The Challenges and Pitfalls in Diagnosing or Misdiagnosing Tuberculosis: Are the Days of TB Skin Tests Over?

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Disclosures

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Outline

- Background
- Population at Risk
- Clinical implications
- Different TB Screening Strategies
- IGRAs in Special Patient Populations

Background

- Among infectious diseases, TB remains the second leading killer of adults in the world
- 1.5 million TB-related deaths in 2010
- 2 billion (one-third the world's population) are thought to be latently infected with *M. tuberculosis*
- In 2011, 62.0% of all TB cases and 82.7% of multi-drug-resistant TB cases in the US occurred in foreign-born individuals

1. Centers for Disease Control and Prevention. www.cdc.gov/tb/events/WorldTBDay/History.htm.

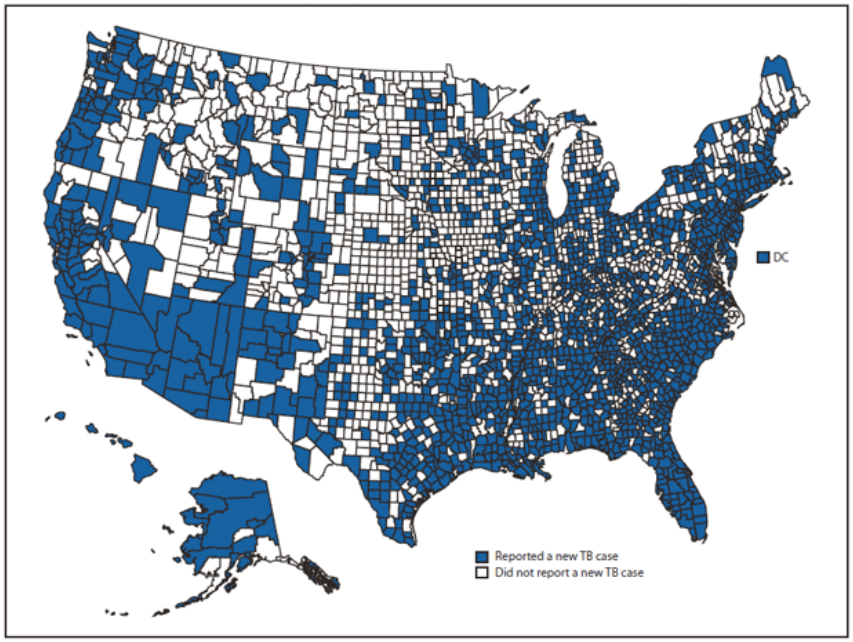
Population At Risk for TB

- Close contacts of persons known or suspected to have TB disease
- Foreign-born from areas of high incidence TB disease (Latin America, Africa, Asia, Eastern Europe, and Russia)
- Persons who travel to areas with a high prevalence of TB disease
- Residents and employees of high-risk congregate settings (correctional facilities, long-term care facilities, homeless shelters)
- HCWs who serve patients who are at increased risk for TB
- Specific populations defined locally as having increased incidence of latent M-TB (possibly including medically underserved, low-income populations, or persons who abuse drugs or alcohol)

TB in the US: 2012 Data

- 9588 new TB cases in US
- TB rate among foreign-born (FB) was 13x higher than US-born
- 4 states account for one-half of all reported cases:
  - California
  - Texas
  - New York
  - Florida

FIGURE 1. Reported new tuberculosis (TB) cases, by county — United States



† Data are current as of March 6, 2013. Data for 2012 are provisional.

1. Centers for Disease Control and Prevention. www.cdc.gov/mmwr/preview/mmwrhtml/mm6211a2.htm?s_cid=mm6211a2_e.

TB in the US: 2013 Data

TB population statistics:

- 54.2% of 6172 FB persons with TB originated from Mexico, Philippines, India, Vietnam, and China
- 6.8% reported as HIV+
- 5.7% reported being homeless in past year
- 3.9% confined to correctional facility at time of diagnosis

1. Centers for Disease Control and Prevention. Trends in Tuberculosis – United States, 2013. *MMWR.* 2014.

Tuberculosis

- Since 2009, most states have decreased funding and staffing for tuberculosis prevention and control[1]
- Sheboygan TB outbreak, April 11, 2013
  - Approximately $5 million to manage the situation[1]
- Los Angeles TB outbreak in Skid Row, March 2013
  - Had to track down 4500 people who may have been exposed[2]
- Jacksonville TB outbreak, April 2012
  - "Worst outbreak CDC has investigated in 20 years"[3]

1. Knox R. NPR, July 18, 2013. www.npr.org/blogs/health/2013/07/18/200871130/tuberculosis-outbreak-shakes-wisconsin-city.
2. Banks S. *Los Angeles Post.* March 8, 2013. http://articles.latimes.com/2013/mar/08/local/la-me-banks-skidrowtb-20130309.
3. Singer S. *The Palm Beach Post.* July 8, 2012. www.palmbeachpost.com/news/news/state-regional/worst-tb-outbreakin-20-years-kept-secret/nPpLs/.

