diagnostic blood tests

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Abstract
The interferon-gamma-release assays were developed to overcome the pitfalls and logistic difficulties of the tuberculin skin test (TST) for the diagnosis of latent tuberculosis infection (LTBI). These blood tests measure the in vitro production of interferon-gamma by sensitized lymphocytes in response to Mycobacterium tuberculosis-specific antigens. Two interferon-gamma-release assays are registered for use in Canada: the QuantiFERON-TB Gold In-Tube assay (Cellestis Inc, Australia) and the T.SPOT–TB test (Oxford Immunotec, United Kingdom). Evaluation of these tests has been hampered by the lack of a gold standard for LTBI, and limited paediatric data on their use. It appears that they are more specific than the TST, and may be useful for evaluating TST-positive patients at low risk of true LTBI. Moreover, they may add sensitivity if used in addition to the TST in immunocompromised patients, very young children and close contacts of infectious adults. A summary of these tests, their limitations and their application to clinical paediatric practice are described.

Key Words: Diagnosis; Interferon-gamma-release assay; Paediatrics; Tuberculosis

For centuries, paediatric tuberculosis (TB) has been a challenge for physicians to diagnose and treat [1-3]. Unlike TB in adults, paediatric TB often presents with nonspecific signs and symptoms. Paediatric TB is usually paucibacillary, and culture confirmation is difficult and not always possible [4-6]. At the same time, the diagnosis of latent TB infection (LTBI), while extremely important in paediatrics, can be challenging due to the limitations in the sensitivity and specificity of the only available screening test to date – the tuberculin skin test (TST) [7]. The test is supported by longitudinal data showing a much higher risk of TB disease in TST-positive individuals. The latter individuals experience a beneficial effect of isoniazid prophylaxis [8]. Current recommendations advocate targeted testing of children at high risk for TB infection or progression of LTBI to TB disease (Refer to Table 1 for testing recommendations) [9]. However, the TST has poor sensitivity (leading to false negatives) in very young children, infants younger than three months of age and immunocompromised patients [10,11]. It can be influenced by many factors such as malnutrition, concurrent viral and parasitic infections, and concurrent medical conditions and diseases [11,12]. It is also known to have poor sensitivity in active and/or disseminated TB infection [13]. The TST also suffers from poor specificity (leading to false-positive tests) in certain uninfected individuals who have been previously vaccinated with Bacillus Calmette-Guérin (BCG) or infected with environmental nontuberculous mycobacteria (NTM). The test is further hampered by poor standardization, inter- and intra-observer variability, and the need for a return visit for interpretation.

TABLE 1
Targeted tuberculosis (TB) screening: Indications for the tuberculin skin test in children

<table>
<thead>
<tr>
<th>Indications</th>
<th>Screening Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contacts of known cases of active TB</td>
<td>TST</td>
</tr>
<tr>
<td>Children with suspected active TB disease</td>
<td>TST</td>
</tr>
<tr>
<td>Children with known risk factors for progression of infection to disease</td>
<td>TST</td>
</tr>
<tr>
<td>Children travelling or residing for three months or longer in an area with a high incidence of TB, especially if the visit is anticipated to involve contact with the local population</td>
<td>TST</td>
</tr>
<tr>
<td>Children who arrived in Canada from countries with a high TB incidence within the previous two years</td>
<td>TST</td>
</tr>
</tbody>
</table>

Adapted from reference [9]
Advances in TB diagnosis: the interferon-gamma-release assays

These tests measure the in vitro production of interferon-gamma by sensitized lymphocytes in response to *Mycobacterium tuberculosis*-specific antigens. The genes encoding these antigens are present in *M tuberculosis*, but are not found in any BCG strain or in several of environmental NTM strains [12]-[14]. Therefore, these tests are substantially more specific (leading to fewer false positives) than the TST. They are also less subjective with respect to interpretation, have the potential for rapid turnaround time, and require only a single visit to complete the testing process.

