Biomarkers for tuberculosis

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National Institute for Infectious Diseases L. Spallanzani, Italy
Singapore, September 26-28, 2019
Conflict of interest

In the last year I have been a consultant or I presented talks for:

Diasorin, Janssen, Qiagen, Quidel
National Institute for Infectious Diseases (INMI) L. Spallanzani, Rome, Italy

HIV: 6,800-7,000 (300 new infection)
HCV: 1,500-2,000
HBV: 800-1,000
Active TB: 300-350, LTBI: 200; HIV-TB: 40
Agenda

- LTBI and limits of the assay for measuring LTBI

- Experimental tests for LTBI:
  - ELISA: IFN-γ response to antigens different from ESAT-6/CFP-10 as HBHA
  - Cytometry: detection of polyfunctional T cell specific response
  - C-Tb: skin test based on ESAT-6/CFP-10

- Transcripts

- Experimental assays to predict active TB development
Natural history of tuberculosis


Elimination of the infection by adaptive or innate immunity

Latent infection

5-10%

Active tuberculosis

5-10%

50-75%

25-50%

90%
TB biomarkers

Correlate of TB infection vs. Natural immunity
20-25% of subjects exposed to M. tuberculosis become LTBI

a. Correlate of TB risk
5-10% of LTBI progress to Active TB

b. Correlate of TB disease

Goletti et al, Respirology 2018

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Integrity of the granuloma is crucial for TB control

Goletti, et al, Expert Rev Anti Infect Ther 2018
Granulomas are independent and dynamic by PET/CT during *M. tuberculosis* infection in macaques

*Lin and Flynn, J Immunol, 2018*
Frequencies of *M. tuberculosis*-specific T cells producing cytokines from individual granulomas of LTBI controls at high or low risk of reactivation.

T cells from granulomas were stimulated with ESAT-6 and CFP-10 peptides.
The End of the Binary Era: Revisiting the Spectrum of Tuberculosis

Lin and Flynn, J Immunol, 2018
LTBI definition from a pragmatic point of view
Efficacy of the preventive therapy in household contacts

Fig. 11. Annual rate of active tuberculosis among Alaskan villagers, averaged for two-year periods after treatment.

Fig. 12. Annual rate of active tuberculosis among Greenland villagers.
Worldwide LTBI: size of the problem

LTBI

1.7 billion
(Houben, Plos Med 2016)

Active TB
10 million

Around 170 fold difference

(Houben, Plos Med 2016)
Limitations of the TST

Reagent:
- Purified protein derivative (PPD) commonly shared among different Mycobacteria (*M. tuberculosis*, *BCG* and *atypical mycobacteria*)

Variability:
- Reproducibility in giving the test
- Subjectivity in reading the test

Logistics
- Repeat visit needed
- 3 days before result
## Tuberculin skin test (TST)

<table>
<thead>
<tr>
<th>Positive TST</th>
<th>Active TB disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M. tuberculosis</strong></td>
<td>Latent TB infection (past or recent exposure to M. tuberculosis)</td>
</tr>
<tr>
<td>NTM</td>
<td>Exposure to environmental mycobacteria</td>
</tr>
<tr>
<td>BCG-vaccination</td>
<td>BCG-vaccination</td>
</tr>
</tbody>
</table>
TST and advantages

High level of standardization of providing the results based on cut-off internationally obtained referring to:

- Exposure (recent or remote)
- Immunodepression
- Age (below 5 years of age..)
- Work context (health care workers)
- Large scale tests (no blood draw)

No high level of standardization for the execution of the assay: it is not a lab test!
IGRAs: tests for LTBI diagnosis

- IFN-γ
  - ESAT-6, CFP-10
    - PBMC
    - Whole Blood
      - T SPOT.TB
      - QuantiFERON TB Plus
IGRA response associates with tuberculosis

