Interferon Gamma Release Assays and the Diagnosis of Latent Tuberculosis

Thomas E Dobbs MD, MPH
Health Officer, District VII/VIII
Mississippi State Department of Health

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Transmission
• Small droplets (<100µm) coughed up by pulmonary TB patients aerosolize and float through the air
• These droplets fall to the ground faster in humid conditions
• Small droplets containing TB bacilli settle in lung alveoli
• Bacilli are ingested by alveolar macrophages
Survival and Proliferation

• Bacilli survive and proliferate within macrophages
• Bacilli kill macrophages, are ingested by new macrophages and continue to proliferate
• Bacilli spread to lymph nodes and spread systemically

Host Immune Response

• Cell mediated immunity (T-Cell directed) coordinates immune response
• Immune system contains/limits bacilli growth

OR

• There is an ineffective immune response and the patient progresses to primary disease

Exposure to Infectious patients

No infection (70%)
Infection (30%)
Early progression (5%)
Containment (95%)
Late progression (5%)
Continued containment (90%)
Natural History of TB Infection
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Natural History of TB Infection

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PPD

- Purified Protein Derivative — developed in 1939 by Florence Siebert in Philadelphia
- Precipitate prepared by filtration of Old Tuberculin
- Mixture of ~170 different proteins
- Intradermal injection leads to delayed type hypersensitivity for those with prior exposure

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Recent TB Timeline

- 1882: Robert Koch discovers TB Bacillus in Berlin
- Tuberculin developed as "cure" for TB
- 1896: Tuberculin (PPD) standardized for diagnosis of TB
- 1940: Tuberculin (PPD) standardized for diagnosis of TB
- 1950: BCG Vaccine
- 1921: FDA approves QFT-Gold
- 2008: FDA approves T-SPOT.TB

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Diagnosing Latent TB

- Tuberculin Skin Testing
- IGRA’s (Quantiferon and T-spot)
- Do not differentiate between Latent and Active Disease!!!
Testing for M. tuberculosis Infection

- Mantoux tuberculin skin test (TST)
  - Skin test that produces delayed-type hypersensitivity reaction in persons with M. tuberculosis infection

- Interferon-gamma Release Assays
  - Blood tests that measures and compared amount of interferon-gamma (IFN-γ) released by blood cells in response to antigens
    - QuantiFERON® TB tests
      - QuantiFERON® Gold
      - QuantiFERON® Gold-IT
      - T-SPOT.TB

Tuberculin Skin Test

ISRA's

ESAT-6, CFP-10, TB7.7 - antigens quite specific to TB (not on M avium or BCG)
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Purpose of Targeted Testing

- Find persons with LTBI who would benefit from treatment to prevent disease
- Find persons with TB disease so that treatment can be started

Groups that are not at high risk for TB should not be tested routinely

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IGRA

- Quantiferon
- T-spot

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Brief History of IGRA's

- Quantiferon – initially developed as a test for Bovine TB in Cattle
- Whole blood incubated with PPD for 16-24 hours
**Evolution of Quantiferon Assay**

- QFT - Whole blood incubated with PPD
- QFT Gold – Whole blood incubated with TB antigens ESAT-6 and CFP-10
- QFT Gold In Tube - Whole blood incubated with TB antigens ESAT-6, CFP-10 and TB 7.7

**How do IGRA’s Differ from TST**

- TST – nonspecific extract of attenuated MTB strain
- QFT Gold IT – ESAT-6, CFP-10, TB 7.7
- T-Spot – ESAT-6, CFP-10

**Problems with TST**

- Non-specific for MTB (other NTM’s and BCG)
- Subjectivity of Reading
- Second visit required
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- TB Ag – Nil > 0.35 IU/ml (and that value > 25% Nil with appropriate controls) = Positive

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- T-spot

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- Methodology of T-Spot
  - 250,000 Peripheral Blood Mononuclear Cells are collected from whole blood and placed in well
  - Cells producing Gamma-interferon due to TB antigen exposure counted and compared to controls
  - Benefit of standardization of cell numbers
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**Sensitivity of IGRA**

- Meta-analysis*:
  - Elispot: 88%
  - QFT: 76%
  - TST: 70%
- IGRA’s possibly more sensitive in immunocompromised


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**Enhanced Specificity vs. TST**

- NTM (MAC) –
  (with exception of M kansasii, M szulgai, M. marinum)
- BCG

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Skin test reactions to Mycobacterium tuberculosis purified protein derivative and Mycobacterium avium sensitin among health care workers and medical students in the United States.


