Diagnosis of latent tuberculosis infection (LTBI)

P. Van Bleyenbergh
Pneumologie, UZ Leuven
Mostly TB bacilli are eliminated or contained by host defenses.

Diagnosis of LTBI

• No diagnostic gold standard!
  (Surrogate endpoint: development of active disease)

• Indirect approach to diagnosis
  → immunological evidence of host sensitization

  1. Tuberculin skin test (TST)
  2. Interferon-γ release assay (IGRA)
Diagnosis of LTBI: evidence of host sensitization

Tuberculin skin test (TST)

- One of the **oldest tests** in current clinical use
  - 1890 Robert Koch
  - 1907 von Pirquet
  - 1908 Mantoux
  - 1930s: widespread use to diagnose LTBI

- **Intradermal injection of purified protein derivate (PPD)**
  - 5 TU of PPD-S (USA)
  - 2 TU of PPD-RT23 (Europe)
Reading a tuberculin skin test

- Interpretation 48-72hrs after injection

- Conversion: recent infection!
  = increase $\geq$10mm of induration within a 2-year period, regardless of age

VRGT, 2003
CDC, MMWR 2004; 53(33): 683-686
## Interpretation of tuberculin skin test results

<table>
<thead>
<tr>
<th>Induration ≥ 5 mm</th>
<th>Induration ≥ 10 mm</th>
<th>Induration ≥ 15 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-positive persons</td>
<td>Recent arrivals (&lt; 5 yr) from high-prevalence countries</td>
<td>Persons with no risk factors for TB</td>
</tr>
<tr>
<td>Recent contacts of TB case</td>
<td>Injection drug users</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Residents and employees* of high-risk congregate settings: prisons and jails nursing homes and other health care facilities, residential facilities for AIDS patients, and homeless shelters</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mycobacteriology laboratory personnel</td>
<td></td>
</tr>
<tr>
<td>Fibrotic changes on chest radiograph consistent with old TB</td>
<td>Persons with clinical conditions that make them high-risk: silicosis diabetes mellitus, chronic renal failure, some hematologic disorders (e.g., leukemias and lymphomas), other specific malignancies (e.g., carcinoma of the head or neck and lung), weight loss of &gt; 10% of ideal body weight, gastrectomy, jejunoileal bypass</td>
<td></td>
</tr>
<tr>
<td>Patients with organ transplants and other immunosuppressed patients (receiving the equivalent of &gt; 15 mg/d Prednisone for &gt; 1 mo)</td>
<td>Children &lt; 4 yr of age or infants, children, and adolescents exposed to adults in high-risk categories</td>
<td></td>
</tr>
</tbody>
</table>

Interpretation of tuberculin skin test results

< 5 mm: negatief (tenzij het om een fout-negatieve interpretatie gaat)

5-9 mm: meestal negatief
positief: in geval van HIV-infectie of ernstige immunodeficïëntie
twijfelachtig: in geval van nauw contact met besmettelijke tuberculosepatiënt
(positief sputum op rechtstreeks microscopisch onderzoek of op kweek)
en bij jonge kinderen (≤ 5 jaar) en oudere personen (≥ 65 jaar)

10-17 mm: positief: in geval van nauw contact met besmettelijke tuberculosepatiënt en/of indien
verhoogd risico op tuberculose-infectie of aanwezigheid van één of meer risicofactoren
voor het ontwikkelen van actieve tuberculose
twijfelachtig: bij afwezigheid van risicofactoren en/of indien antecedenten van recente
(minder dan 5 jaar) BCG-vaccinatie

≥ 18 mm: positief
Performance of the tuberculin skin test

- Waiting period 6-8 weeks
- No differentiation between latent and active disease
- Some operator-related liabilities
  - injection
  - interpretation
- Reactivity may wane over time (booster?)
- False-positive results:
  - specificity varies greatly!!
  - BCG <10 years
  - NTM sensitization
  - Dose PPD >> 2 TU RT23

• BCG received in infancy: effect on TST minimal

• BCG received after infancy: more frequent and more persistent and larger TST reactions

• NTM no clinically important cause of false-positive TST, except when high prevalence of NTM and low prevalence op MTB

Recombinant PPD (DPPD): fewer false-positive reactions secondary to non-tuberculous mycobacteria!
- Waiting period 6-8 weeks
- No differentiation between latent and active disease
- Some operator-related liabilities
  - injection
  - interpretation
- Reactivity may wane over time  (booster?)
- False-positive results:
  - BCG <10 years
  - NTM sensitization
  - Dose PPD >>2 TU RT23

*specificity varies greatly!!*

- False-negative results: cf.