<table>
<thead>
<tr>
<th>Positive IGRA</th>
<th>M. tuberculosis</th>
<th>Active TB disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latent TB infection (past or recent exposure to M. tuberculosis)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Negative IGRA</th>
<th>NTM</th>
<th>Exposure to environmental mycobacteria</th>
</tr>
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<tbody>
<tr>
<td>BCG-vaccination</td>
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<td></td>
</tr>
</tbody>
</table>
Accuracy of TB-immune tests in published studies. HIV-uninfected patients.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity for active TB</th>
<th>Specificity for active TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>TST</td>
<td>65</td>
<td>75</td>
</tr>
<tr>
<td>QFT-IT</td>
<td>80</td>
<td>79</td>
</tr>
<tr>
<td>T-SPOT.TB</td>
<td>81</td>
<td>59</td>
</tr>
</tbody>
</table>

Sester et Sotgiu et al, ERJ 2010
## Accuracy of TB-immune tests in published studies in HIV-infected subjects

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QFT</td>
<td>61%</td>
<td>62-89% (depending on TB load in the country)</td>
</tr>
<tr>
<td>T SPOT-TB</td>
<td>65%</td>
<td>70%</td>
</tr>
</tbody>
</table>

### Sensitivity
- QFT: 61%
- T SPOT-TB: 65%

### Specificity
- QFT: 62-89% (depending on TB load in the country)
- T SPOT-TB: 70%
Need of a new test for LTBI detection
CD8$^+$ T-cell specific response and TB

In HIV-uninfected patients: 15% LTBI patients vs 60% active TB (Rozot, 2013)

In HIV-infected patients: CD8-specific response is associated with active TB (Chiacchio, 2014)
CD8+ T-cell frequency decreases in active TB patients after TB-specific therapy
TB 1: CD4 response in all groups.
TB 2: CD4 response in all groups and CD8 response in active TB

### CD4 response by cytometry

<table>
<thead>
<tr>
<th></th>
<th>TB1-responders</th>
<th>TB2-responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB1-responders</td>
<td>TB LTBI Remote, Recent</td>
<td></td>
</tr>
<tr>
<td>TB2-responders</td>
<td>TB LTBI Remote, Recent</td>
<td></td>
</tr>
</tbody>
</table>

### CD8 response by cytometry

<table>
<thead>
<tr>
<th></th>
<th>TB1-responders</th>
<th>TB2-responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB1-responders</td>
<td>TB LTBI Remote, Recent</td>
<td></td>
</tr>
<tr>
<td>TB2-responders</td>
<td>TB LTBI Remote, Recent</td>
<td></td>
</tr>
</tbody>
</table>

**Table:**

<table>
<thead>
<tr>
<th>Group</th>
<th>CD4</th>
<th>CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TB1</td>
<td>TB2</td>
</tr>
<tr>
<td>TB1 N (%)</td>
<td>19 (83)</td>
<td>21 (91)</td>
</tr>
<tr>
<td>TB2 N (%)</td>
<td>17 (94)</td>
<td>15 (83)</td>
</tr>
<tr>
<td>LTBI Remote</td>
<td>12 (100)</td>
<td>11 (92)</td>
</tr>
<tr>
<td>LTBI Recent</td>
<td>48 (90.5)</td>
<td>47 (89)</td>
</tr>
</tbody>
</table>

Petruccioli et al, J Infection 2016
Sensitivity of QFT-Plus in patients with Active TB

Random effects:
Pooled proportion= 0.92 (95% CI = 0.88 to 0.95)
I² = 57.1% (95% CI = 0% to 77.8%)

Sensitivity (95% confidence interval)

Barcellini L. 2016: 0.88 (0.81-0.93)
Hofland R.W. 2018: 0.90 (0.68-0.99)
Hoffmann H. 2016: 0.96 (0.87-1.00)
Lina Yi 2016: 0.91 (0.86-0.95)
Petruccioli E. 2016: 0.85 (0.66-0.96)
Kamada A. 2017: 0.95 (0.82-0.99)
Petruccioli E. 2017: 0.90 (0.80-0.96)
Takasaki Jin 2017: 0.99 (0.95-1.00)
Telisinghe L. 2017: 0.89 (0.81-0.94)
combined: 0.92 (0.88-0.95)
Sensitivity of QFT-Plus-TB1 in patients with Active TB

