

IGRAs in the Immunosuppressed (They are better than TST)

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Disclosures

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 - Oxford Immunotech

Immunosuppression

- **Increased risk of progression of latent TB infection (LTBI) to active disease**
- **Medical conditions**
 - **Renal disease, cancer, rheumatoid arthritis (RA), transplant recipients, DM, HIV, Crohn's, others**
- **Immunosuppressive therapies**
 - **Corticosteroids, tumor necrosis factor antagonists (anti-TNF), anti-T cell therapies, others**

LTBI Screening

- **TST with 5mm definition of positive**
 - HIV
 - Anti-TNF and other immunosuppressive therapies
- **TST with 10mm definition of positive**
 - Renal disease, diabetes, cancer

Ying and Yang of Diagnostic Tests

- **Energy with TST well-documented**
 - HIV, renal disease, RA, prednisone use, others
- **In high risk setting**
 - Set lower threshold for “positive” result
 - Desire to maximize sensitivity
 - Cost to specificity
- **Well known drawbacks with TST**
 - Placement, readability, reproducibility, others
 - Specificity (particularly at low cut-points)

IGRAs

- **QuantiFERON-TB Gold[®] test (QFT-G)**
 - Detects cell-mediated immunity
 - Whole blood incubated with TB antigens (ESAT-6/CFP-10)
 - INF- γ released from sensitized lymphocytes
- **QFT-In Tube (QFT-IT)[®]**
 - Added third antigen (7.7)
- **T-SPOT.TB[®] assay**
 - Measures number of reactive lymphocytes

IGRA Role in Screening Immunosuppressed

- **Role of IGRA and how to use**
 - Replacement of TST?
 - Supplement to TST?
- **Last 5 years**
 - Large number of head to head studies
 - Rheumatic diseases, HIV, transplant, others

IGRAs in Inflammatory Arthritis

- Rheumatoid arthritis (RA)
 - 0.5-1.0% of population
- TB risk elevated due to disease state
- Further elevation with therapy
 - 40% take prednisone
 - 40% on anti-TNF therapy
- TST anergy well-documented
 - Due to disease state
 - Due to prednisone

Prednisone and Tuberculosis

- *Jick et al. Arthritis Rheum 2006*
- General Practice Research Database, UK
- TB cases 1990-2001 and controls[†]

- Current glucocorticoid use *OR 4.9 (2.9-8.3)
 - ≤ 15 mg/day *OR 2.8 (1.0-7.9)
 - ≥ 15 mg/day *OR 7.7 (2.8-21.4)

*Adjusted for smoking, BMI, lung disease, diabetes, anti-rheumatic therapy, other TB risk factors

[†]Controls matched for age, sex, residence, time clinically followed

RA Biologic Therapies

- **TNF- α inhibition**
 - **Infliximab, adalimumab, and etanercept**
 - **Just approved: certolizumab, golimumab**
- **Newly approved**
 - **CD4 co-stimulation modulator: abatacept**
 - **B-cell (CD20+) antibody: rituximab**
 - **Anti-IL-6 receptor antibody: tocilizumab**

IGRAs in RA

- Greater specificity for TB than TST
 - Does not cross react with BCG or most environmental mycobacterium
- Relative sensitivity with TST for LTBI?
- Cobanoglu et al, *IJTL D* 2007
 - QFT-IT vs. TST (n=109)
 - 10% positive (QFT-IT) Vs. 49% (TST)
 - All BCG vaccinees

IGRAs in Rheumatic Diseases

- **Matulis et al, *Ann Rheum Dis* 2007**
 - Rheum patients treated with anti-TNF or non-biologic treated (n=126)
 - 32% TST+ and 12% QFT-IT+
- **QFT-IT more closely associated with LTBI risk factors than TST**
- **In BCG (–)**
 - 23% TST+ and 21% QFT-IT+
- **6% indeterminate**

IGRAs in anti-TNF Candidates

- Ponce de Leon et al, *J Rheum* 2008
 - Peru, background LTBI prevalence 60%
 - 80% BCG vaccinees in both groups

	RA (n=101)	Controls (n=93)
TST +	27 (27%) [±]	61 (66%) [*]
QFT-IT +	45 (45%) [±]	55 (59%)

[±]p < 0.05 for comparisons

Note: (1) TST + cut-off was ≥ 5 mm in RA and >10 mm in controls, but used only 2 TU dose in TST
(2)BCG 80% similar in RA and control groups.

