LTBI Diagnosis

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Special thanks to David Horne

LTBI Cascade

Alsdurf Lancet ID 2016
Variability in TB Outcomes

80% of U.S. TB cases due to reactivation: PREVENTABLE

~90% will not progress to TB

How do we identify those most likely to develop TB?

Predicting Progression to TB

Need: test to identify progressors

- 7500 rural Chinese, followed 2 yrs
  - Cumulative TB Incidence: 0.3% (TST+/QFT-); 2.0% (TST-/QFT+); 1.6% (TST+/QFT+)

- S. African Study, >6000 participants to identify transcriptional signatures associated with TB progression
- Validated in other settings
- RCT to randomize individuals to LTBI tx based on signature

Diagnosing LTBI

- **No gold standard** for diagnosing LTBI
- All tests assess T-cells for prior exposure to TB antigens

Tuberculin Skin Test (TST)  
Interferon Gamma Release Assays (IGRAs)
Diagnosing LTBI

- **No gold standard** for diagnosing LTBI
- **All** tests assess T-cells for prior exposure to TB antigens

TST/IGRA are **indirect measures** of *M. tb* infection

Require: *infection* and a **functioning immune system for a positive test**

Both may take 2-10 weeks to become positive after an exposure

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**Test Accuracy**

- **Sensitivity**: Probability of a positive test in someone with infection
- **Specificity**: Probability of a negative test in someone without infection
- **Positive Predictive Value**: Probability of infection in someone with a positive test
- **Negative Predictive Value**: Probability of no infection in someone with a negative test
**Test Accuracy**

- **Sensitivity:** $A/A+C$
- **Specificity:** $D/B+D$
- **Positive Predictive Value:** $A/A+B$
- **Negative Predictive Value:** $D/C+D$

**Targeted Testing: Rationale**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TST</td>
<td>80%</td>
<td>97% (60% if BCG vaccinated)</td>
</tr>
<tr>
<td>QFT</td>
<td>80%</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>T-SPOT.TB</td>
<td>90%</td>
<td>&gt;95%</td>
</tr>
</tbody>
</table>

For a US born patient (LTBI prevalence ~3%) with no other risk factors, the PPV* of....

- + TST is 45%
- + QFT is 55%
- + T.SPOT. TB is 58%

*Probability of infection in someone with a positive test*
LTBI Diagnosis: TST

- **Tuberculin - Robert Koch, 1890**
- Inject 0.1 ml of 5 TU PPD tuberculin solution intradermally on volar surface of lower arm
- At 48 to 72 hours, measure induration not erythema
  - transversely to the long axis of the forearm - **Record induration in mm!**
  - Ensure trained healthcare professional measures and interprets the TST

*False positive: BCG, NTM*
*False negative: anergy, immunocompromised*


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TST: Thresholds Based on Risk

- **≥ 5mm**
  - HIV
  - close contact of infectious case
  - fibrotic changes on CXR consistent with old TB
  - severely immunosuppressed (e.g. organ transplant, TNFα blockade)
- **≥ 10mm**
  - Recent immigrants (<5 yrs) from high prevalence countries
  - Residents/employees of high-risk congregate settings
  - TB lab personnel
  - IVDA
  - Children < 4 years of age (screened if there are risk factors)
- **≥ 15mm (no need to screen) - all others**
- Serial Testing

2000 ATS/CDC Guidelines
TST Limitations

- Specificity—poor to good
  - Mycobacteria besides *M. tuberculosis*
  - Bacillus Calmette-Guérin (BCG) vaccine
- Sensitivity (i.e. false negatives)
  - Factors related to testing procedure
  - TB disease (25% negative)
  - Immunosuppressive states
  - Recent viral infection, vaccinations*
  - Very young age
  - Early TB infection (2-10 weeks)
- Two healthcare encounters for one result

