LTBI conception
definitions and relevance for diagnostic products

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Content

1. Use of the test and rationale for improvement

2. Changing paradigm: what does/should a test for LTBI measure?

3. Implications for test development, performance, utilization and design

4. Issues for discussion – implications for TPP

Definition of *latent tuberculosis infection*
1. **Target preventive treatment**
Select for preventive treatment individuals at (high) risk of progression to TB disease.

2. **Estimate LTBI burden**
Measure LTBI prevalence in general population and in at-risk populations.

3. **Estimate trend of LTBI burden (or: transmission)**
Measure recently acquired LTBI in general population and in at-risk populations.

4. **Treatment monitoring**
Test of cure for persons with LTBI receiving treatment.
Use of the test

1. **Target preventive treatment**
Select for preventive treatment individuals at (high) risk of progression to TB disease

2. **Estimate LTBI burden**
Measure LTBI prevalence in general population and in at-risk populations

3. **Estimate trend of LTBI burden (or: transmission)**
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4. **Treatment monitoring**
Test of cure for persons with LTBI receiving treatment
Rationale: scale up preventive treatment

WHO aims at global elimination of TB by 2035

Current approaches insufficient – need to expand preventive treatment of latent infection

Optimize use of current & new tools emerging from pipeline, pursue universal health coverage and social protection

Introduce new tools: a vaccine, new drugs & treatment regimens, and a point-of-care test for treatment of active TB disease and latent TB infection

Current global trend: -1.5%/year

-10%/year by 2025

-5%/year

-17%/year
Preventive treatment

- Various regimens exist, most studies show protective efficacy of 60-80%:
  - Isoniazid 6-9 months
  - Rifampin-isoniazid 3-4 months
  - Rifapentine-isoniazid 3 months

- But there are limitations:
  - Toxicities (e.g. hepatoxicity)
  - Completion & adherence
  - Feasibility
  - Cost
  - Drug resistance?

- Recommended for use in:
  - Globally: HIV+, children exposed to TB
  - Low-incidence countries: other contacts of TB patients, immigrants?

Utilization restricted partly due to diagnostic limitations
Current diagnostics for LTBI: TST

**Tuberculin skin test**

- Read after 48-96 H
- Inter/intra-observer variability
- **Sensitivity** reduced with immune suppression
- Cross-reactions $\rightarrow$ poor **specificity**
  - BCG vaccination
  - Non-tuberculous mycobacteria
- Remains positive for decades $\rightarrow$ Anamnestic response?
Current diagnostics for LTBI: IGRA

Elispot (TB-Spot)

1. **Stage 1**
   - Separated white blood cells are counted and added to microtiter plate wells that have been coated with monoclonal antibodies to interferon gamma (IFN-γ) and TB-specific antigens.
   - IFN-γ is released from sensitized T cells, which is captured by the antibodies.

2. **Stage 2**
   - Wells are washed and conjugated secondary antibodies are added to bind to any captured IFN-γ.
   - Substrate is added to visualize the IFN-γ, producing highly visible spots.

   - The spots can then be counted. One spot is one T cell.

Whole-blood assay (Quantiferon)

1. **Stage One – Blood Incubation and Harvesting**
   - After blood collection, mix the test tube with TB Gold tubes thoroughly by shaking or by turning tubes end-over-end.
   - Incubate tubes upright at 37°C for 16-24 hours.
   - Centrifuge tubes at 1500-2200 g (RCF) for 5-10 minutes.

2. **Stage Two – Human IFN-γ ELISA**
   - Add 50 μL of conjugate solution to each well. Add 50 μL of plasma or standard.
   - Shake covered plate for 1 min.
   - Incubate for 120 minutes at Room Temperature.
   - Wash plate 3 times. Add 100 μL substrate. Incubate 30 min at Room Temperature.
   - Add 50 μL of stop solution. Read absorbance within 5 min at 450 nm (620-650 nm net).

   - Calculate results using QuantIFERON TB Gold In-Tube Analysis Software.

