cases)
- High rates of TB among foreign-born immigrants to US (including rural locales) from high incident countries
- “Targeted tuberculin testing” remains theme of the LTBI guidelines
- One of the main targets must be the foreign-born immigrants from high incident countries
Latent TB Infection (LTBI) Testing
New technology replacing old...
Mantoux Tuberculin Skin Test (TST)

- Standard (old) method of skin testing for *M. tuberculosis* infection
- Produces delayed-type hypersensitivity reaction
- TST is useful for:
  - Detecting LTBI
  - Contact investigation: Determining how many people in a group are infected
  - Evaluating persons who have symptoms of active TB
Low (Old) Tech…TST
Delayed-type Hypersensitivity Reaction @ 48-72 hrs

- Positive: 18 mm **Induration**
- A positive test may be measured up to 7 days out
- A negative reaction can be read accurately @ 48-72 hrs
TST Interpretation

Positive classification based on pre-test probability of TB:

$\geq 5 \text{ mm} = \text{positive}$
- HIV positive
- Household or close contact to patient with infectious, active TB
- CXR consistent with old/healed TB
- Organ transplant or other immunosuppressed patient

$\geq 10 \text{ mm} = \text{positive}$
- Foreign born (e.g. Africa, SE Asia, Hispanic, India, China, E Europe)
- IV drug abusers
- Residents or employee of high risk congregate setting
- Non-immunosuppressive medical conditions known to increase risk of active TB
- Mycobacteriology lab workers

$\geq 15 \text{ mm} = \text{positive}$
- Persons in regions of low TB incidence
Limitations for TST

• Programmatic Issues: Must return for reading/interp.
• Interpretation variability; False positives: NTM…
• BCG Vaccine impacts TST Interpretation
  – Induces 3-19 mm TST reaction in 1st few mos.
    • Reaction wanes significantly by 10 years
    • Reaction size does not correlate with protection
  – +TST likely d/t TB infection in persons from endemic regions
  – Prior BCG, +TST ➞ Treat (usually argument too)

• Booster Phenomenon
  – False negative TST, becomes positive as a result of skin testing
  – Most common situations:
    • Initial TB infection many years previous
    • Prior BCG immunization
  – Two Step Skin Testing (TST x 2, one week apart)
    • Elderly nursing home population
    • Prior BCG immunization
LTBI Testing Upgrade…
Interferon Gamma Release Assay (IGRA)

Measures interferon-gamma (IFN-\(\gamma\)) released by lymphocytes in response to specific TB antigens: ESAT-6, CFP-10

• QuantiFERON\textsuperscript{®} Family:
  – QuantiFERON\textsuperscript{®} -TB test 1999
  – QuantiFERON\textsuperscript{®} - TB Gold 2005
  – QuantiFERON\textsuperscript{®} - TB Gold In-Tube (GIT) 2007
    Added 3\textsuperscript{rd} antigen TB7.7 (RD4) & travel time

• T-Spot.\textit{TB}\textsuperscript{®} Aug 2008:
TST vs IGRA

Presentation of TB antigens
- TST (Multiple = PPD)
- IGRA (Specific = ESAT-6, CFP-10)

IGRA Results include control wells
- Negative (Nil) – no antigen (subtract from pt value)
- Positive – mitogen stimulation

<table>
<thead>
<tr>
<th>IGRA</th>
<th>TST</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>In vitro</em></td>
<td><em>In vivo</em></td>
</tr>
<tr>
<td>Specific antigens</td>
<td>Multiple antigens</td>
</tr>
<tr>
<td>Unaffected by BCG</td>
<td>BCG affects results</td>
</tr>
<tr>
<td>No boosting</td>
<td>Boost occurs</td>
</tr>
<tr>
<td>One patient visit</td>
<td>Two pt visits</td>
</tr>
<tr>
<td>No inter-reader variability</td>
<td>Inter-reader variability</td>
</tr>
<tr>
<td>One standard result for all</td>
<td>Different thresholds based on risk</td>
</tr>
</tbody>
</table>
QFT vs T-Spot.TB

• Quantiferon TB (QFT): Whole blood incubated w/ TB specific antigens. ELISA measures IFN-\(\gamma\) release

