Assays to predict active tuberculosis development from latent tuberculosis infection

Delia Goletti

Translational Research Unit, INMI, Rome, Italy

Cape Town, South Africa, December 3rd, 2015
LTBI
Predictive value of TST and IGRA for incident active tuberculosis in adults

Zellweger et al, AJRCCM 2015

Rangaka et al, TLID 2011
This talk...

Assays to predict active TB development
Assays to predict active TB development

- 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)
- Monocytes proportion in peripheral blood
Monitoring CD27 Expression to Evaluate Tuberculosis development

before HIV

after HIV

TB

Schuetz et al, PloS One 2011
CD27 expression on *M. tuberculosis*-specific CD4^+^ T cells

- Low CD27 expression on circulating Mtb-specific CD4 T-cells associates with active TB disease (Streitz, 2007; Nikitina, 2012)

- At the site of TB disease a down modulation of CD27 is found (Nemeth, 2012; Nikitina, 2012)
Assessment of the novel T-cell activation marker–TB assay for the diagnosis of active TB in children: a prospective proof-of-concept study

**MEDIAN FLUORESCENCE INTENSITY (MFI):**
is a measure of the expression profile of a particular parameter. MFI is used to monitor the expression of a marker

**CD27 MFI of CD4+ T-cells**

**CD27 MFI of CD4+IFNγ+ T-cells**

*Modified, Portevin et al, Lancet Infectious Diseases 2014*
High CD27 MFI ratio associates with active TB only for specific Mtb-recall antigen responses

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

**Cytokine Response**

<table>
<thead>
<tr>
<th></th>
<th>HIV-uninfected</th>
<th>HIV-infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNg</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TNFα</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Phenotype**

<table>
<thead>
<tr>
<th></th>
<th>HIV-uninfected</th>
<th>HIV-infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Petruccioli and Petrone et al, J Infection 2013

Chiacchio and Petruccioli et al, J Infection 2014
Does combination of tests increase the diagnostic accuracy to distinguish active TB from LTBI?

Screening of patients with suspicious active TB

Bifunctional IFN-$\gamma$ and TNF-$\alpha$ CD4$^+$ T cells

Effector memory phenotype

CD27 MFI ratio

Petruccioli et al, in preparation
Assays to predict active TB development

- 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)
- Monocytes proportion in peripheral blood
CD8-T cell response is associated with a recent exposure to TB and active TB disease.
CD8-mediated T cell response in TB

Growing evidence support a role for CD8 T cells in the host defence against Mtb through cytokine secretion and cytotoxic activity (Turner, 1996; Brookes, 2003; Stenger, 1998)

Studies have found TB-specific CD8+ T cells producing IFN-γ to be:

- More frequently detected in active TB disease vs. LTBI (Day, 2011; Rozot, 2013)
- Associated with a recent exposure to TB (Nikolova 2013)
- Detectable in active TB subjects with HIV co-infection and young children (Chiacchio, 2014; Lancioni, 2011)
- Declining when patients are exposed to anti-tuberculosis treatment (Nyendak, 2014)
QuantiFERON TB Gold Plus

- Nil
- TB1
  (TB Antigen tube of the QuantiFERON TB GOLD In tube)
- TB2
  (additional peptides in which there are epitopes recognized by the CD8)
- Mitogen
QFT-Gold In tube vs QFT-Plus

QuantiFERON® Gold In tube

- ESAT-6 polypeptides
- CFP-10 polypeptides
- TB7.7 polypeptides
  - Long peptides (MHC-class II)

Stimulated cell population

- Primarily CD4+ lymphocytes

Peptide length

Improved Product Performance

QFT Plus

- ESAT-6 polypeptides
- Long peptides (MHC class II)
- CFP-10 polypeptides
  - Long peptides (MHC class II)
  - TB7.7 polypeptides
  - Additional 6 short peptides (MHC class I)

- CD4+ lymphocytes
- CD8+ lymphocytes

Increased sensitivity

Application in CD4-low populations
  - HIV+ subjects

Additional clinical information
  - Research tool
QFT-Gold In tube vs QFT-Plus

QuantiFERON® Gold In tube

Blood collection

3 tube test, lithium heparin option

QFT Plus

4 tube test, lithium heparin option

Interpretation of results

<table>
<thead>
<tr>
<th>Test value</th>
<th>IU/mL</th>
<th>QFT result</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB-Nil</td>
<td>≥0.35</td>
<td>Positive</td>
</tr>
<tr>
<td>TB1-Nil</td>
<td>≥0.35</td>
<td>Positive</td>
</tr>
<tr>
<td>TB2-Nil</td>
<td>≥0.35</td>
<td>Positive</td>
</tr>
<tr>
<td>Both TB1- &amp; TB2-Nil</td>
<td>≥0.35</td>
<td>Positive</td>
</tr>
</tbody>
</table>
QFT-Plus multicenter study