Public Health Concerns

- Every 100 contacts not treated will lead to 3 new cases of TB in 1–2 years.

> *"Initiatives that promote further TB awareness, testing, and treatment of latent infection and TB disease among foreign-born persons and racial/ethnic minorities will be critical for future TB elimination efforts."*

Fox GJ, et al. *Eur Respir J*. 2013;41:140-156.
Centers for Disease Control and Prevention. www.cdc.gov/mmwr/preview/mmwrhtml/mm6211a2.htm?s_cid=mm6211a2_e.

Tuberculosis Testing

Currently 2 methods are available for the detection of *M. tuberculosis* infection in the US:

- Mantoux tuberculin skin test (TST)
- Interferon-gamma release assays (IGRAs)

Timeline of Advancements in TB Screening

1907 – Tuberculin skin test developed by Dr. Charles Mantoux

1900

2000

2004 – US launch of QuantiFERON®-TB Gold

QFT® QuantiFERON-TB Gold

2008 – US launch of the T-SPOT®.*TB* test

2001 – US launch of QuantiFERON®-TB

2010 – US launch of approved overnight storage protocol for the T-SPOT®.*TB* test

2007 – US launch of QuantiFERON®-TB Gold (In-Tube Version)

2009 - US launch of Oxford Diagnostics Laboratories; the only national lab dedicated exclusively to the T-SPOT.*TB* test

oxford DIAGNOSTIC LABORATORIES®
National TB Testing Services

Quantiferon assay

- Gray top - Nil tube
  - The Nil tube serves as the negative control, and no interferon gamma production, or very limited interferon gamma production, should be observed with the Nil tube.
- Red top - antigen tube.
  - these antigens, when mixed with blood containing sensitized T cells, will lead to the production and secretion of interferon gamma.
- Purple top - mitogen tube
  - contains phytohemagglutinin, which acts as a positive control for the production of interferon gamma from T cells

Major issue: done on whole blood, patients with low counts have High indeterminate levels…..

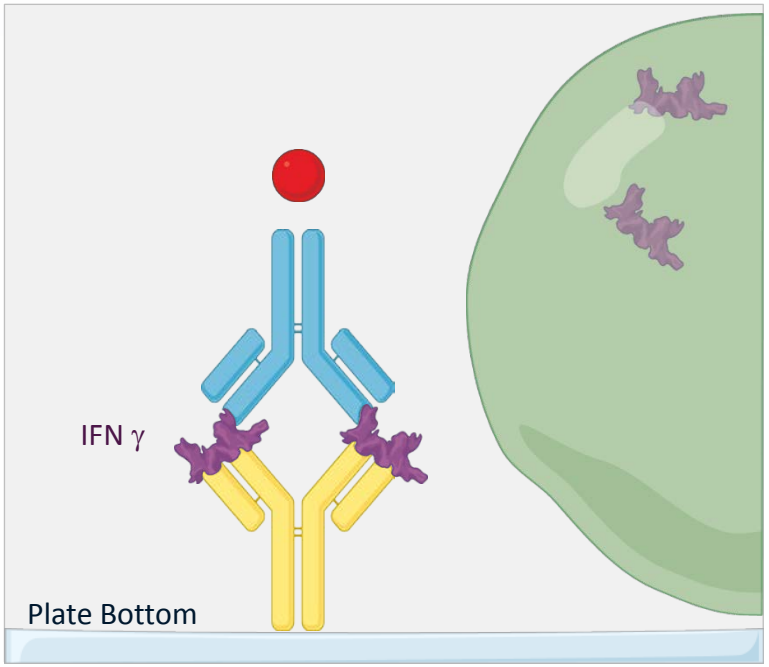
Interpreting QFT results

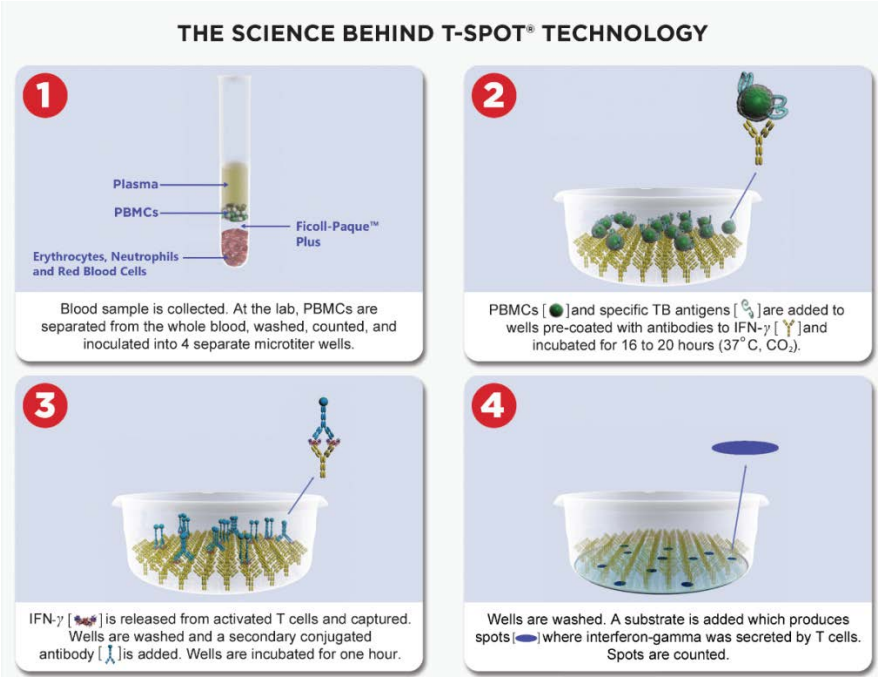
QFT result	Nil [IU/mL]	TB Ag-Nil [IU/mL]	Mitogen-Nil [IU/mL]
Positive	≤8.0	≥ 0.35 and ≥25% Nil value	Any
Negative	≤8.0	<0.35	≥0.5
Indeterminate	≤8.0	≥0.35 and < 25% of Nil value	<0.5
Indeterminate	>8.0	Any	Any

