INTERPRETING INTERFERON GAMMA RELEASING ASSAYS

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Transmission of Tuberculosis and Progression from Latent Infection to Reactivated Disease.

**Infection**

1. Exposure to TB germ
2. TB germ reaches lungs, from there, it can go to other parts of the body.

**Innate Immunity**

- T Cells are being "Primed" & Sensitized to TB Antigens

**Adaptive (cellular) Immunity**

- 3-8 weeks
- Treated LTBI or Treated TB
- Post Primary Disease or Reactivation Tuberculosis
- Latent Infection

If treatment is not given: T treated LTBI or T treated TB
MTB vs. Host

- Mtb – the most successful intracellular bacterium
- It uses & evades the immune system, exists protected in the host in latent form, & infects others
  - ~1/3 world’s people
- DNA evidence of Mtb in humans 9,000 years ago

Tel-Aviv University
“Dynamics of a host-pathogen collusion”


  “A granuloma is defined as an inflammatory…infiltrate that, while capable of limiting growth of *Mycobacterium tuberculosis*, also provides a survival niche from which the bacteria may disseminate. The tuberculosis lesion is highly dynamic and shaped by both immune response elements and the pathogen…To secure transmission to a new host, *M tuberculosis* has evolved to drive T cell immunity to the point that necrotizing granulomas leak into bronchial cavities to facilitate expectoration of bacilli.”
The Immune Response in TB: Basic Terms (Cell-Mediated Immunity)

- **Antigen** - protein that induces an immune response
- **Amino Acid** – building block of protein
- **Peptides** - two or more amino acids joined by a peptide bond (–NH–CO–) *Used in IGRAs as antigen.*
- **Cytokines** - small proteins that are released by cells and affect the behavior of other cells (signaling)
  - **Interferon gamma (IFNγ)** – a cytokine produced by T lymphocytes during the immune response in TB.
    - This is what is detected & measured by the IGRAs
- **Tumor Necrosis Factor alpha (TNFα)** – a cytokine produced by T lymphocytes that prevents Mtb dissemination
To Understand IGRAs:
Understand Immune Response to Mtb

• **Innate immune system** – starts early after infection
  • Does not involve specific immunity
  • Early granuloma forms
  • Overall effect is to promote Mtb replication & dissemination

• **Adaptive immunity** – occurs later
  • Specific, anti-Mtb immunity
  • Its persistence is needed to maintain state of latency (control & containment of Mtb)
  • Memory cells are generated that will recognize Mtb antigens if they encounter them again
  • T Lymphocytes (CD4+ and CD8+) => **IFNγ**, **IL-2**, **TNFα**
Primary Tuberculosis

**Inflammatory infiltrate**
- Mtb Replication
- Mtb Dissemination
- Mtb Growth Control

**Secondary Tuberculosis**

**Protective Granuloma**
- Mtb Growth Control
- Mtb Elimination
- Fibrotic Transformation
- Calcification

**Homeostatic Granuloma**
- Immune Balance
- Latency
- Mtb Sequestration
- Mtb Dormancy
- Mtb Metabolic adaption

**Transmissive Granuloma**
- Immunopathology
- Neutrophil Dominance
- Mtb Recrudescence
- Mtb Growth

**MMP9**

**CCL2**

\[ \text{IFN-}\gamma, \text{TNF-}\alpha, \text{Granulysin} \]

\[ \text{T}_{\text{reg}} \text{cells} \]

\[ \text{IFN-}\gamma/\text{TNF-}\alpha \text{ vs. IL-4/IL-10/TGF-}\beta \]

\[ \text{Immuno suppression} \]

\[ \text{TH1/TH2 dysbalance} \]

\[ \text{Superinfection} \]

\[ \text{Hyperimmunity} \]
TB Infection as a Spectrum

Clinical disease

Disease

Bacterial replication maintained at a subclinical level by the immune system

Active infection

Infection controlled with some bacteria persisting in non-replicating form

Quiescent infection

Infection eliminated in association with T cell priming

Acquired immune response

Infection eliminated without priming antigen-specific T cells

Innate immune response

(original Barry CE et al. Nat Rev. Microbiol 2009, 7, 845-55.)
Tests for TB Infection

QuantiFERON®-Gold In-Tube Test (QFT-GIT)

FDA Approved 10/2007

T-SPOT®. TB test (T-Spot)

FDA Approved 07/2009

Used for > 1 Century
PPD TB Skin Tests & IGRAS

- There is no gold standard for diagnosing LTBI
- These are imperfect tests for diagnosing LTBI
- They indicate a cellular immune response to MTB
- They have a reduced sensitivity (ability to detect LTBI) in immunocompromised persons
- Alone they have a low ability to predict progression to active TB
  - The clinical picture identifies risk factors