Random effects:
Pooled proportion = 0.86 (95% CI = 0.76 to 0.94)
I^2 = 87.1% (95% CI = 68.8% to 92.7%)

Barcellini L. 2016  0.82 (0.74-0.89)
Petruccioli E. 2016  0.78 (0.58-0.91)
Petruccioli E. 2017  0.81 (0.70-0.90)
Takasaki J. 2017  0.99 (0.95-1.00)
Telisinghe L. 2017  0.86 (0.77-0.92)
combined  0.86 (0.76-0.94)

Sensitivity (95% confidence interval)
Sensitivity of QFT-Plus-TB2 in patients with Active TB

Random effects:
Pooled proportion = 0.9 (95% CI = 0.83 to 0.96)
P² = 80.1% (95% CI = 37.5% to 89.8%)
Specificity of QFT-Plus in healthy subjects

Fixed effects:
Pooled proportion = 0.97 (95% CI = 0.96 to 0.98)
I^2 = 0% (95% CI = 0% to 58.5%)
Agenda

☐ LTBI and limits of the assay for measuring LTBI

☐ Experimental tests for LTBI:
  ☐ ELISA: IFN-γ response to antigens different from ESAT-6/CFP-10 as HBHA
  ☐ cytometry: detection of polyfunctional T cell specific response
  ☐ C-Tb: skin test based on ESAT-6/CFP-10
  ☐ Transcripts

☐ Experimental assays to predict active TB development
Heparin-binding hemagglutinin (HBHA)

- Recombinant HBHA produced in *E. coli* is not immunogenic and methylation of HBHA is required for the full immunological properties of the protein.
- A recombinant *M. smegmatis* strain expressing the histidine-tagged recombinant HBHA protein from Mtb (rHBHAm) was developed and used to purify a large amount of protein.
- The methylation pattern of rHBHAm was similar to that observed for nHBHA, as assessed by mass spectrometry analysis.
Modulation of HBHA response is associated with TB development or control

Corbière et al, PloS One 2012

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IFN-γ response to the methilated HBHA of *M. tuberculosis* produced in *M. smegmatis* is significantly reduced in patients with active tuberculosis.
Among HIV-infected subjects, IFN-γ response to HBHA is mainly mediated by CD8\(^+\) T cells.
Response to HBHA is mainly monofunctional

A

CD4

frequency of cytokine response (%)

polyfunctional
monofunctional

p=0.002
p=0.002
p=0.024
p=0.008

HBHA

B

CD8

frequency of cytokine response (%)

polyfunctional
monofunctional

p=0.0006
p=0.002
p=0.016
p=0.0006

LTBI, HIV-LTBi, TB, HIV-TB
IFNγ response to QFT antigens and mHBHA in response in children with LTBI and active TB

45 LTBI
19 active TB
Following TB-specific therapy, most of the non-HBHA-responding children, gained an HBHA-positive response.
IFN-γ response to HBHA in children with active TB before and after successful therapy

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Sali et, J Infection 2018
Model of expected response to HBHA is restored after successful anti-TB therapy
IFN-γ response to HBHA with active TB before and after successful therapy

Wen et al, Eur J Clin Microb Inf Dis, 2017
T cell maturation

![Diagram of T cell maturation with markers and functions](image-url)
CD27 modulation: a potential new biomarker for TB?

\[
\text{RATIO MFI} = \frac{\text{MFI CD27 gate of CD}^+ T \text{ cells}}{\text{MFI CD27 gate of CD}^+ \text{IFN}^+ T \text{ cells}}
\]
CD27 modulation within the CD45RA^- cells helps to discriminate among the different TB stages.