QuantiFERON-TB Gold Package Insert. Cellestis Inc. Valencia CA. *Doc. No. US05990301K July, 2011*

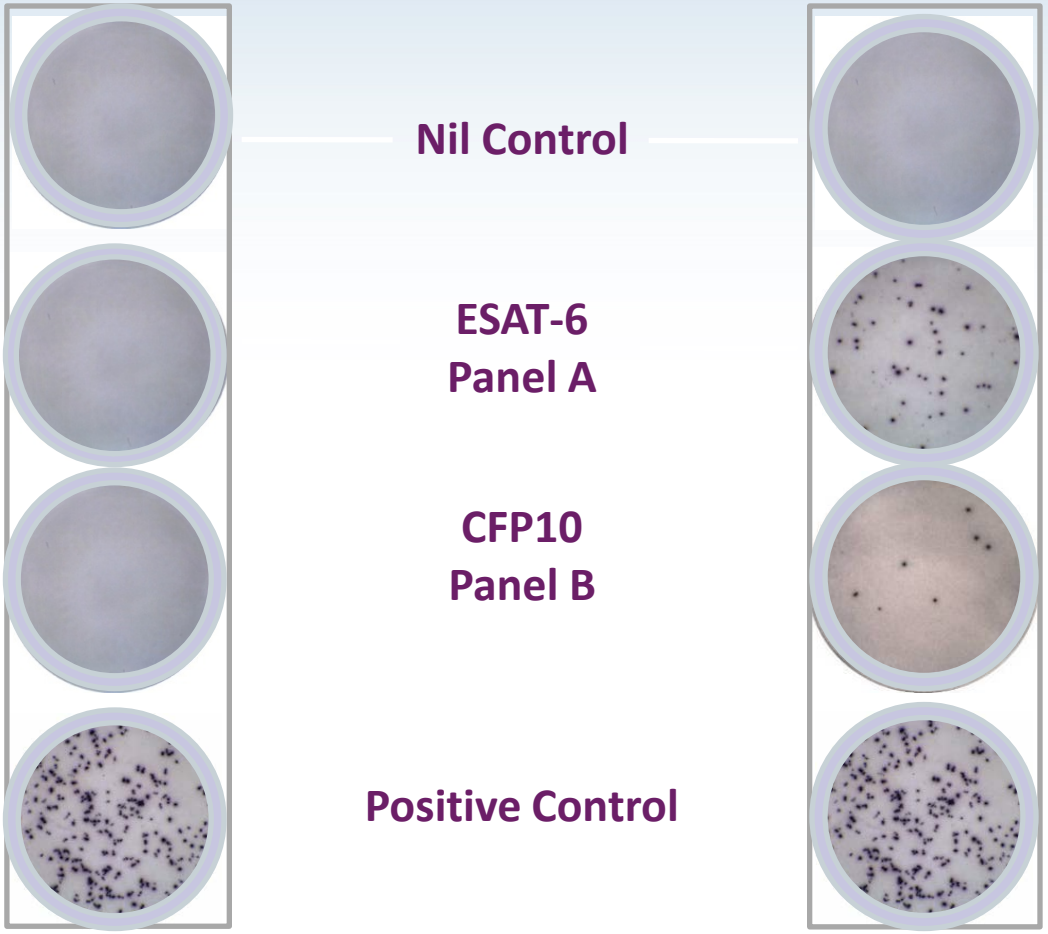
The Science Behind T-SPOT®.*TB* Technology

- Density gradient isolation of mononuclear cells
- Quantitation of cells and adjustment of concentration
- Incubation with specific antigens on ELISPOT microtiter plate





Interpreting T-SPOT®.*TB* Results



Interpreting T-SPOT®.*TB* Results

- The test result is **Positive** if Panel A-Nil and/or Panel B-Nil **≥ 8 spots**.
- The test result is **Borderline** (equivocal) where the higher of Panel A-Nil or Panel B-Nil spot count is **5, 6, or 7** and retesting by collecting another sample is recommended.
- The test result is **Negative** if Panel A-Nil and/or Panel B-Nil **≤ 4 spots**. This includes values less than zero.

