The evolving concept of LTBI diagnosis

tests for incipient TB and tests for persistent infection

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What really matters: prediction of TB disease

Test

negative

positive

Follow up over time ➔

# TB cases

sensitivity

specificity

# TB cases

positive predictive value (PPV)

Test

positive

Follow up over time ➔

# TB cases
IGRA as predictor of TB disease

Meta-analysis of prospective cohort studies
High-risk groups only

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 %

Diel et al. Chest 2012
Number needed to treat

NNT to prevent 1 true case of TB using IGRA

IGRA sensitivity 78%
IGRA specificity 58%

Kik et al., in prep
Number needed to treat

NNT to prevent 1 true case of TB using IGRA

IGRA sensitivity 78%
IGRA specificity 58%

HIV-positive contacts
Cumulative TB incidence = progression to disease

Rangaka, Kik et al, in prep
Number needed to treat

NNT to prevent 1 true case of TB using IGRA

HIV-negative contacts prisoners

Cumulative TB incidence = progression to disease

Kik et al., in prep
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

- Clinical disease
  - Bacterial replication maintained at a subclinical level by the immune system
  - Infection controlled with some bacteria persisting in non-replicating form
  - Infection eliminated in association with T cell priming
  - Infection eliminated without priming antigen-specific T cells

- Disease
  - Active infection
  - Quiescent infection

Barry et al. Nat Rev Microbiol 2009
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

disease

1° progression

infection → unstable → control/elimination

Prd

Prc

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

Esmail et al. Phil Trans Roy Soc 2015
Subclinical active phase

Overview of national TB prevalence surveys 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

35 patients with LTBI (QFN-GIT+, culture -), HIV infected, ART naive (CD4>350)
PET-scans (2-deoxy-2-[18F]fluoro-d-glucose positron emission and computed tomography)
6 months follow-up

→ 10 patients with subclinical disease more likely to progress to active disease

LTBI: changing paradigm

In this stage we cannot predict if and when a precipitating event will occur
→ we cannot predict who will become diseased

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

→ PPVs can be relatively high
What does the test measure?

Conceptually, the test either...

... predicts that disease cannot happen *because there is no persistent infection*

"persistent infection test"

... or predicts that disease will occur *because it has already started*....

"incipient 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
- mRNA?

“incipient TB test”
- bacterial multiplication?
- mRNA?
- inflammatory response?
- CD8 response?
Implications for test performance

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

**PPV** = true positives out of all positives

*Cobelens et al. Lancet Resp Med, accepted*
Performance for anamnestic response (TST?)

- Infection cleared $\rightarrow$ no TB
- Persistent infection $\rightarrow$ no TB
- Incipient TB $\rightarrow$ TB
- Halted progression $\rightarrow$ no TB

PPV for predicting TB disease is very low

Cobelens et al. Lancet Resp Med, accepted
Performance for a test for *persistent infection*

- **Infection cleared → no TB**
  - **True negative**
- **Persistent infection → no TB**
  - **False positive**
  - **True positive**
- **Subclinical TB → TB**
  - **True positive**
  - **False positive**
- **Halted progression → no TB**
  - **False positive**

**Performance for a test for persistent infection**

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

→ **PPV is population-dependent and lower in high-transmission settings (IGRA!)**

*Cobelens et al. Lancet Resp Med, accepted*
Performance for a test for *incipient TB*

Infection cleared $\rightarrow$ no TB  
True negative

Persistent infection $\rightarrow$ no TB
True negative

Incipient TB $\rightarrow$ TB
True positive

Halted progression $\rightarrow$ no TB
False positive

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

$\rightarrow$ PPV is largely population independent ...
Performance for a test for *incipient TB*

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

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

*Cobelens et al. Lancet Resp Med, accepted*
Performance for a test for _incipient TB_

\[ \rightarrow \text{NPV depends on when test is done} \]

\[ \rightarrow \text{NPV will be higher the closer the test is done to the moment TB disease becomes apparent} \]

*Cobelens et al. Lancet Resp Med, accepted*
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

Persistant infection test

Rule-out progression to TB disease

Incipient TB test

Rule-in progression to TB disease
Implications for test utilization

When to rule out, when to rule in?

**Rule out** (= persistent infection test)
- **High probability of progression**, in particular to severe TB disease (e.g. HIV infection, pre-TNFalpha blocking, infants)
- Irrespective of recent exposure

**Rule in** (= incipient TB test)
- **Recent exposure** (e.g. contacts, high transmission settings)
- Irrespective of probability of progression
  → potential for mass test & treat campaigns!
Implications for test design

**Incipient 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

“Risk signatures” may in fact be combinations of persistent infection and incipient TB tests
Conclusions

We need a **TB prediction test**

Positive predictive values for current tests are too low $\rightarrow$ numbers needed to treat too high

A high PPV prediction test probably identifies **incipient TB** rather than persistent infection

A test for incipient TB will be a test for **ruling in** ‘likely progression to TB disease’ in recently exposed individuals

An inexpensive and easy-to-use test for incipient TB could open opportunities for mass test & treat campaigns
Acknowledgements

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A blood RNA signature for tuberculosis disease risk: a prospective cohort study

Summary
Background Identification of blood markers that prospectively infect to tuberculosis disease might lead to interventions to assess whether global gene expression measured in whole blood of signatures of risk of active tuberculosis disease.

First characterization of the CD4 and CD8 T-cell responses to QuantiFERON-TB Plus

RESEARCH ARTICLE

The predictive value of current haemoglobin levels for incident tuberculosis and/or mortality during long-term antiretroviral therapy in South Africa: a cohort study

Abstract
Background: Low haemoglobin concentrations may be predictive of incident tuberculosis (TB) and death in HIV-infected patients receiving antiretroviral therapy (ART), but data are limited and inconsistent. We examined these relationships retrospectively in a long-term South African ART cohort with multiple time-updated haemoglobin
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)

% of “incident” TB cases

0 10 20 30 40 50 60 70 80 90 100

1-3 months 4-8 months 7-12 months 13-18 months 19-24 months 25-36 months

Current diagnostics for LTBI: TST

**Tuberculin skin test**

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

**Elispot**

STAGE 1

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

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

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

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

**Whole-blood assay**

STAGE 1 – Blood Incubation and Harvesting

After blood collection, mix QuantIFERON®-TB Gold tubes thoroughly by shaking or by turning tubes end-over-end.

Incubate tubes upright at 37°C for 18-24 hours.

Centrifuge tubes at 1500-2200 g (RCP) for 5-10 minutes.

Harvest at least 200μL plasma from each tube. Store in ranked microtubes or uncapped microplates.

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 minute. Incubate for 1.5 hours at Room Temperature.
Wash plate 4-5 times. Add 100 μL substrate. Incubate 30 min at Room Temperature.
Add 50 μL of stop solution. Read absorbance within 5 min at 450nm (620-650nm net)

Calculate Results using QuantIFERON®-TB Gold in-Tube Analysis Software.

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 (also variable)

- More specific than TST
  - No cross-reactions with BCG
  - Almost no cross-reactions with NTM

- Correlate 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)?