24H incubation with specific antigens
IFNγ production by individual T-cells

24H incubation with specific antigens
IFNγ measured by ELISA (supernatant)
Current diagnostics for LTBI: IGRA

- **Sensitivity** as good as TST but better in immune suppression (& variable)
- **More specific** than TST → almost no cross-reaction
- Correlates better with TB exposure than TST in low-incidence settings but not in high-incidence settings

**What do IGRA measure?**
- Anamnestic response?
- Recent exposure (→ high risk for disease)?
- Ongoing antigenic stimulation (persistence)?

There is no gold standard for LTBI
IGRA as predictor of TB disease

### Immigrants

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<tr>
<th>Study</th>
<th>PPV</th>
<th>95% CI</th>
<th>n/N</th>
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<td>Clark 2007</td>
<td>0.10</td>
<td>(0.01 – 0.32)</td>
<td>2/20</td>
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<td>Aichelburg 2009</td>
<td>0.08</td>
<td>(0.02 – 0.22)</td>
<td>3/36</td>
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<td>Haldar 2009</td>
<td>0.17</td>
<td>(0.11 – 0.26)</td>
<td>19/110</td>
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<td>Kik 2009</td>
<td>0.03</td>
<td>(0.01 – 0.06)</td>
<td>5/178</td>
</tr>
<tr>
<td>Lee 2009</td>
<td>0.00</td>
<td>(0.00 – 0.22)</td>
<td>0/15</td>
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<td>Lee 2009</td>
<td>0.08</td>
<td>(0.00 – 0.38)</td>
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<td>Diel 2011</td>
<td>0.13</td>
<td>(0.08 – 0.19)</td>
<td>19/147</td>
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<td>Harstad 2010</td>
<td>0.03</td>
<td>(0.01 – 0.05)</td>
<td>6/238</td>
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<td>Jonnalaggada 2010</td>
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<td>Leung 2010</td>
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<td>Kim 2011</td>
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<td>(0.02 – 0.14)</td>
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<td>Song 2011</td>
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<td>Yoshiyama 2011</td>
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<td>Zhang 2011</td>
<td>0.00</td>
<td>(0.00 – 0.21)</td>
<td>0/16</td>
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</table>

### Low-incidence populations

Pooled PPV for progression = 0.068 (0.056 to 0.083)
Chi-square = 50.30; df = 14 (p = 0.0000)
Inconsistency (I-square) = 72.2%
Number needed to treat

NNT to prevent 1 true case of TB using IGRA

IGRA sensitivity 78%
IGRA specificity 58%

Kik & Cobelens, in prep
Number needed to treat

NNT to prevent 1 true case of TB using IGRA

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HIV-positive contacts

Cumulative TB incidence

Kik & Cobelens, in prep
Number needed to treat

NNT to prevent 1 true case of TB using IGRA

IGRA sensitivity 78%
IGRA specificity 58%

HIV-negative contacts prisoners

NNT

Cumulative TB incidence

0% 5% 10% 15% 20% 25% 30%

455
91
46
18

Kik & Cobelens, in prep
The need

So we need a test that has better positive (and negative) predictive value for TB disease occurring in the future

- LTBI test
- TB-risk-stratification-test
  “TB prediction test”

Can high positive predictive values be attained?
LTBI: changing paradigm

Verrall et al. Immunology 2014
LTBI: changing paradigm

(a)

possible predisposing factors
- HIV
- malnutrition
- diabetes
- alcoholism
- pro/anti inflammatory imbalance

possible precipitating factors
- HIV
- anti-TNF therapy
- malnutrition
- Vit D deficiency
- viral infection

infection → unstable → disease → control/elimination

1° progression

control/elimination

Esmail et al. Phil Trans Roy Soc 2015
LTBI: changing paradigm

Esmail et al. Phil Trans Roy Soc 2015
Overview of national TB prevalence survey conducted in Asia, 1990-2012
Proportion of all detected prevalent TB cases that did not report cough

Onozaki et al. TMIH 2015
Subclinical active phase

176 Chinese patients with abnormal X-rays but 5 negative cultures Followed up for TB for 36 months: 93 TB cases (69 culture-confirmed)

In this stage we cannot predict if and when a precipitating event will occur

→ *beyond existing risk classification* we cannot predict who will become diseased

→ PPVs will be relatively low
In this stage we there is active bacterial multiplication with high probability of leading to TB disease

→ PPVs can be relatively high

Esmail et al. Phil Trans Roy Soc 2015
What does the test measure?

Conceptually, the test either...

