Biomarkers for tuberculosis

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National Institute for Infectious Diseases L. Spallanzani, Italy
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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

Active tuberculosis

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

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
Integrity of the granuloma is crucial for TB control
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.

Lin et al, Plos pathogen, 2016
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
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>M. <em>tuberculosis</em></th>
<th>Active TB disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Latent TB infection (past or recent exposure to M. <em>tuberculosis</em>)</td>
<td></td>
</tr>
<tr>
<td>NTM</td>
<td>Exposure to environmental mycobacteria</td>
<td></td>
</tr>
<tr>
<td>BCG-vaccination</td>
<td>BCG-vaccination</td>
<td></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>$M. \textit{tuberculosis}$</th>
<th><strong>Active TB disease</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative IGRA</td>
<td>NTM</td>
<td>Latent TB infection (past or recent exposure to $M. \textit{tuberculosis}$)</td>
</tr>
<tr>
<td></td>
<td>BCG-vaccination</td>
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</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>

Proportions

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

<table>
<thead>
<tr>
<th></th>
<th>Cattamanci</th>
<th>MercaSur</th>
<th>Chan</th>
<th>(Ref. 110)</th>
<th>(Ref. 111)</th>
<th>(Ref. 112)</th>
<th>Current SR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
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<tr>
<td>High-burden TB settings:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>- QFT-GIT</td>
<td>61%</td>
<td>65%</td>
<td>N.D.</td>
<td>69%</td>
<td>(42-75)</td>
<td>(52-77)</td>
<td>(53-94)</td>
</tr>
<tr>
<td>- T SPOT-TB</td>
<td>72%</td>
<td>68%</td>
<td>N.D.</td>
<td>65%</td>
<td>(62-89)</td>
<td>(55-80)</td>
<td>(54-74)</td>
</tr>
<tr>
<td>Low-burden TB settings:</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- QFT-GIT</td>
<td>67%</td>
<td>N.D.</td>
<td>N.D.</td>
<td>59%</td>
<td>(42-82)</td>
<td>N.D.</td>
<td>(46-71)</td>
</tr>
<tr>
<td>- T SPOT-TB</td>
<td>94%</td>
<td>(62-100)</td>
<td>N.D.</td>
<td>60%</td>
<td>(64-97)</td>
<td>(56-100)</td>
<td>(47-98)</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td></td>
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<td>(62-71)</td>
<td>(54-74)</td>
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</tr>
<tr>
<td>- T SPOT-TB</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>63%</td>
<td>(60-71)</td>
<td>(56-74)</td>
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</tr>
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<td></td>
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<td>- QFT-GIT</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>61%</td>
<td>(55-63)</td>
<td>(54-61)</td>
<td>(54-61)</td>
</tr>
<tr>
<td>- T SPOT-TB</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>63%</td>
<td>(58-58)</td>
<td>(54-58)</td>
<td>(54-58)</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
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<td>N.D.</td>
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<td>(62-71)</td>
<td>(54-74)</td>
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</tr>
<tr>
<td>- T SPOT-TB</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>63%</td>
<td>(58-64)</td>
<td>(53-63)</td>
<td>(53-63)</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

Day et al, J Immunol 2011
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 N (%)</th>
<th>TB2 N (%)</th>
<th>(N)</th>
<th>CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB1-responders</td>
<td>19 (83)</td>
<td>21 (91)</td>
<td>ACTIVE TB (23)</td>
<td>4 (17)</td>
</tr>
<tr>
<td>TB1-responders</td>
<td>17 (94)</td>
<td>15 (83)</td>
<td>LTBI REMOTE (18)</td>
<td>3 (17)</td>
</tr>
<tr>
<td>TB1-responders</td>
<td>12 (100)</td>
<td>11 (92)</td>
<td>LTBI RECENT (12)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>TB2-responders</td>
<td>48 (90.5)</td>
<td>47 (89)</td>
<td>TOTAL (53)</td>
<td>11 (21)</td>
</tr>
</tbody>
</table>