Commercially Available IGRAs

QuantiFERON®-TB Gold (In-Tube)[1]

- ELISA technology
- Measures IFN-γ release
- "One and done"
- PI sensitivity: 88.2%
- PI specificity: 99.1%
- "Real world" specificity: 98%-98.9%[3,4]
- 3 specialized tubes
- Provides qualitative results
- No FDA-approved borderline category
- Sample stability: 16 hours
- Can be run in hospital lab
- Available nationally through reference laboratories (eg, Quest)

The T-SPOT®.*TB* Test[2]

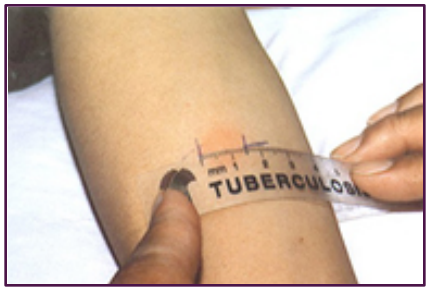
- ELISPOT technology
- Enumerates effector T cells
- "One and done"
- PI sensitivity: 95.6%
- PI specificity: 97.1%
- "Real world" specificity: 98.9% - 99.1%[5,6]
- 1 standard tube
- Provides quantitative and qualitative results
- FDA-approved borderline category
- Sample stability: 32 hours
- Can be run in hospital lab
- Available nationally through Oxford Diagnostic Laboratories®

1. QuantiFERON-TB Gold Package Insert. Cellestis, Inc. Valencia, CA. Doc. No. US05990301K, July 2011.
2. T-SPOT.*TB* Package Insert. Marlborough, MA: Oxford Immunotec; 2010.
3. Schablon A et al. BMC Infect Dis. 2011;(11):245-252.
4. Pai M et al. Ann Intern Med. 2008;149:1-9.
5. Wang SH et al. *Scand J Infect Dis*. 2010 Dec;42(11-12):845-850.
6. Bienek et al. *Int J Tuberc Lung Dis*. 2009 Nov;13(11):1416-1421.

Tuberculin Skin Test (TST) vs Interferon-Gamma Release Assays (IGRAs)

TST

- 2 visits required (minimum)
- Method: injection into skin
- Results affected by BCG
- Results in 48–72 hours
- Subjective results
- Costs unstable



IGRAs

- 1 visit required
- Method: blood draw
- Results not affected by BCG
- Next-day results
- Objective results
- Costs defined and stable

Clinical Implications with a Suboptimal Testing

Case Study

- 41-year-old Indian male with no known history of cancer who was referred to us because of pneumonia. The patient is a nurse at MD Anderson Cancer Center who started working in September 2012.

- PMHx: February 2012, he started having a cough with off and on sputum production. He was diagnosed with bronchitis and received 2 courses of azithromycin with no improvement.

- He was referred to a Pulmonologist and a chest x-ray and a PPD skin test were done in March 2012, which were both negative. He received cefuroxime and later on Levaquin with no improvement.

- At that point, he was diagnosed with possible asthma and he was started on inhaled steroids.

- His cough got a little bit better initially and started to get worse again.

Case Study

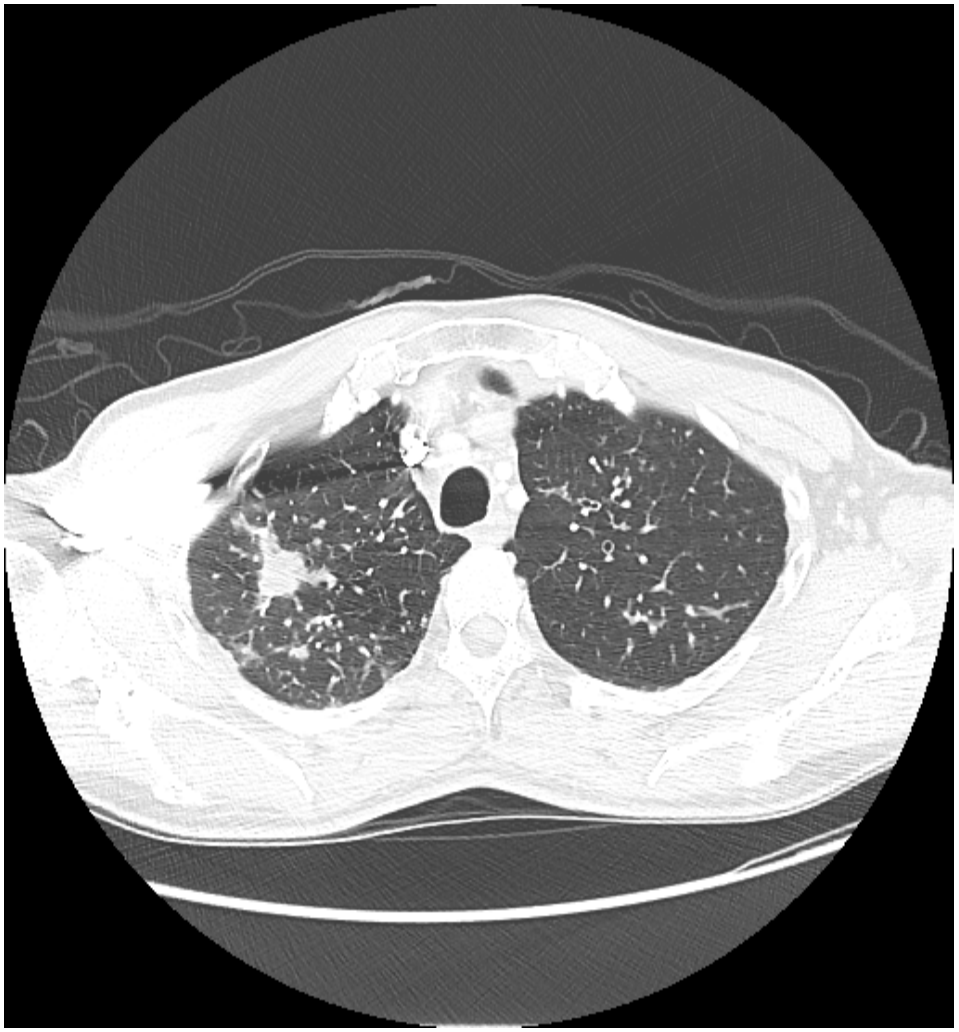
- Meanwhile, patient was hired and started his work at MD Anderson in September 2012.
- He had a PPD skin test done in 2 steps and was read as negative.
- December 2012: Worsening cough and hemoptysis now.
- He saw another pulmonologist on the outside in February 2013 for a second opinion. No chest x-ray was done. The patient was prescribed another course of an antibiotic with no improvement.

