The Use of IGRAs in Children: View From the Laboratory

Beate Heym
Hôpital Ambroise Paré
Université de Versailles-Saint Quentin en Yvelines
France

Tuberculosis in children

Epidemiology
• no accurate figures available
• about 1 million new cases per year ?
• < 5% of all tuberculosis cases in high income countries ?
• 20 – 30% of all tuberculosis cases in low income countries ?
Tuberculosis in children - particular features

• latent infection and active disease less clear cut
• higher risk of progression to disease
• higher risk of extrapulmonary disease
• mostly smear negative (< 20% positive)
• often culture negative (30-50% positive)

Immunity in children - I

reduced microbial killing of the alveolar macrophages

▼ monocyte recruitment to the site of infection
▼ antigen presentation by dendritic cells in the lung
▼ antigen response of naïve T-cells
▼ ability of antigen presenting cells to synthesize Interleukin 12
Immunity in children - II

- functional immaturity of blood-derived dendritic cells
- functional immaturity of CD4 cells to express TH1-effector function -> IFN-\( \gamma \) response limited

Why are IGRAs done in children?

- Diagnosis of latent tuberculosis infection
- Diagnosis of active tuberculosis
- Monitoring of treatment of active tuberculosis
Which tests can we use in children?

- Quantiferon-Gold® IT
- T-Spot.TB® (Tspot)
- Tuberculin skin test (TST)

<table>
<thead>
<tr>
<th>TST</th>
<th>vs</th>
<th>IGRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell types</td>
<td>CD4, CD8</td>
<td>CD4, CD8</td>
</tr>
<tr>
<td>Cytokines</td>
<td>IL-4, IFN-γ</td>
<td>IFN-γ</td>
</tr>
<tr>
<td>Timing</td>
<td>2-5 days</td>
<td>24 h</td>
</tr>
</tbody>
</table>
TST in France

From the fifties until 2007:
BCG vaccination mandatory before entry into kindergarten

🎀 all persons older than 4 years have a positive TST (> 10 mm)

What do these tests measure?

Quantiferon-Gold® IT : whole blood ELISA, Interferon-\(\gamma\) (IFN-\(\gamma\)) concentration after 24h incubation with antigens ESAT-6, CFP10 and TB7.7 of M. tuberculosis

T-Spot.TB® : Ex-vivo enzyme-linked immunoassay, estimates the number of T-cells producing IFN-\(\gamma\) after exposure to antigens ESAT-6 and CFP10 of M. tuberculosis.
Specimen and processing requirements

Quantifieron-Gold® IT: 3 ml of blood, samples must be processed within 12 h of collection.

T-Spot.TB®: 8 ml of blood for children older than 10 years, 4 ml for children between 2 and 10 years, and 2 ml in children younger than 2 years. Samples must be processed within 8 h of collection.

Specimen

3 ml of blood for the QFT-Gold® IT test.

8 ml of blood for children older than 10 years,
4 ml for children between 2 and 10 years,
2 ml in children younger than 2 years for the T-Spot.TB® test

⚠️ It may be difficult to get these volumes, especially in young children.
Interprétation of results: Quantiferon-Gold® IT

**Positive**: measured IFN-\(\gamma\) > 0.35 IU/ml and > 25% above the control.

**Negative**: measured IFN-\(\gamma\) < 0.35 IU/ml.

**Indeterminate**: measured IFN-\(\gamma\) < 0.35 IU/ml and mitogen response < 0.5 IU/ml or measured IFN-\(\gamma\) of ≥ 0.35 IU/ml but < 25% of the nil value or any value of IFN-\(\gamma\) and nil value > 8 IU/ml.

Quantiferon-Gold® IT: indeterminate results I

About 15% indeterminate results

1) mitogen control low or negative
   - too low T cell count
   - too low CD4 count

No correlation with total leucocyte or lymphocyte count in children.

Try the T-spot.TB® test?
Quantiferon-Gold® IT: indeterminate results II

2) negative control too high (high background)
   - virus infection
   - infection by parasites (eosinophils ↑)

Retest after 14 to 21 days.

Interpretation of results: T-Spot.TB®

Results are expressed as number of spot-forming T-cells.

Positive: Panel A-Nil and/or Panel B-Nil > 8 spots.
Negative: Panel A-Nil and Panel B-Nil < 4 spots.
Borderline: Highest of Panel A or Panel B spot count (minus nil) is 5, 6 or 7.
Invalid: if mitogen control < 20 spots
   if Nil > 10 spots
T-Spot.TB®: invalid results

- mitogen control < 20 spots
  - too low T cell count
  - too low CD4 count

- if Nil control > 10 spots
  - virus infection
  - infection by parasites

T-Spot.TB®: inconclusive results

- 2.5 x 10^5 mononuclear cells per well are necessary for the T-spot.TB test

- Normal white blood cell count in children:
  - 4000 - 10000 cells per μl

- Lymphocytes
  - 25 - 30%

- Lymphocytes per ml
  - 1 x 10^6
Which test to use in children?

Quantiferon-Gold® IT:
Less sensitive than T-Spot.TB®
But
Sample less important (3 ml)
May be done the day after sampling
Less training of the technicians
No need of supplementary equipment
Large series are possible
Possibility of automatisation

Which test to use in children?

T-Spot.TB®:
must be done the same day (without T-cell Xtend®)
training of technicians necessary
very time-consuming (< 10 test per day)
needs at least a stereoscope for counting of the spots (immunospot analyzer)
no possibility of automatisation
Costs (in France)

QFT-Gold IT®: one test ~ 30 €
  > 12 tests per series 20 €
T-spot.TB®: one test ~ 50 €
  no series possible
Reimbursement per IGRA test:
  42 €

When should we test children?

When you have the suspicion of latent or active tuberculosis in children who had contact with a person with active tuberculosis.
When should we test children?

To diagnose latent or active tuberculosis in immigrant children from high endemic countries.

To diagnose latent or active tuberculosis in children who are HIV positive or otherwise immunosuppressed.
What about the monitoring of anti-tuberculosis treatment?

129 children: between 2 months and 17 years; 91% BCG+
98 patients 12 healthy contacts
54 LTBI (contact + TST)
32 active TB (clinical signs + positive treatment response)

31 controls

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HC</th>
<th>LTBI</th>
<th>TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>T=0</td>
<td>30%</td>
<td>9%</td>
<td>58%</td>
<td>78%</td>
</tr>
<tr>
<td>T=10d</td>
<td>nd</td>
<td>14%</td>
<td>67%</td>
<td>86%</td>
</tr>
</tbody>
</table>

Starting the treatment lowers the bacterial replication, T cells are less sequestered at the site of infection?

Conclusion

- Very few data are available on IGRAs in children, particularly in children < 5 years.
- Increased frequency of indeterminate assays in children. The reasons for this should be studied in more detail.
- Required blood volumes.
- IGRAs should be done in particular situations like contact investigation, immigrant children and immunosuppressed children.
Herrmann J.L. et al., 2009, Plos One 4: 4130.
Lancioni C. et al., 2011, Am J Respir Crit Care Med. Oct 27. [Epub ahead of print]

Thank you for your attention