- Dual skin testing was performed with PPD and Mycobacterium avium sensitin on 794 health care workers and medical students in the northern and southern US.
- CONCLUSIONS: Infections with NTM are responsible for the majority of 5-14 mm PPD reactions among US-born health care workers and medical students subject to annual tuberculin testing.
Low Risk TST Positives with Subsequent QFT Gold - IT
District VIII MS
TST positive (>10 mm)
N = 49
QFT+
Treat for LTBI
N = 4
-
No treatment
N = 44
Indeterminate
N = 1

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IGRA’s
• The problems related to IGRA’s include:
  - Cost of the test kits
  - Equipment
  - Personnel
  - Need for blood drawing
  - Time barrier for specimen processing and analysis

• Benefits
  - No need for return to clinic
  - Shelters, prisons & jails
  - No false positive from prior BCG vaccination or most NTM's
  - Non-subjective Interpretation (inter-reader variability)

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Time Barriers for IGRA’s
• Quantiferon –
  - Must incubate within 16 hours of collection
  - Incubation 37C 16-24hrs
  - After incubation, may store up to 72 hours (2C-27C)

• T-Spot – Must Process within 8 hours of collection (32 hours if treated with T-cell Extend)
CDC Guidelines for the use of IGRA’s

TST or IGRA
- Contact Investigation
- Periodic Screening (ie Healthcare Workers)

IGRA Preferred Over TST
- Prior BCG
- Clients unlikely to return for reading at 48-72 hours
TST Preferred Over IGRA

- Children < 5 years old

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IGRA and TST May Be Considered

- Improve Sensitivity
  - High risk individuals (contacts <5yo)
  - TB Suspects

- Improve Specificity
  - Low risk TST positive

- Improve Accuracy
  - TST when IGRA result borderline/high nil (or repeat IGRA)

- Improve Acceptance/Compliance
  - Foreign Born with prior BCG

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Canadian Guidelines for Use of IGRA's

2008
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- Similar to CDC Guidelines
- Do not endorse IGRA’s for Serial testing or Children < 18 (2008 Recs though)
- Suggest IGRA for confirmation of positive TST in low risk individuals including low risk contacts

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Use of IGRA’s in Immunocompromised

- HIV – Correlates better with Risk Factors for LTBI than
  - Higher rate of “Indeterminate” results when CD4 < 100
- Immunosuppressive Rx –
  - TST-IGRA+ discordance with steroids
  - IGRA better assoc with TB Risks

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Use of IGRA’s in Children

- Little performance data for children < 5
- Higher proportion of indeterminate results in those < 5 (usually low mitogen response)
- TST recommended for children < 5
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**Risk of TB with Negative IGRA**

- Good negative predictive value – (100% TB contacts with TST positive and negative QFT) (1,2)
- No difference in predictive value in other studies (3)


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**Use of IGRA’s in Setting of Repeat Annual Testing**

- IGRA better correlated to risk
- Lower number of IGRA+ than TST (except in high incidence settings)
- IGRA known to have slight variation on sequential testing with “reversions” to normal


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**Case Example**

- 62 yo hospital transcriptionist
- TST neg since 2002
- No known TB exposure
- 2008 – QFT - Positive
  - 0.16 IU/ml
  - 0.67 IU/ml
  - 8.71 IU/ml
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• TB Ag – Nil > 0.35 IU/ml (and that value > 25% Nil with appropriate controls) = Positive

Case Example

• TST negative
• Repeat QFT – 2 weeks – Negative
• Repeat QFT – 1 year - Negative

Cost Effective?

• Mori T, Harada N. Cost-effectiveness analysis of QuantiFERON TBM2nd generation used for detection of tuberculosis infection in contact investigations. Kekkaku 2005; 80: 675-86.