False-negative tuberculin skin test result

Age <6 months
Age >65 yrs [137]
Cellular immune defects
(e.g. HIV infection, AIDS and lymphoproliferative disorders)

<table>
<thead>
<tr>
<th>Cut-off value</th>
<th>Sensitivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥5 mm</td>
<td>95–99</td>
</tr>
<tr>
<td>≥10 mm</td>
<td>91–95</td>
</tr>
<tr>
<td>≥15 mm</td>
<td>67–80</td>
</tr>
</tbody>
</table>

with Mycobacterium tuberculosis
Application errors (incomplete or subcutaneous tuberculin injection, incorrect quantity of tuberculin, inadequate storage of tuberculin)
Reading errors (too early or too late)

Performance of the tuberculin skin test

Pooled specificity = **0.75** (0.72-0.78)

Pooled sensitivity = **0.65** (0.61-0.68)

Sester M et al. *Eur Resp J* 2011; 37: 100-111
• Modest positive association between tuberculin reactivity and the risk of active TB disease

BUT

• Many confounding factors…
• Mostly ‘passive’ follow-up

• Large majority (>95%) of individuals with positive TST results do not progress to active disease!
Interferon-γ release assay (IGRA)

Behr MA et al. *Science* 1999; 284: 1520-1523
**Interferon-γ release assay (IGRA)**

- IGRA: more specific for *M. tuberculosis complex*

### Strain tested vs. Antigens

<table>
<thead>
<tr>
<th>Tuberculosis complex</th>
<th>ESAT-6</th>
<th>CFP 10</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. tuberculosis</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>M. africanum</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>M. bovis</em></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

### Environmental strains

<table>
<thead>
<tr>
<th>Environmental strains</th>
<th>ESAT-6</th>
<th>CFP 10</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. abscessus</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. avium</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. branderi</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. celatum</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. chelonae</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. fortuitum</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. gordonii</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. intracellulare</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. kansasii</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>M. malmoense</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. marinum</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>M. oenavense</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. scrofulaceum</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. smegmatis</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. szulgai</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>M. terrae</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. vaccae</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. xenopi</em></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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Types of IFN-γ Release Assay

- **Measure Δ IFN-γ concentration**
  - e.g. QuantiFERON®-TB Gold In-Tube
    - Whole Blood stimulated with TB antigens
    - Measure IFN-γ by ELISA

- **Measure Δ # of cells releasing IFN-γ**
  - e.g. T SPOT® (ELISpot)
    - PBMCs stimulated with TB antigens
    - Count spots

What are the (dis)advantages of IGRAs?

<table>
<thead>
<tr>
<th></th>
<th>TST</th>
<th>IGRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-reactivity with BCG</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Cross-reactivity with NTM</td>
<td>Yes</td>
<td>Unlikely</td>
</tr>
<tr>
<td>Negative/positive control</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Reliability/reproducibility</td>
<td>Moderate &amp; variable</td>
<td>High</td>
</tr>
<tr>
<td>Boost effect</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Patient visits</td>
<td>Two</td>
<td>One</td>
</tr>
<tr>
<td>Trained personnel required</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Laboratory infrastructure required</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Time to obtain result</td>
<td>3 days</td>
<td>1-2 days</td>
</tr>
<tr>
<td>Material costs</td>
<td>Low</td>
<td>Moderate to high</td>
</tr>
</tbody>
</table>

Evaluation of IGRAs

Lack of “gold standard” for TB infection!

