Interferon Gamma Release Assays (IGRAs) for the Diagnosis of Latent Tuberculosis Infection

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Conflict of interest statement

I have performed, and am currently performing, studies using the T-Spot. TB IGRA

Outline

• Background
  – Latent tuberculosis infection
  – Sensitivity and specificity
  – Tuberculin skin test
• Interferon-gamma assays
  – How they work
  – Understanding the literature
  – Limitations
• Clinical considerations

Test Sensitivity

# subjects with the disease who test positive

Total # of subjects with the disease

How to calculate sensitivity

• Test a population that you know has the disease you are looking for
  • i.e. test people with culture-proven active tuberculosis
  • Example: the sensitivity of the TST for active TB is about 70%

Figure 3
The pathogenesis of tuberculosis in the infected host

CDN STDs 2000
Specificity

# subjects that do not have the disease who test negative

Total # of subjects who do not have the disease

How to calculate specificity

• Test a population that you know does not have the disease you are looking for

• i.e., test people who have no epidemiologic risk factors for tuberculosis infection or disease

• Example: test health, young CDN born individuals with no TB risk factors

Comparing tests

• Compare the new test to the “gold standard” (i.e., the test that allows you to know whether a subject has or does not have the disease).

• There is no gold standard for latent tuberculosis infection

Confused yet?

Purified Protein Derivative aka “PPD”, “Tuberculin”

• Different formulations used

• Contains approximately 200 antigens

• Many antigens are shared between different mycobacteria
  – M tuberculosis complex
  – M bovis
  – BCG strain of M bovis
  – Environmental mycobacteria

Tuberculin skin testing

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Tuberculin skin testing
- Interobserver variability (2.5 mm)
- Intra-observer variability (1.3-1.9 mm)
- Biologic variability (<3mm)
- False negatives in active disease (30%)
- Requires 2 visits

Sensitivity and specificity
- Sensitivity cited as 97% for latent TB infection in healthy individuals
  - How do we know?
  - Decreased in immunocompromised
- Sensitivity closer to 70% for active disease
- Specificity influenced by BCG vaccination, other mycobacterial exposure

Age at BCG vaccination and positive TSTs
- Infancy: no difference in positivity between vaccinated and unvaccinated after 5 years
- Primary school: 15-25% remain positive after 10 years
- Induration >18mm unlikely to be BCG

Summary
- The TST performs well in healthy, non-BCG vaccinated individuals
  - Decreased specificity if BCG vaccinated, especially later in life
  - Decreased sensitivity in immunocompromised populations
    - Low CD4 count
    - End stage renal disease
    - Chronic corticosteroid use
    - Hematologic malignancies
    - Transplant recipients

INFγ-based tests

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Interferon-gamma release assays (IGRAs)

- **QuantiFERON Gold**
  - ELISA
  - Licensed in Canada, USA
- **T-Spot.**
  - ELISPOT
  - Licensed in Canada, USA license pending

What is an ELISA?

- Enzyme-linked immunosorbant assay
- An enzyme is linked to an antibody direct against the molecule of interest (interferon gamma)
- Excess antibody is washed off and a substrate is added
- The enzyme acts on the substrate and causes a colour change

What is an ELISPOT?

- Enzyme-linked immunospot
- Similar to ELISA except individual positive cells are counted rather than measuring colour change
- In general, ELISPOTs are believed to be more sensitive than ELISAs because:
  - Individual cells are counted
  - Secreted cytokines are measured adjacent to the cell rather than diluted in the supernatant

Antigens used in latest IGRAs

- Two highly-specific proteins for *M. tuberculosis*
- Encoded on the Region of Difference-1 (RD-1) segment of the MTB genome
  - Early secreted antigenic target (ESAT-6)
  - Culture filtrate protein 10 (CFP-10)

Specific antigens for TB?

- ESAT-6 and CFP-10 are also found in:
  - *M. leprae*
  - Wild type *M. bovis* (not the BCG strains)
  - *M. marinum*
  - *M. kansasii*
  - *M. szulgai*
  - *M. flavescens* (Reasonably common in Canada)

Older IGRAs

- Most of the studies more than 3 years old looked at earlier versions of the tests
  - Different antigens
  - Incomplete antigens
- These studies are far less relevant to understanding the current IGRAs
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Summary

• Both assays use RD-1 antigens
• The T-SPOT.TB requires white cell separation and cell counting — i.e., counting of gamma interferon-producing cells as spots at the bottom of a well
• The QuantiFERON measures gamma interferon production in the supernatant
• Theoretically, the T-SPOT.TB should be more sensitive

So how do you evaluate a test when there is no gold standard for latent tuberculosis?