• T-Spot.TB: Lymphocytes (T) incubated w/ specific antigens. ELISPOT-method counts IFN-\(\gamma\) releasing cells
## IGRA Interpretation

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
<th>Gray Zone</th>
<th>Indeterminate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>QFT-TB Gold &amp; IT version</strong></td>
<td>≥0.35*</td>
<td>&lt;0.35*</td>
<td>None</td>
<td>Controls fail:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• High Nil</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Mitogen response</td>
</tr>
<tr>
<td><strong>T Spot.TB</strong></td>
<td>≥8 spots*</td>
<td>&lt;8 spots*</td>
<td>5-7 spots*</td>
<td>Same as above</td>
</tr>
</tbody>
</table>

*TB Ag – Nil, assuming appropriate control response
IGRA CDC Guidelines 2010

• IGRA may substitute for TST
• IGRA preferred:
  – BCG vaccinated persons
  – Clients unlikely to return for TST reading
  – Low risk persons
• TST preferred in children <5
• Clinical judgment required when interpreting IGRA among immunosuppressed, children <5, & TB suspects
• Lab should be reporting quantitative results
Host Factors Creating False Negative TST & IGRA

- HIV (low CD4, no HART)
- <10 wks since TB infection
- Other infections (viral, fungal, bacterial)
- Lymphoma
- Live virus vaccination (eg, measles, smallpox)
- Immunosuppressive Rx
- Overwhelming TB (eg, miliary TB)
- Age (newborn, very old)
Can IGRA Replace TST?

- Contact investigation: YES
- BCG vaccine Hx: YES
- Low risk person: yes
- Screening homeless & other unreliable persons: YES
- Serial Testing: Yes, but…
Real Life with IGRA

• Significant reduction in positive rate vs TST
• Increased frequency of retesting

• Serial testing issues:
  – Unexpected positives that require further review (eg, repeat testing, assessing quantitative results)
  – “Wobblers” = results hovering around cut point
LTBI: TST & IGRA ≠ Gospel

- Reassess TB risk factors
- Review symptoms
- Review CXR... evidence suggest old TB (Upper lobe fibrosis, Gohn lesion, Hilar Ca++)

- LTBI Rx decision should be based on complete certainty that active TB not present
CDC. Updated Guidelines for Using IGRAs to detect M tuberculosis infection, US 2010. MMWR Recommendations and Reports June 25, 2010

LTBI Summary Points

- Screen persons at high risk for TB (eg, foreign born)
- LTBI diagnosis
  - Identify highest risk subgroups
  - Decision to test = Decision to treat
  - Positive results → Chest x-ray (?active TB)
- TST standard reviewed
- IGRA: QFT-Gold, T-Spot. *TB*
  - Acceptable, but imperfect alternative to TST
  - Transition to IGRA from TST continues
Case K-D. P.

- 27 yo Vietnamese male student
- 20 mm TST discovered in pre-employment at hospital
- Reluctantly admitted to...
  - Persistent cough, fevers, sweats, chills, weight loss, & fatigue x 4 weeks
K-D. P. Additional History

- Denied +TST upon arrival in US 1 yr previous
- Avoided student health for TST screening
- No other past medical history
- Denies HIV risk factors; no IV drug use
- Taking no medications
- Denies EtOH consumption
Patients with Active Tuberculosis

- Pulmonary disease 85% of immunocompetent
- Extrapulmonary disease (pleura, lymphatics, etc)
- AIDS patients present atypically
How would you proceed with the diagnostic w/u?
Diagnosis of Active TB

• **TST and IGRA**
  - False negative 10-20% active disease (↑ rate in HIV)
  - Less useful than in diagnosis of latent TB infection

• **Chest x-ray**
  - Non-immunosuppressed
    • Apical infiltrate
    • Cavitation common in advanced disease
    • Other findings (pleural effusion)
  - Immunosuppressed: nonspecific pattern

• **Sputum smear**
  - Acid fast staining (flurochrome enhancement)
  - $10^4$ bugs/ml to be detected on smear
  - 20-50% false negative (↑ in noncavitary disease)
  - Can not distinguish TB from other mycobacteria
Chext X-ray: Can this be TB?