Two commercial interferon-gamma-release assays (IGRAs) using these *M tuberculosis*-specific antigens are now currently registered for use in Canada – the QuantiFERON-TB Gold In-Tube assay (QFT-G-IT; Cellestis Inc, Australia) and the T.SPOT–TB test (Oxford Immunotec, United Kingdom). Brief descriptions of the tests are presented in Table 2. They are not yet widely available at most centres or reference laboratories in Canada. Both tests are also approved by the United States Food and Drug Administration [15][16]. The ELISpot, while similar to the T.SPOT, is an in-house assay not available for commercial use, although it is frequently referenced in studies of the IGRAs.
Role of the IGRAs in children: when should they be used?

While the IGRAs have been well studied in adults and reviewed elsewhere [17], data regarding their use in children are much more limited. Current recommendations for their use in children are best understood in the context of this limited available information. In summary, in low TB incidence settings, there is general support that the tests are more specific and correlate better with gradients of exposure to infectious source cases than the TST [18-21]. However, evaluation of this is hampered by the lack of a gold standard test for the diagnosis of LTBI, and the lack of longitudinal data to validate the predictive value of the IGRAs compared with the TST. Overall agreement between the TST and the IGRA in the diagnosis of LTBI in children appears to be between 55% and 95% [18-20,22-23], and varies depending on age and history of previous BCG vaccination [24]. The majority of discordant results are TST+/IGRA−, and there is concern that the IGRAs may not be as sensitive as the TST for the diagnosis of LTBI in very young and immunocompromised children, and whether the initial infection was remote [24,25]. The 2009 Amer-
Academy of Pediatrics Red Book recommendations on TB states that “IGRAs cannot be recommend-
ed routinely for use in children younger than 5 years of age or for immunocompromised children of any age be-
cause of a lack of published data about their utility with these groups[29].” While there are limited data to sug-
gest that the IGRAs, especially the T.SPOT, may have increased sensitivity over the TST in immunocompro-
mised populations, this again has not been well studied in children and is based on a limited number of adult 
studies[11]. However, the T.SPOT is more difficult to perform, more expensive and requires larger volumes of 
blood than the QFT-G-IT.

There is also variable sensitivity reported in children with active tuberculosis disease, ranging from 50% to 
92% for QFT-G-IT, 81% to 93% for ELISpot, and 40% to 83% for T.SPOT–TB[18,26,27,29]. The wide range in 
reported sensitivity appears to reflect the different performances of these tests across different ages and in 
different settings (endemic versus nonendemic). In very young children with TB, the test may have limited sensi-
tivity, but there are notable cases on record in which the TST is negative and the IGRA is positive[30]. The combi-
nation of the two tests may, therefore, increase sensitivity for the diagnosis of TB, both latent and active, 
in situations in which the TST may be unreliable such as in active and or disseminated TB disease, and in la-
etent disease of very young infants and immunocompro-
mised patients.

Acknowledging these limitations, recommendations on IGRAs for latent TB infection were made by the Cana-
dian Tuberculosis Committee in 2007 and updated in 2008 and 2010[30,31]. Members of the Infectious Dis-
eseas and Immunization Committee of the Canadian Paediatric Society reviewed and agreed with the 2008 
updated guidelines, which include specific recommenda-
dations for the use of the IGRAs for children, as de-
scribed below.

**Canadian TB committee recommendations on the use of IGRAs for children:**

This can be especially useful in the school outbreak setting, in which the population to be screened for con-
tact tracing may be low risk, and yet have false-positive TSTs from BCG or NTM. However, for close contacts of 
the index case, or those contacts who have high or in-
creased risk of progression to active disease if infected, 
a TST (or both TST and IGRA) should be used eight to 
de weeks from the most recent exposure and, if either

is positive, the contact should be considered to have 
LTBI. If both TST and IGRA testing are used, blood 
should be drawn for IGRA on or before the day when 
the TST is read.

This recommendation allows the physician to order an 
IGRA when she/he suspects a false-positive TST result 
in a child or adolescent who has a low risk probability 
of LTBI and no risk factors for progression to active dis-
ease. For example, this may apply to low-risk children 
for whom a TST is performed for school or volunteer 
requirements. In these instances, the IGRA may be use-
ful to confirm diagnosis of LTBI for treatment purposes. 
Any decision not to offer chemoprophylaxis on the ba-
sis of a negative IGRA must be made in consultation 
with a TB specialist.