- **Sensitivity** → Compare to culture
  - Sensitivity: # positives/# culture (+) people tested

- **Specificity** → Subjects at low risk for LTBI
  - Specificity: # negative/# low-risk people tested

- ✓ Accuracy of IGRAs
- ✓ Agreement with TST
- ✓ Positive results vs. exposure
- ✓ Predicting TB disease
## Performance of IGRA test

### Sensitivity

<table>
<thead>
<tr>
<th>Series</th>
<th>Diagnostics</th>
<th>Subject</th>
<th>Studies n</th>
<th>Summary sensitivity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>QFT-G</td>
<td>TB patients, adult</td>
<td>21</td>
<td>0.80 (0.78–0.82)</td>
</tr>
<tr>
<td>2</td>
<td>QFT-G-IT</td>
<td>TB patients, adult</td>
<td>6</td>
<td>0.74 (0.69–0.78)</td>
</tr>
<tr>
<td>3</td>
<td>QFT-G/G-IT</td>
<td>TB patients, child</td>
<td>9</td>
<td>0.82 (0.75–0.87)</td>
</tr>
<tr>
<td>4</td>
<td>QFT-G/G-IT, T.Spot</td>
<td>HIV-infected TB patients</td>
<td>5</td>
<td>0.70 (0.60–0.79)</td>
</tr>
<tr>
<td>7</td>
<td>T.Spot</td>
<td>TB patients</td>
<td>13</td>
<td>0.90 (0.86–0.93)</td>
</tr>
<tr>
<td>8</td>
<td>TST</td>
<td>Healthy subjects</td>
<td>20</td>
<td>0.77 (0.71–0.82)</td>
</tr>
</tbody>
</table>

### Specificity

<table>
<thead>
<tr>
<th>Series</th>
<th>Diagnostics</th>
<th>Subject</th>
<th>Studies n</th>
<th>Summary specificity (96% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>QFT-G/G-IT</td>
<td>Healthy young adults</td>
<td>12</td>
<td>0.98 (0.97–0.99)</td>
</tr>
<tr>
<td>2</td>
<td>QFT-G/G-IT</td>
<td>Healthy young adults, BCG^-</td>
<td>8</td>
<td>0.99 (0.98–1.00)</td>
</tr>
<tr>
<td>3</td>
<td>QFT-G/G-IT</td>
<td>Healthy young adults, BCG^+</td>
<td>8</td>
<td>0.96 (0.94–0.98)</td>
</tr>
<tr>
<td>4</td>
<td>T.Spot</td>
<td>Predominantly BCG vaccinated</td>
<td>8</td>
<td>0.93 (0.86–1.00)</td>
</tr>
<tr>
<td>5</td>
<td>TST</td>
<td>BCG not vaccinated</td>
<td>6</td>
<td>0.97 (0.95–0.99)</td>
</tr>
<tr>
<td>6</td>
<td>TST</td>
<td>BCG vaccinated</td>
<td>6</td>
<td>0.59 (0.46–0.73)</td>
</tr>
</tbody>
</table>

IGRAs:
NPV for presence of LTBI

QFT-GIT pooled: 0.88

T-SPOT.TB pooled: 0.94

IGRAs perform well in contact investigations for TB

- 812 close contacts of culture-confirmed TB pts
- All TST positive (>5mm)
- QFT & T-SPOT

Diel R et al. Chest 2009; 135; 1010-1018
IGRAs perform well in contact investigations for TB

- 812 close contacts of culture-confirmed TB pts
- All TST positive (>5mm)
- QFT & T-SPOT

1. Excellent agreement between QFT and T-SPOT (93.9%)
2. Strong association with measures of exposure and infection risk
3. TST (cut-off >5mm) very poor specificity (64.5%)

Diel R et al. Chest 2009; 135; 1010-1018
IGRAs perform well in contact investigations for TB

Diel R et al. *Chest* 2009; 135; 1010-1018
Use of TST and IGRA in contact tracing
(adults, children ≥5 yrs)

Rule out active TB!