CD8 response by cytometry
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 of QFT-Plus-TB1 in patients with Active TB

Random effects:
Pooled proportion = 0.86 (95% CI = 0.76 to 0.94)
I² = 87.1% (95% CI = 68.8% to 92.7%)

Sotgiu et al, J Infection 2019
Sensitivity of QFT-Plus-TB2 in patients with Active TB

Random effects:
Pooled proportion = 0.9 (95% CI = 0.83 to 0.96)
I² = 80.1% (95% CI = 37.5% to 89.8%)

Barcellini L. 2016 0.85 (0.77, 0.91)

Petruccioli E. 2016 0.85 (0.66, 0.96)

Petruccioli E. 2017 0.90 (0.80, 0.96)

Takasaki J. 2017 0.99 (0.95, 1.00)

Telisinghe L. 2017 0.89 (0.81, 0.94)

combined 0.90 (0.83, 0.96)
Specificity of QFT-Plus in healthy subjects

Fixed effects:
Pooled proportion = **0.97** (95% CI = 0.96 to 0.98)
I² = 0% (95% CI = 0% to 58.5%)

- Moon HW. 2017: 0.97 (0.95-0.98)
- Barcellini L. 2016: 0.97 (0.92-0.99)
- Hofland R.W. 2018: 1.00 (0.83-1.00)
- Hoffmann H. 2016: 0.96 (0.88-0.99)
- Lina Yi 2016: 0.98 (0.95-0.99)
- Petruccioli E. 2017: 1.00 (0.82-1.00)
- Takasaki Jin 2017: 0.98 (0.93-1.00)
- Combined: 0.97 (0.96-0.98)
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
IFN-γ response to the methilated HBHA of *M. tuberculosis* produced in *M. smegmatis* is significantly reduced in patients with active tuberculosis

Delogu et al, PloS One 2011
Among HIV-infected subjects, IFN-γ response to HBHA is mainly mediated by CD8+ T cells

Chiacchio et al, PloS One, 2017
Response to HBHA is mainly monofunctional

Chiacchio et al, PloS One, 2017
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.

HBHA-based IGRAs TO MONITOR TB THERAPY

QFT          HBHA

IFN-γ (IU/ml)
0.0 0.1 0.2 0.3 0.35
0.30
0.2 0.3 0.4 0.5 0.6
0.65
0.7 0.8 0.9 1.0
1.05

pre therapy  post therapy

LTBI

Active TB
IFN-γ response to HBHA in children with active TB before and after successful therapy
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

China

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

CD45RA  +  +  −  −  +  +
CD45RO  −  −  +  +  −  −
CCR7    +  +  +  −  −  −
CD62L   +  +  +  −  −  −
CD28    +  +  +  +/- −  −
CD27    +  +  +  +/- −  −
IL-7Rα  +  +  +  +/- −  −
CXCR3   −  +  +  −  −  −
CD95    −  +  +  −  +  +
CD11a   −  +  +  +  +  +
IL-2Rβ  −  +  +  +  +  +
CD58    −  +  +  +  +  +
CD57    −  −  −  −  +  +

Stemness  Cytotoxicity
Proliferative potential  Tissue tropism
Lymphoid homing  Antigen addiction
Antigen independence  Glycolytic metabolism
Lipid metabolism  Oxidative stress
Low Δψm

Gattinoni et al, Nature Med. 2017
CD27 modulation: a potential new biomarker for TB?

![Graph showing CD27 MFI ratio for TB, cured TB, LTBI, and LTBI+ cured TB patients.