CD45RA^- CD27^+ "CM"

CD45RA^- CD27^- "EM"

TB cured TB LTBI+ cured TB

p<0.0001

p=0.0002

p=0.0003

p=0.0047

Petruccioli et al, J Infection, 2015
Bifunctional IFN-γ and TNF-α CD4 cells responding to RD1 proteins and an effector memory phenotype associate with active TB

<table>
<thead>
<tr>
<th>HIV-uninfected</th>
<th>HIV-infected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytokine Response</strong></td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>TNF-α</td>
</tr>
<tr>
<td>active TB</td>
<td>cured TB</td>
</tr>
<tr>
<td>HIV-uninfected</td>
<td>HIV-infected</td>
</tr>
<tr>
<td><strong>Phenotype</strong></td>
<td></td>
</tr>
<tr>
<td>EM</td>
<td>CM</td>
</tr>
<tr>
<td>Active TB</td>
<td>cured TB</td>
</tr>
</tbody>
</table>

Petruccioli and Petrone et al, J Infection 2013
Chiacchio and Petruccioli et al, J Infection 2014
Combination of tests increases diagnostic accuracy

Sensitivity vs. 1-Specificity

- CD4+IFNγ+CD45RA-CD27+
- CD4+IFNγ+TNFα+ with CD4+IFNγ+CD45RA-CD27+
- CD4+IFNγ+TNFα+ with CD27 MFI ratio with CD4+IFNγ+CD45RA-CD27+

p = 0.185

Petruccioli et al, Diagn Microbiol Infect Dis., 2016
The proportion of HLA-DR$^+$ *M. tuberculosis*-specific CD3+ T-cells co-expressing IFNγ and TNFα associates to active TB

Musvosvi, ERJ, 2018
C-Tb skin, a novel specific skin test based on ESAT-6 and CFP10 antigens

The authors investigated the safety and diagnostic potential of C-Tb compared with established tests in the contact-tracing setting.
C-Tb skin test: accuracy results

<table>
<thead>
<tr>
<th></th>
<th>Negative controls (n=263)</th>
<th>Occasional contacts (n=299)</th>
<th>Close contacts (n=319)</th>
<th>Patients with tuberculosis (n=101)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-Tb skin test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>9 (3%)</td>
<td>49 (16%)</td>
<td>136 (43%)</td>
<td>68 (67%)</td>
</tr>
<tr>
<td>Negative</td>
<td>253 (96%)</td>
<td>250 (84%)</td>
<td>180 (57%)</td>
<td>32 (32%)</td>
</tr>
<tr>
<td>Not done</td>
<td>1 (&lt;0.5%)</td>
<td>0</td>
<td>0</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Quantiferon-Tb Gold In-Tube interferon γ release assay</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>10 (4%)</td>
<td>57 (21%)</td>
<td>122 (42%)</td>
<td>82 (81%)</td>
</tr>
<tr>
<td>Negative</td>
<td>253 (96%)</td>
<td>227 (82%)</td>
<td>166 (57%)</td>
<td>19 (19%)</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>0</td>
<td>2 (&lt;1%)</td>
<td>2 (&lt;1%)</td>
<td>0</td>
</tr>
<tr>
<td>Not done</td>
<td>0</td>
<td>13</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>Tuberculin skin test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>46 (22%)</td>
<td>80 (27%)</td>
<td>162 (51%)</td>
<td>90 (90%)</td>
</tr>
<tr>
<td>Negative</td>
<td>167 (78%)</td>
<td>219 (73%)</td>
<td>154 (49%)</td>
<td>19 (10%)</td>
</tr>
<tr>
<td>Not done</td>
<td>50 (19%)</td>
<td>0</td>
<td>0</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

![Graphs showing comparison between BCG-unvaccinated and BCG-vaccinated groups for C-Tb and TST skin tests](https://example.com/graph.png)