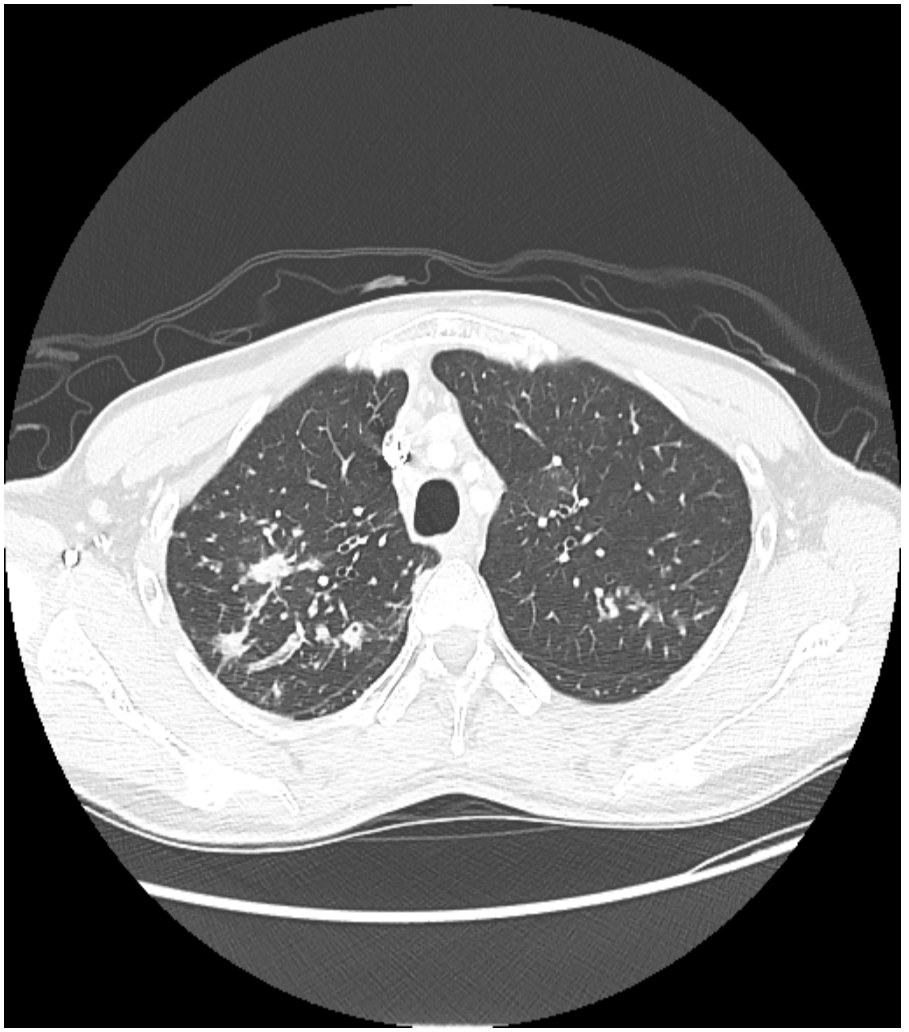
Case Study

- Past Social History:
  - Born and raised in India. He immigrated to Canada in 2005 and then moved to the US few years later.
  - Working at MD Anderson since September 2012 as a nurse on the Stem Cell Transplant Units.
  - Exposure to an active case of TB 35 years ago when he was a child (his uncle had active TB).
  - The patient had multiple PPD skin tests which were negative. He received the BCG vaccine as well.
  - He is married and he has 2 kids (9-month-old & 6 y.o).