- These tests are indirect. They provide immunologic evidence of host sensitization to TB antigens. They depend on cell-mediated immunity (memory T cell response). They don’t distinguish latent infection from treated infection from active disease or from treated active disease.
PPD Skin Tests and IGRAS: Tests for Detecting Mtb Infection

• Cannot be used to monitor success of treatment
• Intended use is as “indirect tests for *M. tuberculosis* infection (including infection resulting in active disease) when used *in conjunction with risk assessment, radiography, and other medical and diagnostic evaluations.*”*

MMWR 2010; 59 (No.RR-5)
Problems with PPD

- Antigens in PPD are also present in BCG vaccine & non-tuberculous mycobacteria => false+ tests
- Proper storage & administration are required
- 2 visits are needed
- Measurement & interpretation of reaction is difficult
- Results are not routinely reported properly or maintained in medical record

Reactions can be severe
IGRAs Were Developed to: Overcome Problems with PPD Skin Test

- Antigens used in the IGRAs are not shared with BCG or most NTMs
- Cross-reacting antigens not completely eliminated, however => possible false + tests
  - M kansasii
  - M szulgai
  - M marinum

- There is no need for a 2nd visit
- Scarring is avoided

Remember these exceptions for the remainder of this presentation.
IGRAS Test for “Mtb Complex”

- Nucleic amplification tests, PCR tests, and IGRAs detect Mtb Complex.
- Mtb Complex includes
  - Mtb = *Mycobacterium tuberculosis*
  - M bovis
  - M Africanum
  - M mcroti
  - M canetti
Underlying Concept of IGRAs: General

- Mixing blood cells from people who were **never** infected with *Mtb* with specific *Mtb* antigens will **not** produce an immune reaction
  - People who were never infected with *Mtb* have **not** developed adaptive immunity (cell mediated immunity, specific immunity) to *Mtb*.
  - Their memory cells were **not** sensitized to *Mtb* and will **not** recognize *Mtb* antigens

- Mixing blood cells from people who **were** infected with *Mtb* with antigens that are specific to *Mtb** will** cause a reaction
  - Their memory cells were sensitized to *Mtb* and will recognize *Mtb* antigens
  - Requires an intact immune system
Underlying Concept of IGRAs: 
Mtb Antigens & Interferon Gamma (IFNγ)

- **ESAT-6** (early secretory antigenic 6 kDa) and **CFP-10** (culture filtrate protein 10) are 2 small antigenic proteins that are secreted by Mtb and not by BCG or most NTMs. (Remember those exceptions)

- **Interferon gamma (IFNγ)** is an important cytokine that is secreted during the immune response to Mtb.

- If we mix blood from people who were infected with Mtb with **ESAT-6** and **CFP-10** we will recreate the immune response to Mtb and **IFNγ** level will rise.
  - **TB Antigen** in QFT GIT
  - **Panel A and B** in T-Spot
  - *Encoded by gene in Region of Difference 1 (RDI)*

Labels in QFT GIT and T-Spot
Underlying Concept of IGRAs: Positive and Negative Controls

- If we mix blood with heparin & dextrose we will have a measure of the background (unstimulated) amount of IFNγ
  - **Negative Control**: NIL
  - **Mitogens** – potent stimulators of T-cell activation and proliferation independent of antigenic specificity.
  - **Phytohemagglutinin** is a useful mitogen to test the adequacy of the immune system.
  - If we mix blood with the mitogen phytohemagglutinin the IFNγ level should rise if the immune system is intact.
  - **Positive Control**

Labels in QFT GIT and T-Spot
Quantiferon Gold-In-Tube (QFT GIT)

- Uses a single mixture (cocktail) of 14 synthetic peptides
  - Represents the entire amino acid sequences of ESAT-6 & CFP-10
    plus parts of TB7.7 (RD4)
- Uses 3 blood collection tubes
  - **Nil** (negative control) contains heparin & dextrose
  - **TB antigen** contains the peptide cocktail
  - **Mitogen** (positive control) contains heparin, dextrose, phytohemagglutinin
- 1 ml. blood is added to each tube (0.8-1.2 ml. is OK)
  - Each tube is shaken 10 times, enough to coat the inside of the tube
  - Purge must be used if butterfly is used to draw blood
  - Blood is incubated for 16-24 hours (can be held for 16 hours)
  - Centrifuged, IFNγ in plasma measured by ELISA, reported as IU/ml
Interpreting Results