Ruhwald et al, Lancet Respiratory Medicine, 2017
Our study has several limitations. C-Tb was developed as a tool to guide treatment for latent tuberculosis infection in people at risk of developing active tuberculosis. Ten participants who had positive C-Tb and QFT results and two who had negative C-Tb and QFT results developed active tuberculosis during follow-up, which corresponds to 2% of 615 contacts. Most of these contacts probably had insipient active tuberculosis at the time of enrolment, despite the absence of symptoms, and the follow-up period was too short to assess the predictive potential of C-Tb. On the basis of the high concordance between tests, we assume that C-Tb has similar positive predictive value to QFT, but we cannot exclude the possibility that the individuals who progressed would be among the 5% with positive QFT and negative C-Tb results. Although a randomised
Agenda

- TB latency
- how do we measure latency? Commercial tests and experimental tests
- How do we predict TB development? Commercial tests and experimental tests
Predictive value of TST and IGRA for incident active tuberculosis in adults

Rangaka et al, TLID 2011

Zellweger et al, AJRCCM 2015
Prognostic value of interferon-γ release assays and tuberculin skin test in predicting the development of active TB (UK PREDICT TB): a prospective cohort study

Abubakar et al, Lancet ID, 2018

<table>
<thead>
<tr>
<th></th>
<th>Progressed</th>
<th>Did not progress</th>
<th>Person-years at risk</th>
<th>Annual incidence per 1000 person-year (95% CI)</th>
<th>IRR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>QuantIFERON-TB Gold In Tube</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test positive</td>
<td>47/1444 (3.3%)</td>
<td>1357/1444 (96.7%)</td>
<td>4649.9</td>
<td>10.1 (7.4-13.4)</td>
<td></td>
</tr>
<tr>
<td>Test negative</td>
<td>20/1493 (0.6%)</td>
<td>4906/1493 (99.4%)</td>
<td>15,926</td>
<td>1.9 (1.2-2.7)</td>
<td></td>
</tr>
<tr>
<td>Positive vs negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T-SPOT.TB</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test positive</td>
<td>52/1235 (4.2%)</td>
<td>1183/1235 (95.8%)</td>
<td>3926.2</td>
<td>13.2 (9.9-17.4)</td>
<td></td>
</tr>
<tr>
<td>Test negative</td>
<td>25/5145 (0.5%)</td>
<td>5120/5145 (99.5%)</td>
<td>16,645.3</td>
<td>1.5 (1.0-2.2)</td>
<td></td>
</tr>
<tr>
<td>Positive vs negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TST-5</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test positive</td>
<td>64/1957 (2.2%)</td>
<td>2883/1957 (97.8%)</td>
<td>9416.8</td>
<td>6.8 (5.2-8.7)</td>
<td></td>
</tr>
<tr>
<td>Test negative</td>
<td>13/3423 (0.4%)</td>
<td>3420/3423 (99.6%)</td>
<td>11,154.6</td>
<td>1.2 (0.6-2.0)</td>
<td></td>
</tr>
<tr>
<td>Positive vs negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TST-10</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test positive</td>
<td>58/1515 (2.7%)</td>
<td>2093/1515 (97.3%)</td>
<td>6822.3</td>
<td>8.5 (6.5-11.0)</td>
<td></td>
</tr>
<tr>
<td>Test negative</td>
<td>19/4229 (0.4%)</td>
<td>4210/4229 (99.6%)</td>
<td>13,749.2</td>
<td>1.4 (0.8-2.2)</td>
<td></td>
</tr>
<tr>
<td>Positive vs negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TST-15</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test positive</td>
<td>52/1485 (3.5%)</td>
<td>1433/1485 (96.5%)</td>
<td>4674.8</td>
<td>11.1 (8.3-14.6)</td>
<td></td>
</tr>
<tr>
<td>Test negative</td>
<td>25/4895 (0.5%)</td>
<td>4870/4895 (99.5%)</td>
<td>15,896.6</td>
<td>1.6 (1.0-2.3)</td>
<td></td>
</tr>
<tr>
<td>Positive vs negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Data are n/N (%), when N is number of participants with the result, and n is number of participants who progressed or did not progress to tuberculosis. IRR = incidence rate ratio. TST-tuberculin skin test. TST-5→TST with threshold ≥ 5 mm. TST-10→TST with threshold ≥ 10 mm. TST-15→BCG. Independent definition of TST: ≥ 15 mm for BCG-vaccinated participant and ≥ 5 mm non-vaccinated participant.