RATIO MFI = \( \frac{\text{MFI CD27 gate of CD4}^+ \text{ T cells}}{\text{MFI CD27 gate of CD4}^+\text{IFN}^+ \gamma \text{ T cells}} \)

Petruccioli et al, J Infection 2015
CD27 modulation within the CD45RA- cells helps to discriminate among the different TB stages

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

HIV-uninfected

HIV-infected

Cytokine Response

Phenotype

Petruccioli and Petrone et al, J Infection 2013

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

\[ p = 0.185 \]

**Sensitivity**

**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^+

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

Safety and efficacy of the C-Tb skin test to diagnose *Mycobacterium tuberculosis* infection, compared with an interferon γ release assay and the tuberculin skin test: a phase 3, double-blind, randomised, controlled trial

Morten Ruhwald, Henrik Aggerbeck, Rafael Vázquez Gallardo, Søren T Hoff, José I Villate, Bettine Borregaard, José A Martínez, Ingrid Kromann, Antón Peras, Luis L Aníbarro, María Luíza de Souza-Galvão, Francisca Sánchez, José Ángel Rodrigo-Pendás, Antonio Nogueira-Julían, Xavier Martínez-Lacasa, María Victoria Tuñez, Virgínia Leiro Fernández, Joan P Millet, Antonio Moreno, Nazaret Cebas, José M Miró, Llanos Roldán, Angelís Oroa, Peter Andersen, Joan A Caylà, the TESEC Working Group

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

Ruhwald et al, Lancet Respiratory Medicine, 2017
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>10 (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>

Ruhwald et al, Lancet Respiratory Medicine, 2017
C-Tb skin test: accuracy results. Similar PPV of the QFT-IT

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

Ruhwald et al, Lancet Respiratory Medicine, 2017
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
Abubakar et al, Lancet ID, 2018

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Progressed</th>
<th>Did not progress</th>
<th>Person-years at risk</th>
<th>Annual incidence per 1000 person-years (95% CI)</th>
<th>IRR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>QuantIFERON-TB Gold + Tube</strong></td>
<td></td>
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<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>20/14926 (0.6%)</td>
<td>4906/14926 (99.4%)</td>
<td>15921.6</td>
<td>1.9 (1.3-2.7)</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>
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</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>16645.3</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>
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<td></td>
<td></td>
</tr>
<tr>
<td>Test positive</td>
<td>64/957 (2.2%)</td>
<td>2882/957 (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>11154.6</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>58/2151 (2.7%)</td>
<td>2093/2151 (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>13749.2</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>1432/1485 (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>15896.6</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>
<td>..</td>
</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: TST with threshold 15 mm; BCG=vaccinated participant; 5mm = 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 \textit{M. tuberculosis} become LTBI

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

b. Correlate of TB disease

Cured TB

Legend

LTBI
Active TB
Cured TB

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

Goletti et al, Respirology 2018
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 at al, Lancet, 2016
Cross-validation performance of the tuberculosis risk signature in the ACS training set by days before tuberculosis diagnosis

<table>
<thead>
<tr>
<th>Time Period</th>
<th>ROC AUC (95% CI)</th>
<th>Sensitivity (95% CI)</th>
<th>Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>By 6 month period</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>54.0% (4.2-13.0)</td>
<td>61%</td>
</tr>
<tr>
<td>By 12 month period</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>Total time period</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