- Subtract the nil plasma concentration of IFNγ from the TB antigen and mitogen tubes.
  - Reported as:
  - TB Antigen minus nil and Mitogen minus nil
## Interpretation: QFT-GIT: No Borderline or Intermediate Results

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>NIL</th>
<th>TB Response (TB Antigen) - Nil</th>
<th>Mitogen Response - Nil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>$\leq 8$</td>
<td>$&gt;0.35$ IU/ml &amp; $\geq 25%$ of Nil</td>
<td>Any</td>
</tr>
<tr>
<td>Negative</td>
<td>$\leq 8$</td>
<td>$&lt;0.35$ IU/ml or $&lt;25%$ of Nil</td>
<td>$&gt;0.5$</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>$\leq 8$</td>
<td>$&lt;0.35$ or $&lt;25%$ of Nil</td>
<td>$&lt;0.5$</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>$&gt;8$</td>
<td>Any</td>
<td>Any</td>
</tr>
</tbody>
</table>

NIL = plasma IFN-gamma concentration in nil tube  
TB Response = plasma IFN-gamma concentration in TB Antigen tube  
*minus* Nil level  
Mitogen Response = plasma IFN-gamma concentration in Mitogen tube  
*minus* Nil level
T-Spot

- Uses 2 separate mixtures of peptides.
  - 1 represents the entire amino acid sequence of ESAT-6
  - 1 represents that of CFP-10
- Blood is drawn into a standard green top tube by standard phlebotomy technique (no need for purges)
- Blood is sent to a central laboratory
- Uses peripheral blood mononuclear cells (PBMCs), which are incubated with controls & 2 separate mixtures of peptides
T-Spot Process

- Peripheral blood mononuclear cells (PBMCs) are separated, washed, counted, & inoculated into 4 separate microfilter wells.
T-Spot Process

A. Wells are pre-coated with antibodies to IFNγ. Captures any IFNγ produced later

B. Either ESAT-6 or CFP-10 is added to stimulate PBMCs

C. Incubated. PBMCs & antigen washed away. Conjugated antibody to IFNγ is added & binds to IFNγ secreted by the PBMCs.

D. Substrate added to produce spots.
T-Spot Process

• A spot is formed from a sensitized T cell secreting the cytokine IFNγ after being stimulated by the specific Mtb antigens ESAt-6 or CFP-10.
• T cells that have not been sensitized to Mtb antigens should not secrete much IFNγ.
• Method used is called ELISpot
  • Enzyme-linked immunospot assay
  • Detects # of cells, visualized as spots, that secrete IFNγ
• TB response = greatest # of spots counted from blood stimulated with ESAT-6 or CFP-10 minus # spots from Nil.
• Mitogen response = # spots counted from blood stimulated with mitogen minus # spots from Nil.
**T-Spot Interpretation of Results:**

**TB Response is for Panel A or Panel B**

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>Nil</th>
<th>TB Response (TB Antigen)</th>
<th>Mitogen Response</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive</strong></td>
<td>≤10 spots</td>
<td>≥8 spots</td>
<td>Any result</td>
</tr>
<tr>
<td><strong>Borderline</strong> - repeat test</td>
<td>≤10 spots</td>
<td>5, 6, or 7 spots</td>
<td>Any result</td>
</tr>
<tr>
<td><strong>Negative</strong></td>
<td>≤10 spots</td>
<td>≤4 spots</td>
<td>≥20 spots</td>
</tr>
<tr>
<td><strong>Invalid</strong> – repeat test</td>
<td>&gt;10 spots</td>
<td>Any result</td>
<td>Any result</td>
</tr>
<tr>
<td><strong>Invalid</strong> – repeat test</td>
<td>≤10 spots</td>
<td>&lt;5 spots</td>
<td>&lt;20 spots</td>
</tr>
</tbody>
</table>

Nil = # spots
TB Response = the greatest # spots (ESAT-6 or CFP-10) *minus* Nil spots
Mitogen Response = # spots *minus* Nil spots
Interpretation of T-Spot Results

- **Negative Result**
  - Nil Control
  - ESAT-6 Panel A
  - CFP 10 Panel B

- **Positive Result**
  - Positive Control
Case 1: Panic!