- Cirillo DM, Borroni E, Barcellini L. Emerging Bacterial Pathogen Unit Ospedale San Raffaele, Milano, IT
- Ruffo Codecasa L, A.O Niguarda, Milano, IT
- Tadolini M, Ospedale Sant’Orsola, Bologna, IT
- Goletti D, INMI Lazzaro Spallanzani, Roma, IT
- Brunetti E, IRCCS San Matteo, Pavia, IT
- Brown J, Free Royal Hospital, London, UK
## Accuracy of IGRA and TST in adults

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity for active TB</th>
<th>Specificity for active TB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Percentage</strong></td>
<td></td>
<td></td>
</tr>
<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*
### Sensitivity of QFT-Plus for active TB detection

<table>
<thead>
<tr>
<th>Active TB patients</th>
<th>Negative</th>
<th>Positive</th>
<th>Indeterminate</th>
<th>Sensitivity (excluding indeterminate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB1</td>
<td>20</td>
<td>96</td>
<td>3</td>
<td>83%</td>
</tr>
<tr>
<td>TB2</td>
<td>15</td>
<td>101</td>
<td>3</td>
<td>87%</td>
</tr>
<tr>
<td>QFT-Plus</td>
<td>14</td>
<td>119</td>
<td>3</td>
<td>88%</td>
</tr>
</tbody>
</table>

Barcellini et al, in preparation
## Specificity of QFT-Plus for active TB detection

<table>
<thead>
<tr>
<th></th>
<th>Negative</th>
<th>Positive</th>
<th>Indeterminate</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB1</td>
<td>104</td>
<td>2</td>
<td>0</td>
<td>98%</td>
</tr>
<tr>
<td>TB2</td>
<td>104</td>
<td>1</td>
<td>1</td>
<td>98%</td>
</tr>
<tr>
<td>QFT-Plus</td>
<td>103</td>
<td>3</td>
<td>0</td>
<td>97%</td>
</tr>
</tbody>
</table>

*Barcellini et al, in preparation*
### Active TB (Total patients 73) N (%)

<table>
<thead>
<tr>
<th></th>
<th>Negative</th>
<th>Positive</th>
<th>Indeterminate</th>
</tr>
</thead>
<tbody>
<tr>
<td>QFT-GIT</td>
<td>12 (16)</td>
<td>59 (81)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>QFT-Plus</td>
<td>8 (11)</td>
<td>62 (85)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>TB1</td>
<td>11 (15)</td>
<td>59 (81)</td>
<td></td>
</tr>
<tr>
<td>TB2</td>
<td>8 (8)</td>
<td>62 (85)</td>
<td></td>
</tr>
</tbody>
</table>

- 4 patients were scored positive only to the QFT-Plus, 3 of them scored positive at the TB2 only.
- The head-to-head comparison of the QFT-GIT and the QFT-Plus demonstrated agreement in 68/73 (93%) results.
Assays to predict active TB development

- 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)
- Monocytes proportion in peripheral blood
Background

- Heparin-binding hemagglutinin (HBHA) of the MTB complex is contained in the cell wall.

- IFN-γ response to HBHA has been associated to LTBI. It may be produced in *M. bovis* or in *M. smegmatis*.

- Histidine methylation is needed. rHBHAs has a similar pattern of methylation as that observed in nHBHA, as assessed by mass spectrometry analysis.
Loss of response to HBHA is associated to active TB development in HIV-uninfected subjects

Risk Stratification of Latent Tuberculosis Defined by Combined Interferon Gamma Release Assays

Véronique Corbière¹, Gaelle Pottier¹, Florence Bonkain², Kinda Schepers³, Virginie Verscheure¹, Sophie Lecher⁴.5.6.7, T. Mark Doherty⁸, Camille Locht⁴.5.6.7, Françoise Mascart⁴.⁹

Corbière et al, PloS One 2012
Assays to predict active TB development

- 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)
- Monocytes proportion in peripheral blood
IL-13 and AIRE differential gene expression pattern prior to TB diagnosis in cases versus controls (HIV-infected cohort)

AIRE: APECED, autoimmune regulator

Sloot et al, EBioMedicine, 2015
IL13 expressing cases show signature of type I IFN signaling prior to diagnosis.

Sloot et al, EBioMedicine, 2015
An IFN-inducible signature associates with active tuberculosis

Barry et al., Cur Opin Immunol, 2013
Genome-wide expression profiling identifies type 1 INF-response pathways in Active TB

Ottenhoff et al, PloS One 2012
A prognostic Correlate of Risk (COR) discriminates TB cases from controls up to 18 months before diagnosis.