* MMR, oral polio, varicella give TST same day or wait weeks

Booster Phenomenon

- Ms. A may have been exposed and infected with M.tb sometime in the 3 months OR this may represent a booster phenomenon
- Positive TST after prior negative TST without TB exposure
  - Due to recall of waned cell-mediated immunity
  - Maximal if interval 1-5 weeks although may persist for >1 year
  - More common in elderly, BCG-vaccinated, sensitization due to NTM
- IF boosted TST reflects true LTBI→ risk of progression lower than w/new conversion
- For annual TST screening programs, the initial test (if negative) should have 2nd TST 1-3 weeks later
- IGRA may be “boosted” by TST administration
  - May increase IFN response enough to go from negative to positive
  - IGRA boosting occurred at 7 days, but not 3 days, post-TST (van Zyl-Smit, AJRCCM 2009)
Interferon-gamma Release Assays (IGRAs)

Collect 1mL of blood in 3 tubes

Incubate within 16 hr, at 37°C for 16–24 hr

Collect plasma for ELISA

Measure [IFN-γ]/Interpret

QuantiFERON-GIT

TSPOT.TB

Interferon-gamma Release Assays (IGRAs)

QuantiFERON-Plus

4th Generation QuantiFERON, FDA approved in 2017, uses same test principle, procedure and technology

Added 2nd antigen tube (TB1, TB2), kept ESAT6/CFP10

- Nil – same grey color, Mitogen – same purple
- TB1 – green, CD4 only
- TB2 – yellow: detect both CD4 and CD8
- OR... Standard lithium heparin tube→ 16 hours to transfer to 4 tubes

Why CD8+ antigens? May incite stronger response in recent infection and remain relatively intact in immunocompromised, children Lancioni AJRCCM 2012

Multicenter study of QFT Gold + in patients with active TB Home IJLTD 2018

*164 participants with active TB
*QFT-GIT sensitivity 94%
*QFT-Plus sensitivity 93%
*Kappa 0.89
Antigens: TST vs. IGRAs

*M. tuberculosis* antigens shared with NTM & BCG

Antigens specific to *M. tuberculosis*
ESAT-6 & CFP-10

 juga et al, 2008: 88, 510-517

May get false positives due to infection with these uncommon pathogens

QFT-Plus Interpretation

<table>
<thead>
<tr>
<th>Nil (IU/ml)</th>
<th>TB1 minus Nil (IU/ml)</th>
<th>TB2 minus Nil (IU/ml)</th>
<th>Mitogen minus Nil (IU/ml)*</th>
<th>QFT-Plus Result</th>
<th>Report/interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤8.0</td>
<td>≥0.35 and ≥25% of Nil</td>
<td>Any</td>
<td>Any</td>
<td>Positive¹</td>
<td>M. tuberculosis infection likely</td>
</tr>
<tr>
<td></td>
<td>Any</td>
<td>≥0.35 and ≥25% of Nil</td>
<td>Any</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;0.35 or ≥0.35 and ≥25% of Nil</td>
<td>&lt;0.35 or ≥0.35 and &lt;25% of Nil</td>
<td>≥0.50</td>
<td>Negative</td>
<td>M. tuberculosis infection NOT likely</td>
</tr>
<tr>
<td></td>
<td>&lt;0.35 or ≥0.35 and ≥25% of Nil</td>
<td>&lt;0.35 or ≥0.35 and &lt;25% of Nil</td>
<td>&lt;0.50</td>
<td>Indeterminate¹</td>
<td>Likelihood of M. tuberculosis infection cannot be determined</td>
</tr>
<tr>
<td>&gt;8.0⁴</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
QFT-Plus Interpretation

Either TB1-Nil or TB2-Nil considered positive

Indeterminates

Two main reasons for an indeterminate result:
- **Low Mitogen** response (a weak immune response to a strong stimulant)
- **High Nil** (background level of IFN-gamma)

<table>
<thead>
<tr>
<th></th>
<th>NIL</th>
<th>TB1</th>
<th>TB2</th>
<th>MIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>-</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>Positive</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Negative</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
**Indeterminates**

- Optimally, an indeterminate result tells you that MTB infection data cannot be obtained from the QFT-IT test
  - Low lymphocyte count
  - Low lymphocyte activation potential
- Optimally, an improvement over the TST in which “anergy” cannot be diagnosed
- Repeat test with valid result (pos/neg) in 68% (Banach IJTLD 2011)