“Typical Pattern” of Active TB (Post-primary or Reactivation)

- “Patchy” consolidation (100%)
- Apical/posterior segments of upper lobes & superior segments of lower lobes (90%)
  - Lung Ca usually anterior vs TB
  - “TB takes a backseat to Cancer”
Additional Patterns of Active TB (pp)

- Cavitation (40%)
- Endobronchial spread (20-25%)
- Other, More Rare
  - Miliary (2-5%)
  - Bronchostenosis
  - Tuberculoma
  - Pleural effusions
  - Bronchiectasis
“Atypical Pattern”: Primary TB (Mostly Children, HIV)

- Distribution: any lobe involved (slight lower lobe predominance)
- Air-space consolidation
- Cavitation is uncommon (<10%)
- Adenopathy is common, predilection for right side
- Miliary pattern
- Pleural effusions
# Radiographic Patterns: Pulmonary TB

<table>
<thead>
<tr>
<th>TB Pattern</th>
<th>“Typical” (Post-Primary)</th>
<th>“Atypical” (Primary)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infiltrate</td>
<td>90% upper</td>
<td>Upper : Lower</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 : 40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Usually upper in</td>
</tr>
<tr>
<td></td>
<td></td>
<td>children</td>
</tr>
<tr>
<td>Cavitation</td>
<td>Common</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Adenopathy (Only)</td>
<td>Uncommon</td>
<td>Children common</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adults ~30%</td>
</tr>
<tr>
<td>Effusion</td>
<td>May be present</td>
<td>May be present</td>
</tr>
</tbody>
</table>
Old/Healed TB

- Ca++ granuloma–Ghon lesion
- Ca++ granuloma and hilar node calcification–Ranke complex
- Apical pleural thickening
- Fibrosis and volume loss
Take Home: TB Radiology

- Typical or atypical radiograph...
- Chest CT often adds little to the plain CXR for diagnosing typical TB

➡️ TB can always fool you!
K-D. P. Sputum AFB Smear

What else do you order?
Diagnosis of Active TB II

- Culture & susceptibility (Rapid culture system)
  - Liquid media, rapid growth system (MGIT)
  - When growth index sufficient, DNA extraction/probe
    - UI lab: ~21 days considering all positive cultures
    - Faster if 3+ sputum smear (10-18 days)
    - DNA probe takes one additional day
  - INH, Rif, PZA, & Ethambutol adapted; susceptibility results
    ~ 8-14 days after culture positive notification

- Nucleic Acid Amplification Tests (NAAT):
  - Advantage: immediate identification
  - Less cost prohibitive than previously
  - Formerly used…
    - Amplicor: only for AFB smear positive samples
    - M. tuberculosis Direct (MTD): both smear pos. & negative spec.
Nucleic Acid Amplification Tests (NAAT)

- Detects *M. tuberculosis* rRNA or DNA (eg, PCR)
- Identification results w/in 24 hrs routinely (Sometimes w/in 48 hrs)
- Sensitivity $\approx$ Culture…but order culture for drug susceptibility data
- Clinical advantages
  - Enhances the diagnostic value of sputum sample
  - Immediate confirmation of *M. tuberculosis* impacts treatment decisions (eg, NTM vs. Mtb frequent issue in IA)
- Caveats
  - Detects dead as well as living organisms;
  - Reports of NAAT+ sputum in patients who completed successful Rx
  - Application in specimens from extrapulmonary sources not well defined (eg, pleural, CSF, LN)
TB Lab 2013

1. Process Specimen
   - 1 day

2. AFB Microscopy

3. Inoculate Media
   - 2 to 6 weeks

4. Culture Positive

5. Species Identification
   - 2 to 3 weeks

   - Nucleic Acid Amplification Tests (NAAT)

6. Drug Susceptibility

7. Molecular Detection of Drug Resistance

Metchock-CDC
NAAT = Gene Xpert Test

- Routinely used by UI & State Hygienic Lab
- ID Mtb & mutations in $rpoB$ (rifampin resistance)
- **Automated** commercial system
  - Decontamination, digestion, DNA extraction, amplification, & detection in same cartridge
  - RT PCR w/ molecular beacons: 5 probes for $rpoB$ & 1 positive control
  - Results w/in 2 hours
- Technically simple; Minimal hands-on
- Closed system (no biohazard issues)
- Multi-use platform