Case Study

- May 2013: he went back to India for vacation. He saw an internist who did a Ziehl-Neelsen stain on 3 sputum specimens which came back positive for AFB. His chest x-ray showed bilateral infiltrates.





Baseline CT Scan of Chest on 5/2013

QUANTIFERON - TB GOLD (NEGATIVE) **Positive ***

NIL CONTROL	0.07	IU/mL
MITOGEN CONTROL	6.88	IU/mL
TB ANTIGEN	1.62	IU/mL

The Nil tube value is used to determine if the patient has a preexisting immune response which could cause a false-positive reading on the test. In order for a test to be valid, the Nil tube must have a value of less than or equal to 8.0 IU/mL.

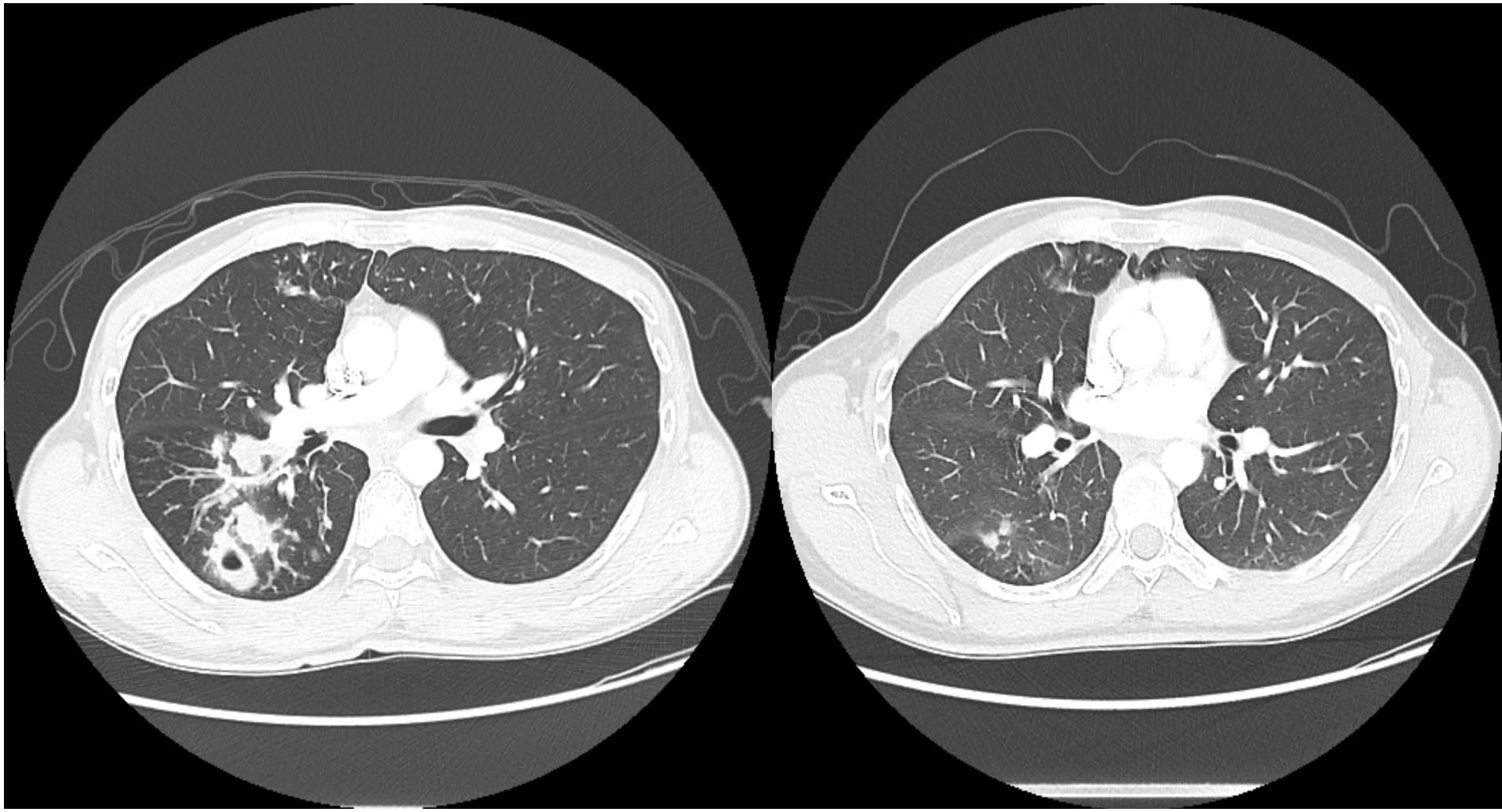
The mitogen control tube is used to assure the patient has a healthy immune status and also serves as a control for correct blood handling and incubation. It is used to detect false-negative readings. The mitogen tube must have a gamma interferon value of greater than or equal to 0.5 IU/mL higher than the value of the Nil tube.