**COR Classifier (qRT-PCR)**
- 247 transcript pairs
- 47 PCR primers
- 16 genes

**Progressor +**
(TB Case)

**Non-Progressor -**
(Control)

Prognostic performance: 70% Sensitivity & 84% Specificity for incident TB disease occurring within 1 year of sampling.

Diagnostic performance: 87% Sensitivity & 97% Specificity for prevalent TB disease at time of sampling

(applied to published datasets; HIV- South African adults)

**Zak, Scriba, Penn-Nicholson, Hanekom, many others - submitted**
Assays to predict active TB development

- 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)
- Monocytes proportion in peripheral blood
Peripheral white blood cell subpopulation ratios vary according to TB clinical status

- Several studies indicate that circulating immune cells are activated and recruited to the Mtb-infected lungs to form the granuloma where the Mtb proliferation is controlled by an active interaction of lymphocytes and infected macrophages.

- It has been shown that peripheral white blood cell subpopulation ratios varied according to TB clinical status in a limited number of BCG-vaccinated individuals from an area with a high TB burden (Rakotosamimanana, 2013).

- Observations from an HIV-positive or HIV-exposed population in South Africa also suggest an association between the ratio of circulating immune cells and the risk of TB disease (Naranbhai, 2014).
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.

**TABLE 2 Descriptive paired analysis of cohorts with regard to the development of tuberculosis symptoms**

<table>
<thead>
<tr>
<th></th>
<th>sHC</th>
<th>hHC</th>
<th>p-value</th>
<th>Bonferroni correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects n</td>
<td>12</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes</td>
<td></td>
<td></td>
<td>0.39</td>
<td>NS</td>
</tr>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(cells ×10⁹ L⁻¹)</td>
<td>2.7 (1.2–5.9)</td>
<td>3.8 (0.8–10.2)</td>
<td>0.42</td>
<td>NS</td>
</tr>
<tr>
<td>Percentage</td>
<td>46.1 (13.0–100.0)</td>
<td>47.0 (12.8–100.0)</td>
<td>0.72</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(cells ×10⁹ L⁻¹)</td>
<td>2.2 (1.5–7.4)</td>
<td>2.85 (1.3–8.4)</td>
<td>0.02*</td>
<td>0.14</td>
</tr>
<tr>
<td>Percentage</td>
<td>37.6 (18.5–77.5)</td>
<td>37.8 (18.5–74.2)</td>
<td>0.47</td>
<td>NS</td>
</tr>
<tr>
<td>Monocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(cells ×10⁹ L⁻¹)</td>
<td>0.68 (0.38–1.04)</td>
<td>0.47 (0.19–0.86)</td>
<td>0.04*</td>
<td>0.28</td>
</tr>
<tr>
<td>Percentage</td>
<td>8.5 (6.4–16.5)</td>
<td>6.1 (2.7–12.5)</td>
<td>&lt;0.001*</td>
<td>&lt;0.01*</td>
</tr>
</tbody>
</table>

Data are presented as median (range), unless otherwise stated. sHC: symptomatic household contacts; hHC: healthy household contacts; NS: nonsignificant. *: statistically significant values.
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.

**TABLE 3** Parameters analysed to predict risk of progression to active tuberculosis in household contacts

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Crude HR (95% CI)</th>
<th>p-value</th>
<th>Adjusted HR (95% CI)</th>
<th>p-value</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.97 (0.92–1.02)</td>
<td>0.23</td>
<td>0.98 (1.02–1.03)</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.99 (0.94–1.04)</td>
<td>0.66</td>
<td>0.97 (0.53–1.79)</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>TST ≥14 mm</td>
<td>5.5 (1.2–25.3)</td>
<td>0.03*</td>
<td>5.72 (1.22–26.80)</td>
<td>0.03*</td>
<td></td>
</tr>
<tr>
<td>Monocytes ≥7.5%</td>
<td>6.08 (1.61–22.91)</td>
<td>&lt;0.01*</td>
<td>6.25 (1.63–23.95)</td>
<td>&lt;0.01*</td>
<td>108.73</td>
</tr>
<tr>
<td>Monocyte/lymphocyte ratio</td>
<td>4.49 (1.19–16.94)</td>
<td></td>
<td>4.97 (1.30–18.99)</td>
<td>0.02*</td>
<td>113.39</td>
</tr>
<tr>
<td>Monocytes ≥7.5% + TST ≥14 mm</td>
<td>8.78 (1.82–42.32)</td>
<td>&lt;0.01*</td>
<td>8.46 (1.73–41.22)</td>
<td>&lt;0.01*</td>
<td>80.81</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.
Assays to predict active TB development

- 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)
- Monocytes proportion in peripheral blood
My thanks and conclusion...

A lot to do still.......