Table 2: Incidences and rate ratios for individual tests
TB biomarkers

Correlate of TB infection vs. Natural immunity
20-25% of subjects exposed to *M. tuberculosis* become LTBI

a. Correlate of TB risk
5-10% of LTBI progress to Active TB

b. Correlate of TB disease

c. Correlate of Response to TB Treatment
3-5% of relapses after TB cure

Legend
LTBI
Active TB
Cured TB

Goletti et al, Respirology 2018
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Experimental assays to predict active TB development

Correlate of risk (COR)

- PET-CT scan
- HBHA response modulation (ELISA, citometry)
- Monocytes proportion in peripheral blood

- CD27 expression down modulation in IFN-γ Mtb-specific CD4 T cells (citometry)
- CD8-specific response
- HBHA response modulation (ELISA, citometry)
- IL-13 expression (gene expression)

From vaccine studies: HLA-DR, antibody response, IFN-γ production in response to in vitro stimulation to BCG
A blood RNA signature for tuberculosis disease risk: a prospective cohort study

Zak et al., Lancet, 2016

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Cross-validation performance of the tuberculosis risk signature in the ACS training set by days before tuberculosis diagnosis

<table>
<thead>
<tr>
<th></th>
<th>ROC AUC (95% CI)</th>
<th>Sensitivity (95% CI)</th>
<th>Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>By 6 month period</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-180</td>
<td>0.79 (0.75-0.82)</td>
<td>71.2% (66.6-75.2)</td>
<td>61%</td>
</tr>
<tr>
<td>181-360</td>
<td>0.771 (0.75-0.79)</td>
<td>62.9% (59.0-66.4)</td>
<td>61%</td>
</tr>
<tr>
<td>361-540</td>
<td>0.726 (0.70-0.76)</td>
<td>47.7% (42.9-52.5)</td>
<td>61%</td>
</tr>
<tr>
<td>541-720</td>
<td>0.540 (0.49-0.59)</td>
<td>29.1% (23.1-35.9)</td>
<td>61%</td>
</tr>
<tr>
<td>&gt;720</td>
<td>0.496 (0.43-0.56)</td>
<td>54% (2.4-13.0)</td>
<td>61%</td>
</tr>
<tr>
<td><strong>By 12 month period</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-360</td>
<td>0.779 (0.76-0.80)</td>
<td>66.1% (63.2-68.9)</td>
<td>61%</td>
</tr>
<tr>
<td>360-720</td>
<td>0.547 (0.62-0.673)</td>
<td>37.5% (33.9-41.2)</td>
<td>61%</td>
</tr>
<tr>
<td><strong>Total time period</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.743 (0.73-0.76)</td>
<td>58.4% (56.1-60.7)</td>
<td>61%</td>
</tr>
</tbody>
</table>

Sensitivity values are reported at a specificity of 80.0% (95% CI 78.6-81.4). ROC AUC: area under receiver operating characteristic curve. ACS: adolescent cohort study.

**Table 1**: Cross-validation performance of the tuberculosis risk signature in the ACS training set by days before tuberculosis diagnosis.
Positive Predictive Value according to Sens/Spec for risk of progression

- **Optimum TPP**
  - PPV: ~16%

- **16-gene transcriptomic COR**
  - PPV: ~7%

- **Minimum TPP**
  - PPV: ~6%

- **TST / IGRA**
  - PPV: ~2% / ~3%

**cumulative incidence**: 2%

**IPT effectiveness**: 50%

Petruccioli et al, ERJ 2016
Number Needed to Test & Treat according to Sens/Spec for risk of progression

- Optimum TPP - NNT: ~13
- 16-gene transcriptomic COR - NNT: ~37
- Minimum TPP - NNT: ~40
- TST / IGRA - NNT: ~250 / ~85

cumulative incidence: 2%
IPT effectiveness: 50%

NNTT captures clinician/PH perspective (If treating all test+, how many do I need to test and treat to prevent one case?)