<table>
<thead>
<tr>
<th>Signature</th>
<th>Original no. of genes</th>
<th>Model</th>
<th>Discovery population</th>
<th>Discovery HIV status</th>
<th>Discovery setting</th>
<th>Discovery approach</th>
<th>Intended application</th>
<th>Discovery TB cases</th>
<th>Discovery non-TB controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andersen36TT</td>
<td>42</td>
<td>Disease risk score*</td>
<td>Children</td>
<td>HIV positive and negative</td>
<td>South Africa, Malawi</td>
<td>Elastic net using genome-wide data</td>
<td>TB vs LTBI</td>
<td>87</td>
<td>40</td>
</tr>
<tr>
<td>BATF2</td>
<td>1</td>
<td>N/A</td>
<td>Adults</td>
<td>HIV negative</td>
<td>UK</td>
<td>SVM using genome-wide data</td>
<td>TB vs healthy (acute vs convalescent samples)</td>
<td>46</td>
<td>31</td>
</tr>
<tr>
<td>Gjoen7TT</td>
<td>7</td>
<td>LASSO regression*</td>
<td>Children</td>
<td>HIV negative</td>
<td>India</td>
<td>LASSO using 159 pre-selected genes</td>
<td>TB vs healthy controls and other diseases</td>
<td>47</td>
<td>36</td>
</tr>
<tr>
<td>Giddens9TT</td>
<td>3</td>
<td>Disease risk score*</td>
<td>Adults</td>
<td>HIV positive and negative</td>
<td>South Africa, Malawi</td>
<td>Forward Selection-Partial Least Squares using genome-wide data</td>
<td>TB vs LTBI</td>
<td>293 (TB + non-TB)</td>
<td>69</td>
</tr>
<tr>
<td>Huang11TT</td>
<td>13</td>
<td>SVM (linear kernel)</td>
<td>Adults</td>
<td>HIV negative</td>
<td>UK</td>
<td>Common genes from elastic net, L1/2 and LASSO models, using genome-wide data</td>
<td>TB vs healthy controls and other diseases</td>
<td>10</td>
<td>69</td>
</tr>
<tr>
<td>Kaforou21TT</td>
<td>27</td>
<td>Disease risk score*</td>
<td>Adults</td>
<td>HIV positive and negative</td>
<td>South Africa, Malawi</td>
<td>Elastic net using genome-wide data</td>
<td>TB vs LTBI</td>
<td>285 (TB + non-TB)</td>
<td>69</td>
</tr>
<tr>
<td>Maertzdorf14TT</td>
<td>4</td>
<td>Random forest*</td>
<td>Adults</td>
<td>HIV negative</td>
<td>India</td>
<td>Random forest using 860 selected target genes</td>
<td>TB vs healthy</td>
<td>118</td>
<td>76</td>
</tr>
<tr>
<td>NPC31TT</td>
<td>1</td>
<td>N/A</td>
<td>Adults</td>
<td>Not stated</td>
<td>Brazil</td>
<td>Differential expression using genome-wide data</td>
<td>TB vs healthy</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td>Glenn17TT</td>
<td>17</td>
<td>Sum of standardised expression</td>
<td>Adults</td>
<td>HIV negative</td>
<td>UK</td>
<td>Differential expression of nuclear factor, erythroid 2-like 2-mediated genes</td>
<td>TB vs healthy controls and other diseases</td>
<td>10</td>
<td>69</td>
</tr>
<tr>
<td>Rajen5TT</td>
<td>5</td>
<td>Unassigned sum*</td>
<td>Adults</td>
<td>HIV positive</td>
<td>Uganda</td>
<td>Differential expression using genome-wide data</td>
<td>TB vs healthy (case finding among RIFU)</td>
<td>80 total (1:2 cases:controls)</td>
<td>46</td>
</tr>
<tr>
<td>Rae3TT</td>
<td>3</td>
<td>SVM (linear kernel)</td>
<td>Adults</td>
<td>HIV negative</td>
<td>UK</td>
<td>Stability selection, using genome-wide data</td>
<td>Incipient TB vs healthy</td>
<td>46</td>
<td>31</td>
</tr>
<tr>
<td>Singhania20TT</td>
<td>20</td>
<td>'Modified' disease risk score*</td>
<td>Adults</td>
<td>HIV negative</td>
<td>UK, South Africa</td>
<td>Random forest using modular approach</td>
<td>TB vs healthy controls and other diseases</td>
<td>Discovery set not explicitly stated</td>
<td>46</td>
</tr>
<tr>
<td>Sulimani2TT</td>
<td>2</td>
<td>ANKR0222 - GOBP1 (0)</td>
<td>Adults</td>
<td>HIV positive</td>
<td>Gambia, South Africa</td>
<td>Pair ratios algorithm using genome-wide data</td>
<td>Incipient TB vs healthy</td>
<td>79</td>
<td>328</td>
</tr>
<tr>
<td>Sulimani15TT</td>
<td>4</td>
<td>(GADD45α + NFKB) - (CDK1 + DLK)</td>
<td>Adults</td>
<td>HIV positive</td>
<td>Gambia, South Africa</td>
<td>Pair ratios algorithm using genome-wide data</td>
<td>Incipient TB vs healthy</td>
<td>45</td>
<td>141</td>
</tr>
<tr>
<td>Sweeney45TT</td>
<td>8</td>
<td>(GBP5 + DUSP9) / 2 = KIF2</td>
<td>Adults</td>
<td>HIV positive and negative</td>
<td>Meta-analysis</td>
<td>Significance thresholding and forward search in genome-wide data</td>
<td>TB vs healthy controls and other diseases</td>
<td>266</td>
<td>081</td>
</tr>
<tr>
<td>Walter45TT</td>
<td>51</td>
<td>SVM (linear kernel)</td>
<td>Adults</td>
<td>HIV negative</td>
<td>USA</td>
<td>Support vector machines, using genome-wide data</td>
<td>TB vs LTBI</td>
<td>24</td>
<td>24</td>
</tr>
</tbody>
</table>