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**TSPOT Interpretation**

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>TB Response*</th>
<th>Nil</th>
<th>Mitogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>≥ 8 spots</td>
<td>≤ 10 spots</td>
<td>any</td>
</tr>
<tr>
<td><strong>Borderline</strong></td>
<td>5, 6, or 7 spots</td>
<td>≤ 10 spots</td>
<td>any</td>
</tr>
<tr>
<td>Negative</td>
<td>≤ 4 spots</td>
<td>≤ 10 spots</td>
<td>&gt; 20 spots</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>&lt; 5 spots</td>
<td>≤ 10 spots</td>
<td>&lt; 20 spots</td>
</tr>
<tr>
<td></td>
<td>any</td>
<td>&gt; 10 spots</td>
<td>any</td>
</tr>
</tbody>
</table>

*TB Response is the higher number of spots resulting from stimulation of PBMCs with two separate cocktails of peptides representing ESAT-6 or CFP-10, minus the number of spots resulting from incubation of PBMCs with saline (i.e., Nil).
LTBI Diagnosis Guidelines

Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children

What they cover: Recommended tests for TB (LTBI and TB disease)
6 specific recommendations for TST vs IGRA based on...
   - Likelihood of LTBI
   - Likelihood of Progression from LTBI to TB disease

RISK OF TB INFECTION \times RISK OF PROGRESSION = RISK OF TB DISEASE

New Guidelines

Lewinsohn CID 2017
Preferred Test = IGRA, especially if likely BCG vaccinated

Specificity preferred
Screening Guidelines (age > 5 yrs)

Preferred Test = either IGRA or TST or Both

Sensitivity prioritized

Lewinsohn CID 2017

TST & IGRA Agreement

- Take advantage of poor test agreement in patients at high risk of TB progression if latently infected (e.g. HIV-positive, TNF-alpha blocker therapy)
- Dual testing: if TST negative then perform IGRA and treat for any positive tst

Ghassemieh AJRCCM 2016
Screening Guidelines (age > 5 yrs)

**Preferred Test = IGRA (TST ok too)**
- if 1st test +, perform 2nd
- Also look at IGRA values

**Specificity Prioritized**

Lewinsohn *CID* 2017

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**Case 2**

54 yo man (US born) who works in hospital cafeteria & is without known TB exposures is tested as part of annual TB screening. QFT results were:

<table>
<thead>
<tr>
<th>Time</th>
<th>Nil</th>
<th>TB Ag</th>
<th>Mitogen</th>
<th>Ag-Nil</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 year ago</td>
<td>0.12</td>
<td>0.42</td>
<td>7.1</td>
<td>0.30</td>
<td>Negative</td>
</tr>
<tr>
<td>Last week</td>
<td>0.36</td>
<td>0.77</td>
<td>8.2</td>
<td>0.41</td>
<td>Positive</td>
</tr>
</tbody>
</table>

A. The patient converted in the past year
B. Results from 1 year ago were likely false negative
C. Unclear situation → place TST and treat based on result
D. No treatment, no additional testing at this time
54 yo man (US born) who works in hospital cafeteria & is without known TB exposures is tested as part of annual TB screening. QFT results were:

A. The patient converted in the past year
B. Results from 1 year ago were likely false negative
C. Unclear situation → place TST and treat based on result
D. No treatment, no additional testing at this time
Many potential sources of variability

IFN-γ may vary by +0.24 IU/ml when result between 0.25-0.80 (Metcalf AJRCCM 2013)

S. Africa study of serial QFTs – “converters” who had levels < 0.7 IU/ml had same TB risk as those with levels <0.2 IU/ml (Nemes AJRCCM 2017)

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Use all the information…assess Ag-nil value

Preferred test: TST

Rationale

– Limited evidence suggests TST more sensitive in children
– Prioritize sensitivity over specificity
– Allows for serial testing with TST during “window prophylaxis”

Can use IGRAs in immunocompetent children > 2 years; some experts down to 1 year of age - Esp. if prior BCG vaccination

Lewinsohn CID 2017; AAP 2018 Red Book; Amina 2018
**LTBI Tests & Immunocompromise**