The TB antigen tube is coated with the M. tuberculosis specific antigens. For a test to be considered positive, the TB antigen tube value minus the Nil tube value must be greater than or equal to 0.35 IU/mL.

Confirmation of Diagnosis

- SEQUENCE IDENTIFICATION
- COLLECTED: 05/31/2013 ; 0610
- SOURCE: SPUTUM
- Primary Panel for Mycobacterium tuberculosis (MTB) by Broth Method
- Susceptibility testing:
- ANTIBIOTICS.....Concentration.....Interpretations....
- ==========
- Ethambutol.....(5.0)mcg/mL..... S
- Ethambutol.....(8.0)mcg/mL..... S
- Isoniazid.....(0.1)mcg/mL..... S
- Isoniazid.....(0.4)mcg/mL..... S
- Pyrazinamide.....(300)mcg/mL..... S
- Rifampin.....(1.0)mcg/mL..... S
- ==========
- ---------- FINAL REPORT ----------
- MYCOBACTERIUM TUBERCULOSIS COMPLEX (MY TBC)! from AFB
- Culture on same accession number.
- Identified by 16S ribosomal DNA sequencing.

- DIRECT SPECIMEN AFB SMEAR
- DIRECT SPECIMEN AFB SMEAR ACC# 13-151-05296
- COLLECTED: 05/31/2013 ; 0610 STARTED: 05/31/2013 ; 1201
- SOURCE: SPUTUM 05/31/2013 1631
- FEW TO MODERATE ACID FAST BACILLI SEEN IN DIRECT SMEAR!



5/2013; before therapy

10/2013; while on therapy

May- June 2013

May:

- Patient diagnosed with MTB; 6 employees exposed, repeat PPDs and/or respiratory query on August 2, 2013. ***No patients exposure***

- Patient diagnosed with MTB; 97 employees exposed, repeat PPDs and/or respiratory query on August 9, 2013. ***No patients exposure***

- Employee diagnosed with active TB; 70 employees exposed; employees completing PPDs and/or respiratory queries presently. ***No patients exposure***

Issues Affecting TST Utility

- **False-Positive Results**
  - Foreign-born persons (BCG vaccinated) account for 60% of all TB cases in US
  - Exposure to other mycobacteria
  - Unknown cause
- **False-Negative Results**
  - Miliary TB
  - Immunosuppression
    - AIDS
    - Cancer
    - Anti-TNF
    - Transplant
- **Noncompliance**
  - Failure to return for TST interpretation

Centers for Disease Control and Prevention. *MMWR Morb Mortal Wkly Rep*. 2010;59:291.

Updated CDC Guidelines

CDC guidelines allow the use of IGRA or TST for screening healthcare workers:

- "An IGRA or a TST may be used without preference for periodic screening of persons who might have occupational exposure to M. *tuberculosis* (eg, surveillance programs for healthcare workers)."
  - IGRA preferred testing for groups with low rates of return
  - IGRA preferred testing for individuals who have received BCG
- "Prior to implementing IGRAs, each institution and tuberculosis-control program should evaluate the availability, overall cost, and benefits of IGRAs for their own setting."

Updated Guidelines for Using Interferon Gamma Release Assays to Detect Mycobacterium tuberculosis Infection - 2010. CDC MMWR 59 RR-5.

IGRAs in Special Patient Population

ESTIMATED NUMBER OF IMMUNOCOMPROMISED PERSONS LIVING IN THE US

Condition	Estimated Number of Persons Living with Condition in the United States
HIV infection	1.2 million
Immune-mediated inflammatory disorders	
RA	1.5 million
IBD	1.1 million
SLE	320,000
Systemic sclerosis	49,000
Spondyloarthropathies	2.4 million
Vasculitis	1 million
End-stage renal disease	0.87 million
Hematologic malignancies including HSCT recipients and candidates	1 million
Solid organ transplant candidates	120,000
Total	10 million

Redelman-Sidi G, Sepkowitz K. Am J Respir Crit Care Med. 2013 Aug 15;188(4):422-31.

Issues Affecting LTBI Diagnosis

- LTBI is difficult to diagnose in immunocompromised patients
- Risk for progression to active TB is greater in immunocompromised patients
- No gold standard exists to diagnose LTBI
  - TST performs particularly poorly in immunocompromised patients because of an increased likelihood for false negative results

Redelman-Sidi G, Sepkowitz K. Am J Respir Crit Care Med. 2013 Aug 15;188(4):422-31.