Petruccioli et al, ERJ 2016
Blood transcriptional signatures for incipient TB

Gupta et al, BioRxiv, 2019
Diagnostic accuracy of eight best performing transcriptional signatures for incipient tuberculosis (TB) shown in receiver operating characteristic space, stratified by months to disease.

Based on pre-test probability of 2%, the 8 signatures achieved PPV ranging from 6.8-9.4% over 24 months, rising to 11.1-14.3% over 3 months.
The sensitivity of all eight signatures declined with increasing disease-free time interval. Using a threshold derived from two standard deviations above the mean of uninfected controls giving specificities of >90%, the eight signatures achieved sensitivities ranging 24.7-39.9% over a 24 month interval, rising to 47.1-81.0% over 3 months.

Based on pre-test probability of 2%, the eight signatures achieved positive predictive value ranging from 6.8-9.4% over 24 months, rising to 11.1-14.3% over 3 months.

When using biomarker thresholds maximising sensitivity and specificity with equal weighting to both, no signature met the minimum World Health Organization (WHO) Target Product Profile parameters for incipient TB biomarkers over a two-year period.

Blood transcriptional biomarkers reflect short-term risk of TB and only exceed WHO benchmarks if applied to 3-6 month intervals.
Characterization of progressive HIV-associated tuberculosis using 2-deoxy-2-[\(^{18}\)F]fluoro-d-glucose positron emission and computed tomography: a tool to identify sub-clinical TB?

Esmail et al., Nature Medicine, 2016
Elevated proportion of peripheral monocytes plus an elevated TST are potential biomarkers for identifying contacts of TB patients at highest risk of developing active TB.
Loss of response to HBHA is associated to active TB development in HIV-uninfected subjects

Corbière et al, PloS One 2012
Among the QFT-IT+, HIV-infected subjects, is the lack of HBHA in vitro response predictive of active TB development?

<table>
<thead>
<tr>
<th>HIV-LTBI (QFT-IT+)</th>
<th>HBHA-</th>
<th>HBHA+</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 yr follow-up for active TB development</td>
<td>14% (2/14)</td>
<td>0% (0/6)</td>
</tr>
</tbody>
</table>

2 LTBI diagnosis at enrollment:
1 start INH and ART, stop INH after 1 month, active TB development after 4 months of ART start
1 start INH after years of ART. He finished 6 months INH. After 1 year he developed active TB

Delogu et al, Scan J of Immunol, 2016
Correlate of TB disease
Quantification of circulating Mycobacterium tuberculosis antigen peptides allows rapid diagnosis of active disease and treatment monitoring.

Liu et al, PNAS, 2017
Trascripts associated to different TB stages in HIV-infected subjects
Complement Component C1q as Serum Biomarker to Detect Active Tuberculosis

Lubbers et al, Front Immunol, 2018

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Complement Component C1q as Serum Biomarker to Detect Active Tuberculosis

Lubbers et al, Front Immunol, 2018
TB biomarkers: correlates of response to therapy

b. Correlate of TB disease

c. Correlate of Response to TB Treatment

3-5% of relapses after TB cure

Goletti et al, Respirology, 2018
TABLE 1 Definitions of cured tuberculosis (TB), recurrent TB, re-infection and relapse

**Cured TB**
Smear- or culture-negative sputum specimens in the last month of treatment and on at least one previous occasion, according to WHO guidelines.

**Recurrent TB disease**
Refers to a repeat occurrence of TB disease in a patient that occurs as a result of either relapse or re-infection. Recurrent TB occurs after the previous/initial episode has been classified as clinically cured according to WHO guidelines.

**Re-infection**
Recurrent TB disease may occur as a result of re-infection, whereby a patient is exogenously infected with a *Mycobacterium tuberculosis* strain that is either the same or distinct from the organism that caused the original infection.