TST
( cut-off can be chosen low [5mm] to increase sensitivity)

NEGATIVE
NO LTBI

POSITIVE
IGRA

NEGATIVE
NO LTBI

POSITIVE
LTBI

(BCG vaccinated, ...)

NEGATIVE
NO LTBI

POSITIVE
LTBI

NEGATIVE
NO LTBI

POSITIVE
LTBI
IGRAs: NPV for progression to active TB

Diel R et al. *Chest* 2012; 142: 63-75

Pooled NPV (IGRA) 0.997
Pooled NPV (TST) 0.994
IGRAs: PPV for progression to active TB

• Most IGRA-positive individuals do not progress to active TB disease (RR 2-3, weak to moderate association)

• No tests for LTBI with high prognostic value available

• Proportion of IGRA-positive individuals generally lower than proportion of TST-positive individuals → less pts for preventive chemotherapy!

Diel R et al. Chest 2012; 142: 63-75
IGRA for serial testing: much confusion, not superior to TST

- IGRA lower prevalence of positive tests for one-time screening, in low-incidence settings

- Serial testing: much higher rate of conversions and reversions → ‘wobble’-effect

- Not all conversions and reversions are stable

- Most IGRA conversions seem not to be false-positive

Fong KS et al. *Chest* 2012; 142: 55-62
Dorman SE et al. *Am J Resp Crit Care Med* 2014; 189: 77-87
Some concerns about issues with reproducibility


<table>
<thead>
<tr>
<th>Source of variability</th>
<th>Impact on assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source of variability</td>
<td>QFT</td>
</tr>
<tr>
<td>Manufacturing source</td>
<td></td>
</tr>
<tr>
<td>Between-lot variability</td>
<td>↑ ↓</td>
</tr>
<tr>
<td>Preanalytical sources</td>
<td></td>
</tr>
<tr>
<td>Time of blood draw (a.m. vs p.m.)</td>
<td>↑ p.m.</td>
</tr>
<tr>
<td>Skin disinfection</td>
<td>?</td>
</tr>
<tr>
<td>Traumatic blood draw</td>
<td>?</td>
</tr>
<tr>
<td>Blood vol (0.8–1.2 ml)</td>
<td>↓ NA</td>
</tr>
<tr>
<td>Shaking of tubes (gentle to vigorous)</td>
<td>↑ NA</td>
</tr>
<tr>
<td>T-cell and APC counts</td>
<td>?</td>
</tr>
<tr>
<td>Transportation temp</td>
<td>?</td>
</tr>
<tr>
<td>Delay in incubation (0–16 h)</td>
<td>↓</td>
</tr>
<tr>
<td>Incubation time (16–24 h)</td>
<td>Possible effect</td>
</tr>
<tr>
<td>Plasma separation delays (seconds to hours)</td>
<td>?b</td>
</tr>
<tr>
<td>Plasma storage (+4—80°C)</td>
<td>No effect</td>
</tr>
<tr>
<td>Analytical sources</td>
<td></td>
</tr>
<tr>
<td>Within-run imprecision</td>
<td>↑ ↓</td>
</tr>
<tr>
<td>Between-run imprecision</td>
<td>↑ ↓</td>
</tr>
<tr>
<td>Between-operator imprecision</td>
<td>↑ ↓</td>
</tr>
<tr>
<td>Between-laboratory imprecision</td>
<td>↑ ↓</td>
</tr>
<tr>
<td>Immunological sources</td>
<td></td>
</tr>
<tr>
<td>Boosting by PPD</td>
<td>↑</td>
</tr>
<tr>
<td>Modulation by PAMP</td>
<td>↑ ↓</td>
</tr>
</tbody>
</table>

Sources of IGRA Variability

Phlebotomy, Tube Order, and Time of Blood Draw

Immunomodulation and Boosting

Manufacturing Defects

Incubation of Processing Delay

Incubation Duration
IGRAs: time interval to conversion

- Interval for positive conversion following exposure to a patient with active TB is unclear
  - **TST**: 2-12 weeks → 8 weeks
  - **IGRA**:
    - NICE guidelines (UK): 6 weeks
    - CDC guidelines (USA): 8-10 weeks
    - ERS guidelines (EUR): 8 weeks

- Recent study:
  "IGRA conversion generally occurred 4-7 weeks after exposure, although it could be as late as 14-22 weeks!"