Gupta et al, BioRvix, 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.
Concise whole blood transcriptional signatures for incipient tuberculosis: A systematic review and patient-level pooled meta-analysis

- 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

Rakotosamimanana et al, ERJ 2015

<table>
<thead>
<tr>
<th>TABLE 3 Parameters analysed to predict risk of progression to active tuberculosis in household contacts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>----------------------------------------</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Lymphocytes</td>
</tr>
<tr>
<td>TST ≥14 mm</td>
</tr>
<tr>
<td>Monocytes ≥7.5%</td>
</tr>
<tr>
<td>Monocyte/lymphocyte ratio</td>
</tr>
<tr>
<td>Monocytes ≥7.5% + TST ≥14 mm</td>
</tr>
</tbody>
</table>

n=274. HR: hazard ratio; AIC: Akaike information criterion per Cox model; TST: tuberculin skin test. *: statistically significant; †: adjusted for age, sex and TST ≥14 mm; ‡: adjusted for age, sex and lymphocyte count.
Loss of response to HBHA is associated to active TB development in HIV-uninfected subjects
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></td>
<td>14%</td>
<td>0%</td>
</tr>
<tr>
<td>3 yr follow-up for active TB development</td>
<td>(2/14)</td>
<td>(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
Correlate of TB disease

b. Correlate of TB disease
Quantification of circulating Mycobacterium tuberculosis antigen peptides allows rapid diagnosis of active disease and treatment monitoring.
Trascripts associated to different TB stages in HIV-infected subjects

Esmail et al, PNAS 2018
Complement Component C1q as Serum Biomarker to Detect Active Tuberculosis

Lubbers et al, Front Immunol, 2018
Complement Component C1q as Serum Biomarker to Detect Active Tuberculosis

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

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.

---

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>
</tr>
<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>DNA detection (PCR; GeneXpert MTB/RIF test)</td>
<td>R</td>
</tr>
<tr>
<td>RNA detection (isocitrate lyase mRNA; <em>M. tuberculosis</em> rRNA; sets of mRNA signatures)</td>
<td>R</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/Ki67 expression of <em>M. tuberculosis</em>-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: monocytic 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.
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

Day et al, J Immunol 2011
Bifunctional IFN-γ and TNF-α CD4 cells responding to RD1 proteins and an effector memory phenotype associate with active TB

Cytokine Response

Phenotype

HIV-uninfected

HIV-infected

Active TB     cured TB     LTBI

Active TB     LTBI

EM

CM

E. Petruccioli
L. Petrone

T. Chiacchio
E. Petruccioli

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)

Anthony S. Fauci and Robert W. Eisinger, Am J Trop Med, 2018

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

Translational Research Unit
Teresa Chiacchio
Gilda Cuzzi
Linda Petrone
Elisa Petruccioli
Valentina Vanini
Tonino Alonzi

INMI
Enrico Girardi
Fabrizio Palmieri
Andrea Antinori
Vincenzo Schininà
Giuseppe Ippolito