Screening TNF Candidates

- Inflammatory disease patients, Denmark
 - QFT-Gold (not in-tube) vs. TST
 - N=234, RA 51%, most with BCG history
- 66 (28%) TST + and 18 (8%) QFT-G +
 - QFT-G associated with TB risk factors
- Prednisone use
 - TST negative results
 - Indeterminate QFT-G (overall was 5%)

US Comparison Studies

- 179 patients, TST vs. TSPOT.TB
 - Boston, US born women, 80% with RA
 - Taking TNF and/or DMARD +/- steroids

TST +	TSPOT +
2 (1%)	10 (6%)

- No concordance among positives
 - Few to no TB risk factors among the TSPOT+
- False versus true positive TSPOT?

Characteristics	LTBI	with	No. with	LTBI
	Prevalence	Characteristic	LTBI	Prevalence
	% (95% CI)	No. × 1,000	No. × 1,000 (95% CI)	% (95% CI)
All participants	1.8 (1.3–2.4)	231,227	4,154 (3,073–5,607)	18.7 (13.5–23.9)
Sex				
Female	1.5 (1.0–2.2)	112,019	1,761 (1,202–2,575)	14.4 (8.7–23.9)
Male	2.1 (1.3–3.4)	119,208	2,380 (1,482–3,802)	22.7 (16.3–30.1)
Age group, yr				
1–14	0.3 (0.1–1.1)*	53,781	173 (49–610)	11.9 (5.2–24.4)
15–24	0.6 (0.2–1.6)*	33,597	193 (68–540)	12.8 (5.1–28.0)
25–44	1.2 (0.7–2.2)	68,841	826 (453–1,500)	20.6 (14.6–28.3)
45–64	3.4 (2.0–5.8)	48,189	1,650 (963–2,797)	25.3 (17.6–33.9)
≥65	4.8 (2.8–8.0)	26,819	1,288 (762–2,148)	11.9 (5.2–24.4)
Race/ethnicity				
Non-Hispanic white	1.1 (0.6–2.0)	17,441	1,960 (1,120–3,418)	17.9 (11.4–24.4)
Non-Hispanic black/African American	5.7 (4.2–7.8)	29,193	1,661 (1,212–2,263)	20.0 (13.7–26.3)
Mexican/Mexican American	2.5 (1.6–3.8)	12,372	307 (200–470)	19.1 (16.2–22.0)
Other	1.5 (0.4–5.2)*	15,249	221 (60–794)	18.6 (10.9–26.3)
Poverty income index				
Poverty income index ≥1	1.4 (1.0–2.1)	171,561	2,469 (1,714–3,550)	16.5 (11.8–21.2)
Poverty income index <1	2.8 (1.9–4.0)	3,751	1,052 (728–1,516)	20.3 (13.0–27.6)
Education level				
<High school	2.5 (1.7–3.5)	81,900	2,003 (1,391–2,874)	19.2 (14.9–23.5)
High school graduate	1.6 (0.9–2.8)	46,842	740 (413–1,316)	17.9 (8.9–26.9)
Beyond high school	1.6 (1.0–2.7)	85,094	1,371 (824–2,273)	18.3 (9.5–27.1)