Boehme et al, *NEJM* 2010;363:1005-15
Gene Xpert Disadvantages

- Proprietary instrument & cartridges
- Not customizable by user
- Doesn’t detect all $rpoB$ mutations
- Silent mutations can result in false prediction of resistance

Boehme et al, *NEJM* 2010;363:1005-15
TB Lab 2013

Process Specimen → 1 day → AFB Microscopy → Inoculate Media → 2 to 6 weeks → Culture Positive → Molecular Detection of Drug Resistance

Nucleic Acid Amplification Tests (NAAT)

Species Identification → 2 to 3 weeks → Drug Susceptibility

Metchock-CDC
CDC Molecular Detection of Drug Resistance (MDDR)

• Sept 2009 to present
• DNA sequencing (ABI3130xl)
• MDR TB
  – Rifampin: rpoB
  – INH: inhA, katG
  – Ethambutol: embB
  – Pyrazinamide: pncA
• 2^{nd} line drugs (XDRTB) also available
Results for Molecular Detection of Drug Resistance; Conventional Drug Susceptibility Test in progress.

<table>
<thead>
<tr>
<th>Locus (region) examined</th>
<th>Result</th>
<th>Interpretation (based on in-house evaluation of 254 clinical isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpoB (RRDR)</td>
<td>Mutation TCG&gt;TTG Ser531Leu</td>
<td>Rifampin resistant. (100% of isolates in our in-house evaluation of 254 clinical isolates with a mutation at this codon are RMP-R.)</td>
</tr>
<tr>
<td>inhA (promoter)</td>
<td>No mutation</td>
<td>Cannot rule out INH resistance. (89% of INH-R isolates in our in-house evaluation of 254 clinical isolates have a mutation at one or both of these loci.)</td>
</tr>
<tr>
<td>katG (ser315 codon)</td>
<td>No mutation</td>
<td>Cannot rule out ethambutol resistance. (79% of EMB-R isolates in our in-house evaluation of 254 clinical isolates have a mutation at this locus.)</td>
</tr>
<tr>
<td>embB (Met306,Gly406)</td>
<td>No mutation</td>
<td>Cannot rule out PZA resistance.</td>
</tr>
<tr>
<td>pncA (promoter, coding region)</td>
<td>No mutation</td>
<td>Cannot rule out fluoroquinolone resistance. (96% of FQ-R isolates in our in-house evaluation of 254 clinical isolates have a mutation at this locus.)</td>
</tr>
</tbody>
</table>
| gyrA (QRDR)             | No mutation | Cannot rule out resistance to injectable drugs (kanamycin, capreomycin, amikacin). (In our in-house evaluation of 254 clinical isolates:  
- 89% of AMK-R isolates have a mutation in the rrs locus;  
- 88% of KAN-R isolates have a mutation in the rrs locus; an additional 29% of KAN-R isolates have a mutation in the eis locus;  
- 46% of CAP-R isolates have a mutation in the rrs locus; an additional 5% of CAP-R isolates have a mutation in the tlyA locus.) |
| rrs (1400 region)       | No mutation | Cannot rule out resistance to injectable drugs (kanamycin, capreomycin, amikacin). (In our in-house evaluation of 254 clinical isolates:  
- 89% of AMK-R isolates have a mutation in the rrs locus;  
- 88% of KAN-R isolates have a mutation in the rrs locus; an additional 29% of KAN-R isolates have a mutation in the eis locus;  
- 46% of CAP-R isolates have a mutation in the rrs locus; an additional 5% of CAP-R isolates have a mutation in the tlyA locus.) |
| eis (promoter)          | No mutation | |
| tlyA (entire ORF)       | PENDING** | |

*A negative results (e.g., no mutation) does not rule out contributory mutations present elsewhere in the genome.