Patient Population	CDC Guidelines*	Comments
HIV-infected persons	• Consider sequential testing with TST and an IGRA in high-risk patients[†] • Any positive result should be considered evidence of LTBI	• TST performance is limited in patients with $CD4^+$ cell count < 200 cells/mm^3 • Correlation between IGRAs and clinical risk factors for LTBI: *strong evidence* • Increased likelihood of indeterminate results for both IGRAs • T-SPOT.TB performance less affected by low $CD4^+$ lymphocyte count
Patients with hematologic malignancy, including HSCT candidates	• Consider sequential testing with TST and an IGRA in high-risk patients[†] • Any positive result should be considered evidence of LTBI	• TST performance is limited • Correlation between IGRAs and clinical risk factors for LTBI: *weak evidence* • T-SPOT.TB may be less affected by presence of neutropenia and/or lymphopenia and may be preferable
Solid organ transplant candidates	• Consider sequential testing with TST and an IGRA in high-risk patients[†] • Any positive result should be considered evidence of LTBI	• TST of value if obtained before transplantation • Correlation between IGRAs and clinical risk factors for LTBI: *weak evidence* • Underlying liver disease appears to increase likelihood of indeterminate results of both IGRAs in candidates for liver transplantation

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High Risk for TB Reactivation in HIV+ Patients

Performance of T-SPOT®.*TB* test for aid in diagnosis of active/probable TB in HIV-1 patients

Patient Group	No.	Sensitivity	Specificity
All HIV w/ active/probable TB	30	90.3%	100%
CD4 T-cell count < 300 cells/µL	22	95.4%	100%
CD4 T-cell count < 200 cells/µL	14	92.9%	100%
CD4 T-cell count < 100 cells/µL	8	87.5%	100%

NPV of the assay for the diagnosis of active TB in HIV pts with clinical and radiologic signs of infection was 98.2%

Clark SA, Martin SL, Pozniak A, et al. Clin Exp Immunol. 2007;150:238-244.

High Risk for TB Reactivation in HIV+ Patients

- 19 newly diagnosed HIV patients with active TB

	Sensitivity
T-SPOT®.TB Test	89% (17/19)
QuantiFERON®-TB Gold In-Tube	68% (13/19)

- T-SPOT.*TB* test positive results were independent of CD4 T-cell counts

"In conclusion, in HIV-infection, immune responses in the TST and QFT-G-IT are both strongly related to the degree of immunodeficiency, while the T-SPOT.TB seems to function independently of the level of CD4+ T-cell depletion."[1]

Leidl L, Mayanja-Kizza H, Sotgiu G, et al. ERJ Express. 2009

Patient Population	CDC Guidelines	Comments
Patients with hematologic malignancy, including HSCT candidates	• Consider sequential testing with TST and an IGRA in high-risk patients • Any positive result should be considered evidence of LTBI	• TST performance is limited • Correlation between IGRAs and clinical risk factors for LTBI: *weak evidence* • T-SPOT.TB may be less affected by presence of neutropenia and/or lymphopenia and may be preferable

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Hematological Malignancies

- 95 patients had testing with TST, QFT-GIT, and T-SPOT.*TB*

	TST	QFT-GIT	T-SPOT.*TB*
Positive	10	17	25*
Negative	85	73	69
Indeterminate	NA	5	1

* p=0.03 vs. QFT-GIT positive

Compared to TST, "*Blood tests identified significantly more patients as being infected with MTB …although diagnostic agreement varied …we recommend tailoring application of the new blood IFN-assays for LTBI in different high-risk groups and advise caution in their current use in immunosuppressed patients.*"

Richeldi L, Losi M, D'Amico R, et al. Chest. 2009;136:198-204.

High Risk for Progression/Reactivation: Hematological Malignancies

	Normal WBC Count (n = 68)	Pathological WBC Count (n = 70)
T-SPOT Positive	44.6%	44.3%
Indeterminate	6.2%	2.8%
TST % Positive	25.9%	14.5%

- **T-SPOT.*TB* results were not affected by white blood cell (WBC) count and were more closely correlated with exposure than TST**

"T-SPOT.TB test maintains sensitivity and performance in immunosuppressed patients…and can identify infected patients anergic to the tuberculin skin test." (p. 33)[1]

Piana F, Codecasa LR, Cavallerio P, et al. Eur Respir J. 2006;28:31-34.

Patient Population	CDC Guidelines	Comments
Solid organ transplant candidates	• Consider sequential testing with TST and an IGRA in high-risk patients • Any positive result should be considered evidence of LTBI	• TST of value if obtained before transplantation • Correlation between IGRAs and clinical risk factors for LTBI: *weak evidence* • Underlying liver disease appears to increase likelihood of indeterminate results of both IGRAs in candidates for liver transplantation

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Summary of Guidelines

- The CDC, American Academy of Pediatrics, ATS, and the IDSA recommend:
  - Allow use of IGRAs in place of (but not in addition to) TST in any situation in which TST is recommended.
  - In IC patients, such as patients with HIV, if initial testing by TST or IGRA is negative, performance of both tests should be considered, with a positive result on either sufficient to diagnose LTBI.

- The European Centre for Disease Prevention and Control recommends in IC patients, including HIV-infected patients, using TST and IGRAs in combination to diagnose LTBI.

Advantages of IGRAs

- Results are numerical, and thus less subject to reader bias.
- No need for a follow-up visit for reading of results.
- Not affected by BCG vaccination status as they use TB-specific antigens that are not present in BCG.


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