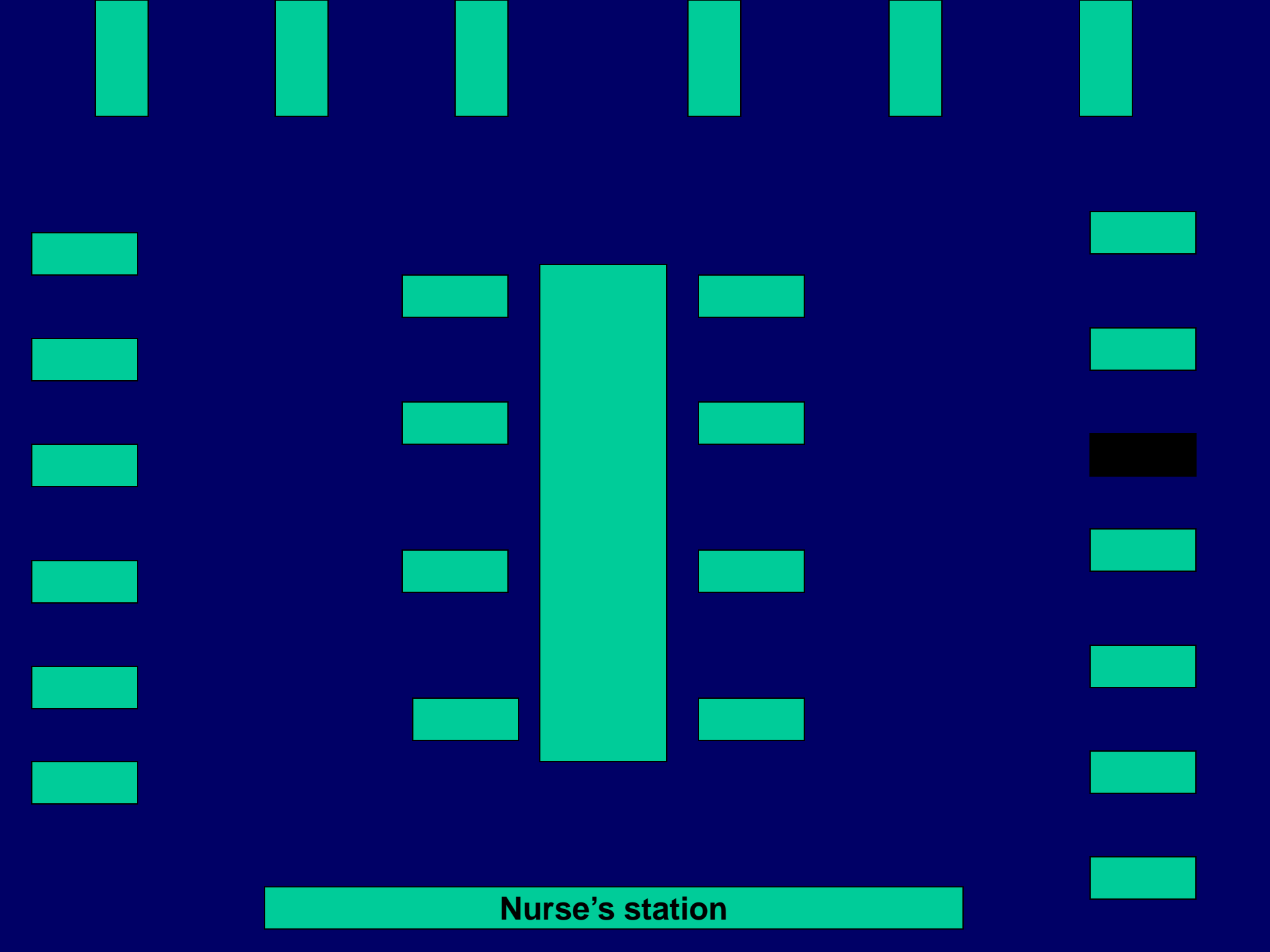
For definition of abbreviations, see Table 1.

* Estimates and 95% CIs are unstable and may not accurately reflect the true proportion because of the small number of cases.

IGRAs in Current anti-TNF Users

- **Baseline and at 14 weeks post-TNF therapy start**
 - ELISPOT using ppd or CFP-10
 - All anti-TNFs decreased # of reactive lymphocytes
 - Poor sensitivity for patients already on anti-TNFs?
- **Matulis study with decreased QFT-IT positivity in patients on anti-TNFs**
 - Decreased mitogen responses





Nurse's station

Risk Factors for Positive TST

<u>Factor</u>	<u>Adj. OR (95% CI)</u>	<u>P-value</u>
Foreign born	3.2 (1.2-8.4)	0.02
Recent TST	2.8 (1.0-7.9)	0.05

TST not associated with case contact

Risk Factors for Positive QFT-G

<u>Factor</u>	<u>Adj. OR (95% CI)</u>	<u>P-value</u>
*Contact	3.5 (1.1-11.0)	0.03
Age	1.04 (1.00-1.09)	0.03
*Proximity	2.0 (1.0-3.8)	0.05

TST Negative / QFT-G Positive

Multivariate analysis

<u>Factor</u>	<u>Adj. OR (95% CI)</u>	<u>P-value</u>
Contact	15.3 (1.2-192.7)	0.03
Age	1.08 (1.01-1.17)	0.02

*From model comparing this outcome to concordant negative results, and incorporating covariates of contact, age, and foreign birth.