**Relapse**
Defined as a second (or third) episode of active TB disease due to re-emergence of the original infection, as determined by genotypic analysis of the prevailing tubercle bacilli.

![Graph showing bacterial burden and culture detection threshold over time with infection/disease status categories: Latent infection, TB disease, TB relapse.](image)

Goletti et al, ERJ, 2018
Available tests to evaluate TB cure

<table>
<thead>
<tr>
<th>Test</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microbiology</strong></td>
<td></td>
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<tr>
<td>Microscopy</td>
<td>S</td>
</tr>
<tr>
<td>Culture</td>
<td>S</td>
</tr>
<tr>
<td>Molecular test</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Immunology</strong></td>
<td></td>
</tr>
<tr>
<td>Immune cell counts</td>
<td>R</td>
</tr>
<tr>
<td>Immune cell profiles</td>
<td>R</td>
</tr>
<tr>
<td>CD38/HLA-DR/CD47 expression of M. tuberculosis-specific T-cells</td>
<td>R</td>
</tr>
<tr>
<td>M-MDSC</td>
<td>R</td>
</tr>
<tr>
<td>Levels of inflammatory molecules (IP-10; CRP; β2-microglobulin; a seven-molecule signature)</td>
<td>R</td>
</tr>
<tr>
<td>T-cell response</td>
<td>R</td>
</tr>
<tr>
<td><strong>Radiology</strong></td>
<td></td>
</tr>
<tr>
<td>Radiography</td>
<td>S</td>
</tr>
<tr>
<td>CT scan</td>
<td>S</td>
</tr>
<tr>
<td>PET/CT scan</td>
<td>R</td>
</tr>
</tbody>
</table>

CT: computed tomography; PET: positron emission tomography; AFB: acid-fast bacilli; S: standard; R: research; M-MDSC: monocyctic myeloid-derived suppressor cells; IP: interferon-γ induced protein; CRP: C-reactive protein; IGRA: interferon-γ release assay; HBHA: heparin-binding haemagglutinin; ESAT: early-secreted antigenic target; CFP: culture filtrate protein.

Goletti et al, ERJ, 2018
Longitudinal monitoring of the frequencies of CD38 in Mtb-specific CD4+ T cells is a useful biomarker for TB cure

CD8+ T-cell frequency decreases in active TB patients after TB-specific therapy
Bifunctional IFN-γ and TNF-α CD4 cells responding to RD1 proteins and an effector memory phenotype associate with active TB

HIV-uninfected

**Cytokine Response**

- Active TB
- Cured TB
- LTBI

**Phenotype**

- N
- E
- CM
- EM

HIV-infected

**Cytokine Response**

- Active TB
- LTBI

**Phenotype**

- EM
- CM

Petruccioli and Petrone et al, J Infection 2013

Chiacchio and Petruccioli et al, J Infection 2014
Persisting positron emission tomography lesion activity and *M. tuberculosis* mRNA after tuberculosis cure

From Anthony Fauci: we need to “reimagine” our research response to TB and bring TB research into the 21st century (Moscow, 2017)

AS Fauci outlined how we might “reimagine” our research response to TB and bring TB research into the twenty first century with the application of new diagnostic, therapeutic, and vaccine platforms. The current situation with TB research contrasts dramatically with the unprecedented advances in HIV/AIDS research made in the > 36 years since HIV was first reported.

<table>
<thead>
<tr>
<th></th>
<th>HIV</th>
<th>TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td>HIV-kit available, rapid and accurate, in low income countries included</td>
<td>Need to be improved</td>
</tr>
<tr>
<td>Therapy</td>
<td>30 drugs approved</td>
<td>Less than 10</td>
</tr>
<tr>
<td>Biomarkers for TB treatment monitoring, cure and relapse</td>
<td>Available</td>
<td>Partly available</td>
</tr>
</tbody>
</table>
Thank you!

Grazie!
Thank you

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