Erkens CGM et al. *ERJ* 2010; 36: 925-949

IGRAs: Take home messages (1)

1) IGRAs do not differentiate between LTBI and active disease
   IGRAs can never rule out active disease
   → usefulness lies within LTBI diagnosis

2) IGRAs are more specific than the TST
   → preferred in BCG vaccinated persons
   → all positive TSTs should be confirmed with an IGRA

3) IGRAs detect infected persons that the TST does not
   → IGRAs should be used instead of, or in addition to the TST in immunosuppressed persons
IGRAs:
Take home messages (2)

4) IGRAs bring logistical advantages of only one patient visit
   → IGRA use is considered in hard to reach populations

5) Using IGRAs is more cost effective than not using them
   → Using only the TST is the most expensive strategy

6) IGRA use in children is still an area under debate
   → IGRA use not advocated at age <5y
Use of interferon-gamma release assays in support of TB diagnosis

ECDC GUIDANCE

Issue date: March 2011

Tuberculosis
Clinical diagnosis and management of tuberculosis, and measures for its prevention and control

This is the full version of NICE clinical guideline 117. It contains details of the methods and evidence used to develop the guideline. It updates and replaces the full version of "Tuberculosis: clinical diagnosis and management of tuberculosis, and measures for its prevention and control" that was developed by the National Collaborating Centre for Chronic Conditions and published by the Royal College of Physicians in March 2009. The updated recommendations have been developed by the Centre for Clinical Practice at NICE following the NICE short clinical guideline process.
Online TST/IGRA interpreter
(www.tstin3d.com)

The Online TST/IGRA Interpreter
Version 2.6

The following tool estimates the risk of active tuberculosis for an individual with a tuberculin skin test reaction of 5-9 mm, based on clinical profile, and is intended for adults tested with standard tuberculin (3TU PPD, or 2TU RT-23) and IGRA. For more details about the algorithm used, go to the About page. The current version of the algorithm contains modifications of the original version, which was detailed in a paper by Menzies, et al. (2008). For further information, see references, or contact dick.menzies@mcgill.ca.

Please select the best response for each field:

- **TST Size:** 5-9 mm
- **IGRA Result:** Positive
- **Age:** 24 years old
- **Age at immigration (If person immigrated to a low TB incidence country):** N/A
- **Country of birth:** Belgium
- **BCG status:** Never vaccinated or unknown

Please select all the conditions that currently apply to the patient (if none of these conditions apply, please leave boxes unchecked):

- AIDS
- Abnormal chest x-ray: fibrocavitary disease
- Chronic renal failure requiring hemodialysis
- Diabetes Mellitus (all types)
- Recent TB infection (TST conversion ≤ 2 years ago)
- Sarcoidosis
- Tumor Necrosis Factor (TNF)-alpha inhibitor (e.g. Infliximab/Enbrel)
- Young age when infected (≤ 4 years)
- Abnormal chest x-ray: granuloma
- Carcinoma of head and neck
- Cigarette smoker (>1 pack/day)
- HIV infection
- Transplantation (requiring immune-suppressant therapy)
- Treatment with glucocorticoids
- Underweight (< 90% ideal body weight or a body mass index (BMI) < 20)

Results

**Printable version**

Below are the results for a patient with a TST reaction of 5-9 mm and a Positive IGRA test, who is 24 years old, born in Belgium, whose BCG status is Never vaccinated or unknown, who has had no contact with active TB, and who can be characterized by:

- Treatment with glucocorticoids

The likelihood that this is a true positive test (PPV) is: 99.93%

The annual risk of development of active tuberculosis disease is estimated to be 0.49%

The cumulative risk of active tuberculosis disease, up to the age of 80, is 7.76%

If treated with INH, the probability of clinically significant drug-induced hepatitis is 2.3%, and the associated probability of hospitalization related to drug-induced hepatitis is 0.6%.

Still some unanswered questions...