Other Immunosuppressed Settings

- **Modena, Italy**
- **Very few patients with BCG**
- **Three groups tested**
 - **Liver transplant candidates (n=120)**
 - **HIV (n=116)**
 - **Hematologic malignancy (n=95)**

Table 2—Results of TST, TS.TB, and QFT-IT in the Different Groups of Immunocompromised Patients

Assay Results	LTC Group (n = 120)	HIV Group (n = 116)	HM Group (n = 95)
TST			
Positive	20 (16.7)*	6 (5.2)	10 (10.5)†
Negative	100 (83.3)	110 (94.8)	85 (89.5)
TS.TB			
Positive	32 (26.7)	4 (3.5)	25 (26.3)‡
Negative	87 (72.5)	112 (96.5)	69 (72.6)
Indeterminate	1 (0.8)	0	1 (1.1)
QFT-IT			
Positive	28 (23.3)	5 (4.3)	17 (17.9)
Negative	80 (66.7)	104 (89.7)	73 (76.8)
Indeterminate	12 (10.0)	7 (6.0)	5 (5.3)

Values are given as No. (%).

*p = 0.007 vs TS.TB positive and p = 0.02 vs QFT-IT positive in the LTC group.

†p < 0.001 vs TS.TB positive and p = 0.02 vs QFT-IT positive in the HM group.

‡p = 0.03 vs QFT-IT positive in the HM group.

Hematologic Malignancy

- N=138, Italy. Contacts to smear-positive TB case

TABLE 1 Comparison of tuberculin skin test (TST) to T-SPOT.TB™ test results in entire patient group

	T-SPOT.TB™			Insufficient T-cells	Total
	Positive	Negative	Indeterminate		
TST+	21	3*	0	0	24 (17.4)
TST-	34	57	5	2	98 (71.0)
No result	6	8	1	1	16 (11.6)
Total	61 (44.2)	68 (49.3)	6 (4.3)	3 (2.2)	138

Data are presented as absolute numbers or n (%). TST+: TST-positive; TST-: TST-negative. *: two bacille Calmette–Guérin vaccinated.

Recent Studies in HIV Setting

- **336 HIV+ patients, Atlanta,GA⁺**
 - **2.1% TST+**
 - **2.7% QFT-IT+**
 - **4.2% TSPOT+**
- **Indeterminate results**
 - **14% TSPOT**
 - **1.8% QFT-IT**
 - **Assoc. with CD4 < 200**

Recent Studies in HIV Setting

- **286 HIV+ patients, Germany***
 - 13% TST+
 - 20% QFT-IT+
 - 25% TSPOT+
- **Indeterminate results**
 - 2.9% TSPOT
 - 0.4% QFT-IT

*Stephan C et al. AIDS 2008

Recent Studies in HIV Setting

- 830 HIV+ patients with QFT-IT, Austria*
- Who developed active TB?
 - 7/44 QFT-IT+
 - 1/739 QFT-IT negative
- 5.7 % indeterminate
 - Associated with low CD4

*Aichelburg MC et al. CID 2009

Summary

IGRAs in the Immunocompromised

- **LTBI sensitivity***
 - IGRAs > TST
- **Recent data for QFT-IT**
 - Increased sensitivity and similar to T.SPOT.TB
 - QFT-Gold less sensitive

1. Hamdi H et al. *Arthritis Res Ther.* 2006;8:R114.

2. Lalvani A, Millington KA. *Autoimmun Rev.* 2008. Epub ahead of print.

Summary

IGRAs in the Immunocompromised

- **Anergy occurs with all tests**
 - IGRAs appear less affected than TST by immunosuppressive therapies
 - Caveat for those already on anti-TNF therapy?
- **Indeterminate results**
 - Depends on the setting
 - T-SPOT.TB < QFT-IT < QFT-G

How to Screen

- **Should IGRAs be used in screening?**
 - **Absolutely**
- **Replace TST vs. used in conjunction with TST**
 - **Effort to maximize sensitivity**
 - **Danger of using IGRA to “confirm” positive TST result**
 - **TSPOT instead of QFT-IT?**
- **IGRAs and TST are diagnostic aids**

Screening for Latent Tubercular Infection (LTBI)

- Screen *before* patient is immunocompromised
- History of TB risk factors
 - Foreign birth or extended living abroad
 - Previous contact with TB case
 - Previous LTBI diagnosis or treatment
 - Incarceration, homelessness, IV drug use