- ...predicts that disease cannot happen *because there is no persistent infection*
- ...or predicts that disease occurs *because it has already started*...

"persistent infection test"

"incipient/subclinical TB test"
This dichotomy matters because it has implications for:

- Test development
- Test performance
- Test utilization
- Test design
Implications for test development

“persistent infection test”
- CD4 response
- Other?

“incipient/subclinical TB test”
- bacterial multiplication?
- inflammatory response?
- CD8 response?
- Other?
Implications for test performance

- **a** probability that infection is cleared
- **b** probability that infection leads to subclinical/incipient TB
- **c** probability that subclinical/incipient TB leads to TB disease
- **d** probability that infection existed before the (recent) exposure

PPV = true positives out of all positives
Performance for anamnestic response (TST?)

- Infection cleared → no TB
- Persistent infection → no TB
- Subclinical TB → TB
- Halted progression → no TB

- False positive
- False positive
- True positive
- False positive

Exposure

Precipitating event
Infection cleared $\rightarrow$ no TB

Persistent infection $\rightarrow$ no TB

Subclinical TB $\rightarrow$ TB

Halted progression $\rightarrow$ no TB

True negative

False positive

False positive

True positive

Performance for a test for persistent infection

PPV depends on b and c (risk of disease progression)
PPV depends on d (previous exposure)

$\Rightarrow$ PPV is population dependent (IGRA)
Performance for a test for subclinical TB

PPV depends on $c$ (probability of spontaneous halting of disease progression)

→ PPV is largely population independent ...
Performance for a test for subclinical TB

- Infection cleared $\rightarrow$ no TB
  - True negative

- Persistent infection $\rightarrow$ no TB
  - True negative

- Subclinical TB $\rightarrow$ TB
  - True positive

- Halted progression $\rightarrow$ no TB
  - False positive

exposure
precipitating event

... but test is only positive AFTER the precipitating event
Performance for a test for subclinical TB

- Infection cleared → no TB
- Persistent infection → no TB
- Subclinical TB → TB
- Halted progression → no TB

... but test is only positive AFTER the precipitating event →

→ NPV depends on when test is done

→ NPV will be higher the closer the test is done to the moment TB disease becomes apparent
Subclinical TB test: RNA signatures

16-gene RNA signature in 6363 South African adolescents followed for incident TB

Prediction improves as sample was tested closer to the timepoint of TB diagnosis

Zak et al. Lancet 2016
Implications for test utilization

Rule-out progression to TB disease

Rule-in progression to TB disease
Implications for test utilization

1. When to rule out, when to rule in?
   - **Rule out:**
     - **High probability of progression**, in particular to severe TB disease (e.g. HIV infection, pre-TNFalpha blocking, infants)
     - Irrespective of recent exposure
   - **Rule in:**
     - **Recent exposure** (e.g. contacts, high transmission settings)
     - Irrespective of probability of progression

2. **Incipient/subclinical TB:** test may need to be repeated

3. Positive test: check for active TB
   - **Incipient/subclinical TB:** can we safely treat with a single drug (e.g. isoniazid)?

4. **Test reversion** after successful preventive treatment
   - Expected of Incipient/subclinical TB test
   - Also of test for persistent TB?
     - does preventive treatment eradicate persisters?
Implications for test design

**Incipient/subclinical TB test**
- Rule in test with potential and intended use at large scale
- Low number-needed-to-treat, but high number-needed-to-test
- May need to be repeated within individuals
  → Important for test to be low-cost

A single biomarker that will show high sensitivity AND high specificity is not likely to exist
  → opportunities for **combining** persistent TB and incipient/subclinical TB markers in single assay
  → “**risk signatures**” may be such combinations
Issues for discussion – implications for TPP

• Do we believe all this?

• How do we deal in the TPP with the issue of rule out versus rule in?

• What is the time period for which the required NPV and PPV should be attained?

• Should the test be able to differentiate from active TB?

• Should the test have a (semi)quantitative read-out?
  – E.g. to indicate whether full-course treatment is needed or only preventive treatment?

• Should we require reversion to negative after successful preventive treatment?
TST+ individuals

Time period

- Rieder. IUATLD 2003
- D’Arcy Hart & Sutherland. BMJ 1977
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GRAZIE !