**Assay is being repeated (12/2/2012).

Testing performed using in-house developed assays.
Summary
Diagnosis of Active Pulmonary TB 2017

• (TST)
• Chest x-ray
• Sputum
  – AFB smear examination
  – NAAT on smear
  – Culture & susceptibilities
  – Consider CDC → MDDR testing

Note: Start treatment immediately in suspect cases to reduce risk of further transmission
Concluding Points

• History & CXR should seek to distinguish active vs. latent TB infection

• Pre-test probability must drive interpretation of test results

• Radiology findings in pulmonary TB
  - Atypical chest X-rays occur
  - Chest CTs assist TB dx minimally, but can suggest alternative diagnosis

• New TB tests:
  - LTBI: IGRA (QFT-G, T-Spot)
  - Active TB: NAAT & MDDR

• Active TB: Obtain drug susceptibility along with culture (Consider CDC → MDDR)

• Call TB expert if suspect drug resistant disease
TB Nomenclature

• Latent TB Infection (~90% TB infections):
  – Positive TST or IGRA (eg, QFT-G, T-Spot)
  – No symptoms
  – Negative or chronic CXR changes
  – Can not transmit disease to others.

• Active TB Infection (~10% TB infections):
  – TST or IGRA may be positive
  – Symptoms present
  – CXR changes & sputum smear positive in most cases
  – Disease transmission to others

• Treatment for both latent and active infections

• Avoid terms: Prophylaxis, Preventive therapy
TB Pathogenesis
Progression to Disease

Infection (LTBI)

- 3-4% First Year
- 1-2% Second Year
- ~0.1% per year thereafter

Disease
(Active Infection)

No Active Disease (~90%)
<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Risk Estimate (vs. control w/ +TST)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced HIV</td>
<td>9.9</td>
</tr>
<tr>
<td>Anti-TNF Rx</td>
<td>7.9</td>
</tr>
<tr>
<td>Old, healed TB</td>
<td>5.2</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3.1</td>
</tr>
<tr>
<td>Tobacco abuse</td>
<td>2.7</td>
</tr>
<tr>
<td>Chronic Renal Failure</td>
<td>2.4</td>
</tr>
<tr>
<td>Silicosis</td>
<td>1.7</td>
</tr>
<tr>
<td>Underweight (10% &lt; IBW)</td>
<td>1.6</td>
</tr>
<tr>
<td>Gastrectomy</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Targeted TB Testing
Decision to Test = Decision to Treat

- Patients at highest risk for progression to active TB
- Patients with medical conditions that increase risk for active TB
- Patients in whom active TB is more prevalent
Targeted TB Testing
Decision to Test = Decision to Treat

- Patients at highest risk for progression to active TB
- Patients with medical conditions that increase risk for active TB
- Patients in whom active TB is more prevalent
Targeted TB Testing
Decision to Test = Decision to Treat
Patients at highest risk for progression to active TB

- HIV infection, or risk factors for HIV infection
- Receiving TNFα antagonist (ie, RA, Crohn’s)
- Fibrotic lesion on CXR c/w prior pulmonary TB
- Close contact of persons with infectious TB (e.g., pulmonary, laryngeal TB)
- New TB infection (TST conversion within prior 2 years)
- IV drug abuser (HIV negative)
Targeted TB Testing
Decision to Test = Decision to Treat

- Patients at highest risk for progression to active TB
- Patients with medical conditions that increase risk for active TB
- Patients in whom active TB is more prevalent
Targeted TB Testing

Decision to Test = Decision to Treat

Medical conditions ↑ risk for progression to active TB

- Diabetes mellitus
- Tobacco abuse *(Recent add)*
- Silicosis
- Jejunoileal bypass surgery or gastrectomy
- Solid organ transplant (e.g. renal, heart)
- Chronic renal failure/hemodialysis
- Head/neck carcinoma
- Hematologic malignancies (e.g. leukemia, Hodgkin’s)
- Immunosuppressed, particularly steroid treatment (≥15 mg/day, ≥ 1 month)
- Substantial weight loss: >10% ideal body weight
Targeted TB Testing
Decision to Test = Decision to Treat

- Patients at highest risk for progression to active TB
- Patients with non-HIV medical conditions that increase risk for active TB
- Patients in whom active TB is more prevalent
Targeted Skin Testing
Decision to Test = Decision to Treat
Patients in whom active TB is more prevalent

- Recent arrivals (< 5 years) from high TB prevalence countries (Africa, SE Asia, Pacific Isles, Latino, E. Europe, Russia)
- Resident or employee of high-risk congregate settings: prisons/jails, nursing homes/other long term facilities, hospitals/other health care facilities, residential facilities for AIDS patients, and homeless shelters
- Mycobacteriology lab workers