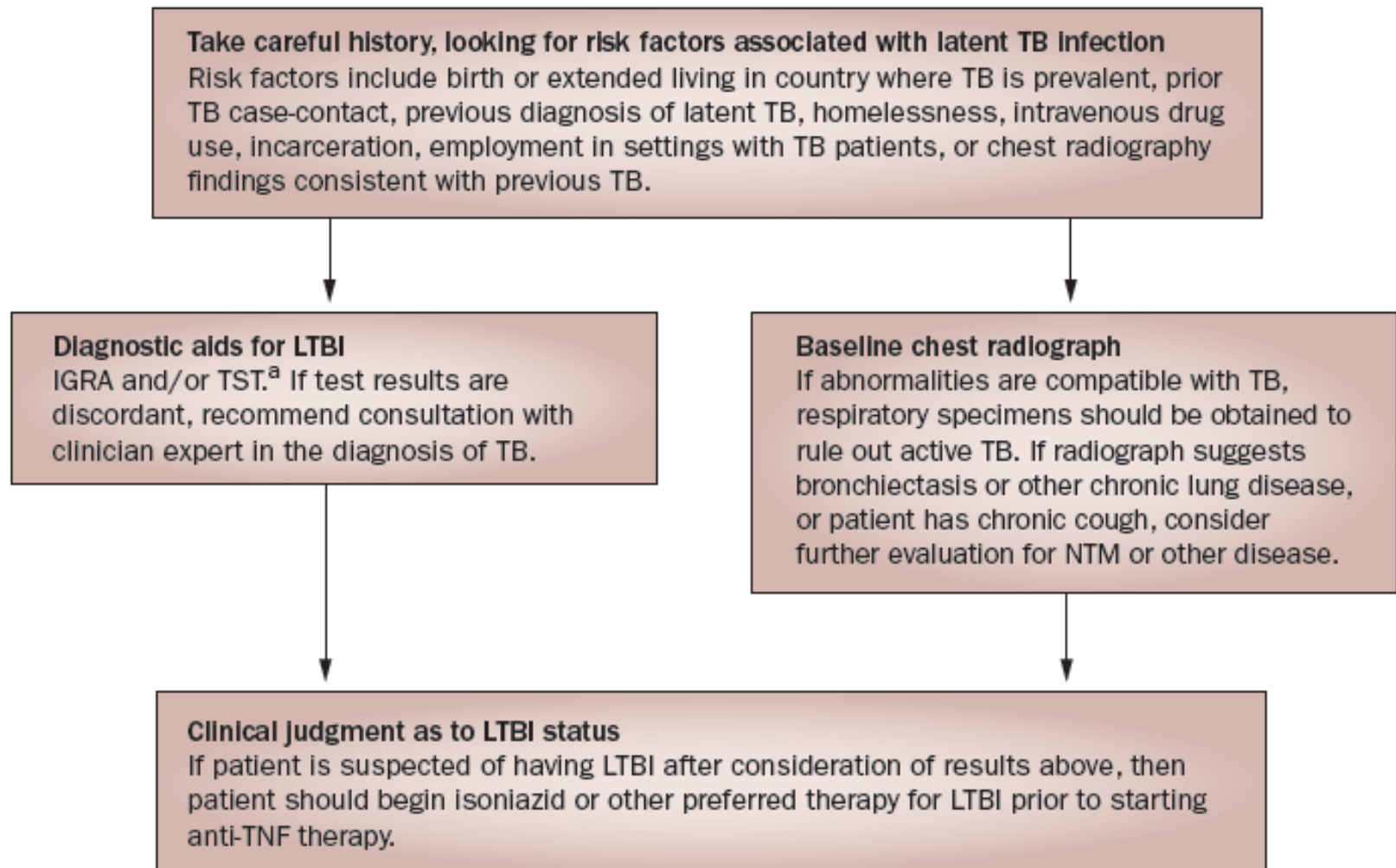


Figure 1 | Proposed schemata for TB screening in patients administered or scheduled to receive TNF antagonists. ^aA screening strategy employing both TST and IGRA could be considered in an effort to maximize the sensitivity in detecting LTBI in this setting. Abbreviations: IGRA, interferon γ release assay; LTBI, latent tuberculosis infection; NTM, nontuberculous mycobacteria; TB, tuberculosis; TNF, tumor necrosis factor; TST, tuberculin skin test.