- A 50 year old woman who has lived in Detroit her whole life had a QFT-GIT done, which was reported “positive”
- She has NO risk factors for being infected with TB
- She has NO risk factors for progressing to TB after infection
- Her only medical problem is hypertension
- She has no TB symptoms
- The blood was drawn in a facility that does not do phlebotomy often for QFTs
- Her primary care doctor ordered a chest x-ray that is normal
# Results for Case 1

<table>
<thead>
<tr>
<th>Nil</th>
<th>Mitogen - nil</th>
<th>TB Antigen - nil</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 IU/ml</td>
<td>&gt;10 IU/ml</td>
<td>0.36 IU/ml</td>
</tr>
</tbody>
</table>

Remember: To be valid the nil result should be <8, so this is a valid test.

Remember: Cut point for QFT is 0.35. > .35 is “positive”

The official report: **Positive**

- **What should you do now?**
  - A. Treat for LTBI.
  - B. Reassure her that treatment for LTBI is not indicated without symptoms of TB.
  - C. Inform her the test is not valid.
  - D. Repeat the QFT.
Case 2: Do I really have to be evaluated for this?

- A 45 year old woman, life long resident of Detroit, was recently diagnosed HIV/AIDS. Her father had TB when she was an adolescent. She is being treated with Triumeq, which has a significant drug drug interaction with Rifampin and Rifapentine. She is doing well and her CD4+ count is normal. She is not very compliant with medications and even stopped the Triumeq for 2 months.
- She has no TB symptoms. Hypertension is out of control because she forgets to take her medicine.
- Chest x-ray is normal.
# Results for Case 2

<table>
<thead>
<tr>
<th>Nil</th>
<th>Mitogen - nil</th>
<th>TB Antigen - nil</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 IU/ml</td>
<td>0.3 IU/ml</td>
<td>0.53 IU/ml</td>
</tr>
</tbody>
</table>

- **What should you do now?**
  - A. Consider this to be a borderline result because it is <1
  - B. Treat with INH 300 mg. daily for 9 months
  - C. Do a T-Spot
  - D. Treat with INH 900 mg. 2 days a week by DOT for 9 months

Remember: To be valid the mitogen result should be $\geq 0.5$ for a negative or an indeterminate result with a normal nil. Otherwise any result over 0 is valid.

Remember: Cut point for QFT is 0.35. $\geq 0.35$ is “positive”. There is no borderline result.

The official report: **Positive**
Case 3: No, I don’t want to deal with this.

- This 38 year old African man from Somalia is a contact to an active case of TB. His wife has highly infectious pulmonary TB
- He has no risk factors for progressing to TB after infection
- He is asymptomatic and has a normal chest x-ray
- There are 2 children at home. One has a + T-Spot and is receiving INH and Rifapentine for LTBI.
- He delays getting his T-Spot, but finally agrees to it.
Results for Case 3

<table>
<thead>
<tr>
<th>Nil</th>
<th>0 IU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panel A</td>
<td>6 spots</td>
</tr>
<tr>
<td>Panel B</td>
<td>7 spots</td>
</tr>
<tr>
<td>Mitogen</td>
<td>&gt;10 spots</td>
</tr>
</tbody>
</table>

Remember: The reported spots in Panel A and B is the # of spots minus the nil

Borderline is 5, 6, or 7 spots in EITHER panel A or B.

The official report: **Borderline**

- What should you do now?
  A. Begin treatment with INH 900 mg and Rifapentine 900 mg weekly for 12 doses by DOT
  B. This is not a positive test. Wait another month and repeat it.
  C. Reassure the patient that he does not need treatment
  D. Obtain a QFT
Case 4: I will take your advice

- This is a 35 year old woman, born in the United States. Her parents are from India and she has traveled frequently to India, including trips for many months.
- She is a health care worker and requires TB testing annually. She cares for psychiatric patients. For the past 5 years she has had negative results on yearly TB testing.
- She has not been to India or other high burden area since 6 months prior to her last TB test.
- She has had no contact with anyone with TB or symptoms of TB.
- She has no risk for progressing to TB after infection.
- She has no TB symptoms and chest x-ray is normal.
Results for Case 4

<table>
<thead>
<tr>
<th>Nil</th>
<th>0 spots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panel A</td>
<td>9 spots</td>
</tr>
<tr>
<td>Panel B</td>
<td>4 spots</td>
</tr>
<tr>
<td>Mitogen</td>
<td>&gt;10 spots</td>
</tr>
</tbody>
</table>

What should you do now?

A. Begin treatment for LTBI with Rifampin 600 mg daily for 4 months
B. Obtain a QFT
C. Reassure her that treatment for LTBI is not indicated
D. Consider this a false + test, perhaps due to M. marinum

Remember:

Positive is 8 spots or more in **EITHER** panel A or B.

The official report: **Positive**
Star Pupils