Hint: You can’t just compare it to the TST

Clinical assessment of IGRAs

• Measure the correlation between the TST and IGRA with:
  – Active TB
  – Outbreak setting (stratify according to exposure)
  – High risk setting (e.g. healthcare worker)
  – BCG vaccination
• Add in additional variables such as immunocompromised status
• Unable to measure true sensitivity and specificity

T-SPOT.TB in an outbreak setting

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HIV positive

TABLE 1. BASELINE CHARACTERISTICS OF YOUNG ADULTS ATTENDING VOLUNTARY COUNSELING AND TESTING FOR HIV INFECTION IN WAKHLENGA TOWNSHIP, SOUTH AFRICA

<table>
<thead>
<tr>
<th>HIV infected (n = 72)</th>
<th>HIV uninfected (n = 84)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, % (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>51 (72)</td>
<td>58 (69)</td>
</tr>
<tr>
<td>Apig, %</td>
<td>10 (29)</td>
<td>10 (12)</td>
</tr>
<tr>
<td>Tuberculin positive, % (n)</td>
<td>29 (40)</td>
<td>15 (18)</td>
</tr>
<tr>
<td>Detoxified BCG scarring, % (n)</td>
<td>8 (11)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Any</td>
<td>30 (42)</td>
<td>31 (37)</td>
</tr>
</tbody>
</table>


TST decrease sig.
IGRA decrease not sig.

Hemodialysis patients

Table 3: Logistic regression analysis: Factors associated with a positive TSPOT TB test. The and expert physician visual inspection of current or past tuberculosis infection.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>p Value</th>
<th>OR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSPOT TB</td>
<td>1.00 (0.29 to 3.50)</td>
<td>0.21</td>
<td>1.00 (0.29 to 3.50)</td>
<td>0.21</td>
</tr>
<tr>
<td>History of TB</td>
<td>7.45 (0.24 to 28.0)</td>
<td>0.02</td>
<td>7.45 (0.24 to 28.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Radiographic pattern of TB</td>
<td>5.52 (1.37 to 20.8)</td>
<td>0.04</td>
<td>5.48 (1.29 to 23.1)</td>
<td>0.03</td>
</tr>
<tr>
<td>History of BCG scar</td>
<td>1.06 (0.26 to 4.73)</td>
<td>0.95</td>
<td>1.06 (0.26 to 4.73)</td>
<td>0.95</td>
</tr>
<tr>
<td>High-risk occupation*</td>
<td>0.79 (0.32 to 2.20)</td>
<td>0.66</td>
<td>0.79 (0.32 to 2.20)</td>
<td>0.66</td>
</tr>
<tr>
<td>History of BCG scar</td>
<td>1.36 (0.53 to 3.46)</td>
<td>0.50</td>
<td>1.36 (0.53 to 3.46)</td>
<td>0.50</td>
</tr>
<tr>
<td>Expert visual inspection</td>
<td>2.34 (1.12 to 5.0)</td>
<td>0.03</td>
<td>2.34 (1.12 to 5.0)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*High-risk occupation: Definition of high-risk occupation is not provided in the text.

Passalent et al., CJASN 2006

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What does a positive IGRA mean in terms of active TB risk?
• + TST carries a 5-15% lifetime risk of active disease
• Most of the risk is in the first 2 years after infection
• There are no natural history studies with IGRAs

Discordant results
• TST +, IGRA –
  – Secondary to BCG or false negative IGRA?
• TST -, IGRA +
  – Poor sensitivity of the TST or false positive IGRA?
  • You cannot tell with certainty

Clinical uses of IGRAs

Serial testing
• Small studies
  – IGRA response increased, decreased, or stayed the same……
• One study of HCWs in India
  – QuantiFERON test results showed reversion, conversion, or did not change…just like the TST
  • Continue to use TST

Immigrant screening
• In general, screening of immigrants with TST is not recommended, unless they have underlying disease
• IGRAs will not significantly increase program efficiency
• If going to screen, use TST

Contact tracing
• QuantiFERON and T-SPOT.TB similar to TST in non-BCG vaccinated, better in BCG vaccinated
• But, considerable discordance found with both tests

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Contact tracing recommendations

- Not enough evidence to recommend replacing the TST with IGRA
- IGRA should not be used alone
- If IGRA and TST both used, and patient high risk, treat if either is positive
- If TST + and patient considered low risk, may do an IGRA to confirm

Immunocompromised

- In general, IGRAs appear to be more sensitive than the TST
  - But, IGRAs have a higher percentage of “indeterminate” results
- T-SPOT.TB likely more sensitive than the QuantIFERON in immunocompromised

Immunocompromised recommendations

- Jury still out
- Some say evidence is enough to recommend them, at least in conjunction with TST
- Others say they is not enough evidence to recommend their use

Summary

- At present, the role for IGRAs is relatively limited
  - Natural history of + IGRA is unknown
  - Discordant results are common
  - Discordant results not well defined
  - If used with TST, treat if either is positive in high risk
    - Willing to accept discordance in low risk
  - A new study is out every week

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