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 - Ken Saag, Josef Smolen, Dan Furst

QuantiFERON[®]-TB Gold

Principle of the Test:



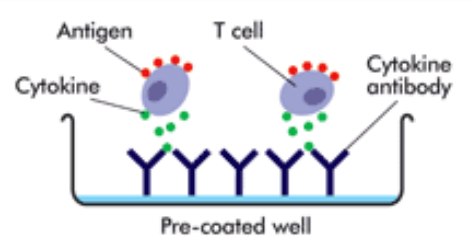
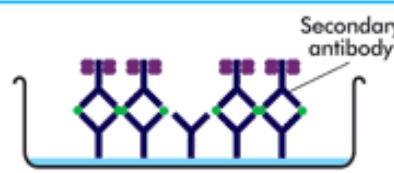
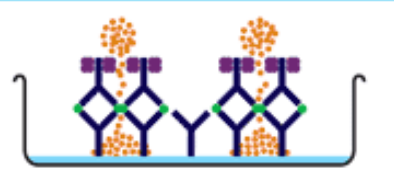
Compare IFN- γ levels of the antigen wells to the 2 controls wells.

TB Antigen - Nil	Mitogen - Nil	Result
≥ 0.35 IU and $> 25\%$ of Nil	Any	Positive
< 0.35 IU and Nil ≤ 0.8 or	≥ 0.5	Negative
< 0.35 IU and $< 25\%$ of Nil result	< 0.5	Indeterminate
Nil > 0.8 but peptide less than 50% above Nil	Any	

Species Specificity of ESAT-6 and CFP-10

Tuberculosis complex	Antigens		Environmental strains	Antigens	
	ESAT	CFP		ESAT	CFP
M tuberculosis	+	+	M abcessus	-	-
M africanum	+	+	M avium	-	-
M bovis	+	+	M branderi	-	-
BCG substrain			M celatum	-	-
gothenburg	-	-	M chelonae	-	-
moreau	-	-	M fortuitum	-	-
tice	-	-	M gordonii	-	-
tokyo	-	-	M intracellulare	-	-
danish	-	-	M kansasii	+	+
glaxo	-	-	M malmoense	-	-
montreal	-	-	M marinum	+	+
pasteur	-	-	M oenavense	-	-
			M scrofulaceum	-	-
			M smegmatis	-	-
			M szulgai	+	+
			M terrae	-	-
			M vaccae	-	-
			M xenopi	-	-

T-Spot.TB™

Step 1	Collect the blood sample and centrifuge to separate Peripheral Blood Mononuclear Cells (PBMCs)	
Step 2	Wash and count the PBMCs	
Step 3	Add PBMCs to wells with antigens and incubate overnight (37°C, CO ₂)	
Step 4	Wash and add secondary antibody	
Step 5	Add substrate and count any resulting spots. One spot = one T cell	



Nil Control



Infection



Infection



Positive Control

CDC Recommendation Nuances

- **Similar to HIV-infected TB screening guidelines**
- **No role for anergy panel testing**
- **Routine “two-step” testing not recommended**
 - **Specificity issue in countries with high BCG prevalence**
- **Repeat screening?**
 - **Did not formally address**
 - **No role for annual screening**
 - **Repeat if potential exposure**

Other Studies in non-BCG vaccinated populations

- Inflammatory disease patients (N=398), Italy
 - RA (51%), psoriatic arthritis (24%), ankylosing spondylitis (15%), other (10%)
- Most (79%) on immunosuppressive Rx at time of screening
 - Prednisone, methotrexate, anti-TNF
- Few with BCG (4%)

Results

- **Positive results**
 - TST 74 (19%) QFT-IT 52 (13%)
- **Both associated with ≥ 1 TB risk factors**
- **QFT-GIT indeterminate in 5 (1.2%)**
- **Concordance high between TST and QFT-IT**
 - 87.7%

Immunosuppressive Rx affects likelihood of positive results

	QFT-IT positive		TST positive	
	OR (95% CI)		OR (95% CI)	
Steroids	0.4 (0.2-0.9)		0.3 (0.2-0.6)	
DMARDs	1.1 (0.5-2.3)		2.7 (0.9-3.4)	
Anti-TNF	0.9 (0.4-2.0)		0.3 (0.1-0.6)	

- **Vassilopoulos D et al. J Rheum 2008**
 - **TST vs. T.Spot.TB (n=70 RA, AS, other)**
 - **TST+ (39%) > T.Spot.TB+ (23%)**
- **Discordant results**
 - **TST+ / T.Spot.TB – associated with BCG**
 - **T.Spot.TB + / TST– associated with prednisone**