Immigrant children who should be targeted for LTBI 
screening include those younger than 15 years of age 
who have lived in a country with high TB incidence and 
have immigrated within the past two years, and children 
with risk factors for progression to disease, as outlined 
in Table 3.

In an immunocompromised child, the TST should still 
be the initial test used to detect LTBI. If the TST is posi-
tive, the child should be considered to have LTBI. How-
ever, given the known problem with false-negative TST 
results in immunocompromised populations, a physi-
cian still concerned about the possibility of LTBI in an 
immunocompromised child with a negative initial TST 
result may perform an IGRA test.

- The IGRAs may be used as a supplementary diag-
nostic aid in combination with the TST to help sup-
port the diagnosis of active TB.

In the absence of a positive TST or culture confirma-
tion, a positive IGRA could potentially support the 
diagnosis of TB based on typical clinical, radiologi-
cal or other laboratory findings. However, the IGRA 
must never be used in isolation to make a diagno-
sis of TB disease. Every effort must still be made to 
obtain a microbiological confirmation of active TB. A 
negative IGRA test (or TST) does not rule out active 
TB at all ages, but especially in young children.

- The IGRAs may be used in the setting of contact 
investigation to confirm a positive TST in contacts 
who, on the basis of an assessment of the duration 
and degree of contact with an active infectious case, 
are believed to have a low pretest probability of re-
cently acquired LTBI, and who have no other high or
increased risk factors for progression to active disease if infected.

- The IGRA may be performed in a TST-positive, immunocompetent child with relatively low risk of being infected with TB, and of progressing to active disease if infected. Persons with a positive IGRA result may be considered for treatment of LTBI.

- Routine or mass screening of all immigrant children for LTBI, with either TST or IGRA, is not recommended. However, targeted screening for LTBI after arrival in Canada is recommended for foreign-born children and travellers with risk factors for reactivation of LTBI.

- The IGRA can be used, in addition to the TST, to diagnose LTBI infection in an immunocompromised patient.

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### TABLE 3
**Risk factors for the development of active tuberculosis (TB) among persons infected with *Mycobacterium tuberculosis***

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Estimated risk of TB relative to persons with no known risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High risk</strong></td>
<td></td>
</tr>
<tr>
<td>AIDS</td>
<td>110–170</td>
</tr>
<tr>
<td>HIV infection</td>
<td>50–110</td>
</tr>
<tr>
<td>Transplantation (related to immunosuppressive therapy)</td>
<td>20–74</td>
</tr>
<tr>
<td>Silicosis</td>
<td>30</td>
</tr>
<tr>
<td>Chronic renal failure requiring hemodialysis</td>
<td>10–15</td>
</tr>
<tr>
<td>Carcinoma of head and neck</td>
<td>16</td>
</tr>
<tr>
<td>Recent TB infection (two years or less)</td>
<td>15</td>
</tr>
<tr>
<td>Abnormal chest x-ray – fibronodular disease</td>
<td>16–19</td>
</tr>
<tr>
<td><strong>Increased risk</strong></td>
<td></td>
</tr>
<tr>
<td>Treatment with glucocorticoids</td>
<td>4.9</td>
</tr>
<tr>
<td>Tumour necrosis factor-alpha inhibitors</td>
<td>1.5–4</td>
</tr>
<tr>
<td>Diabetes mellitus (all types)</td>
<td>2.0–3.6</td>
</tr>
<tr>
<td>Underweight (90% or lower of ideal body weight; for most persons, this corresponds to a body mass index of 20 kg/m² or lower)</td>
<td>2–3</td>
</tr>
<tr>
<td>Young age when infected (zero to four years of age)</td>
<td>2.2–5.0</td>
</tr>
<tr>
<td>Cigarette smoker (one pack/day)</td>
<td>2–3</td>
</tr>
<tr>
<td>Abnormal chest x-ray – granuloma</td>
<td>2</td>
</tr>
<tr>
<td><strong>Low risk</strong></td>
<td></td>
</tr>
<tr>
<td>Infected person, no known risk factor, normal chest x-ray (low risk reactor)</td>
<td>1</td>
</tr>
</tbody>
</table>

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References


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