Presence of TB Risk Factors

- Immunosuppression
  - IGRA & TST$$
  - IGRA & TST$$
  - IGRA

- Immunosuppression
  - IGRA & TST$$
  - IGRA & TST$$
  - IGRA

$$TB$ risk factors include: a history of contact to a case of active TB; birth or extended living in regions where the TB prevalence is greater than 2/100,000 per year; history of working or living in jails, prisons, healthcare facilities providing care to TB patients, or homeless shelters; or history of intravenous drug use.

$$Immunosupression includes poorly controlled rheumatoid arthritis or other inflammatory immune mediated disease, current use of biologic or non-biologic disease modifying therapies, or current use of immunosuppressives or other conditions.

$$In regions of BCG use (or individuals with BCG history), consider a dual strategy of using both commercially available IGRA (Quantiferon®-in-Tube® and T-Spot.TB®) in lieu of the TST.

For patients with risk factors and immunosuppressed in whom false negative results are more likely, consider repeat screening with one or both tools.

TB: tuberculosis; IGRA: interferon-gamma release assay; TST: tuberculin skin test

**Are IGRAs Useful for Assessing Tx Response?**

- Systematic Review of IGRAs and association with tx responses for LTBI and active TB

- 15 studies that evaluated LTBI responses
- No consistent pattern using reversions or quantitative IFN-gamma levels

*Current IGRAs not useful to assess Tx response*

Clifford *Tuberculosis* 2015
IGRA vs. TST

QFT-Plus *in-vitro* T.SPOT.TB

- specific Mtb antigens (no BCG cross-reactivity)
- no boosting
- 1 patient visit
- phlebotomy
- stimulate within hours
- results possible in 1 day
- complex laboratory test
- built in negative/positive controls
- one cutoff regardless of risk

TST *in-vivo*

- PPD (BCG cross-reactivity)
- boosting
- 2 patient visits
- intracutaneous injection
- injected = done
- results in 2–3 days
- point-of-care test
- serial testing advantages
- risk-based cutoffs

Both:

**Indirect tests of infection**

**Cross-reactive with NTM**

**2-10 week delay after exposure**
**New Guidelines: Summary**

<table>
<thead>
<tr>
<th>Group</th>
<th>Testing Strategy</th>
<th>Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Likely to be infected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Risk of Progression (TST ≥ 5mm)</td>
<td>Acceptable: IGRA OR TST</td>
<td>Prevalence of BCG vaccination</td>
</tr>
<tr>
<td></td>
<td>Consider dual testing where a positive result from either result would be considered positive</td>
<td>Expertise of staff and/or laboratory</td>
</tr>
<tr>
<td></td>
<td>Children ≤ 5 years of age</td>
<td>Test availability</td>
</tr>
<tr>
<td></td>
<td>Preferred: TST</td>
<td>Patient perceptions</td>
</tr>
<tr>
<td></td>
<td>Acceptable: IGRA OR TST</td>
<td>Staff perceptions</td>
</tr>
<tr>
<td></td>
<td>Consider dual testing where a positive result from either would be considered positive³</td>
<td>Programmatic concerns</td>
</tr>
<tr>
<td>Likely to be infected Low to Intermediate Risk of Progression (TST ≤ 10mm)</td>
<td>Preferred: IGRA where available</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acceptable: IGRA or TST</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Testing for LTBI is not recommended</td>
<td></td>
</tr>
<tr>
<td></td>
<td>If necessary:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preferred: IGRA where available</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acceptable: Either IGRA OR TST</td>
<td></td>
</tr>
<tr>
<td></td>
<td>For serial testing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acceptable: Either IGRA OR TST</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Consider repeat or dual testing where a negative result from either would be considered negative³</td>
<td></td>
</tr>
<tr>
<td>Unlikely to be infected (TST &gt; 15mm)</td>
<td></td>
<td></td>
</tr>
</tbody>
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Lewinsohn *CID* 2017

**Summary**

- All LTBI tests have limitations
  - Screening in Low Risk Individuals → Don’t Do
  - Dual Testing → Useful for maximizing sensitivity OR specificity
- Assess IGRA values (Ag-nil)
  - Nemes: Ag-nil < 0.7
  - Especially when serial testing
- AAP Redbook recommends IGRAs down to age 2 (and many experts down to age 1 or younger)
- NO LTBI test used to diagnose active TB
  - Rare exceptions
Resources


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David Horne
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Chris Spitters
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