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The old and the new: Immune-based diagnosis of childhood tuberculosis

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RESOURCES

Is tuberculosis still a public health challenge in Canada?

Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis* and less commonly *Mycobacterium bovis*, remains an infection of tremendous clinical and public health importance in Canada. The diagnosis and management of tuberculosis in children, especially in children under 5 years of age, are often particularly complex. Changes in immigration patterns from tuberculosis endemic regions, an increase in immunocompromised children due to underlying disease, treatment, or transplants, and rapidly changing patterns of microbial resistance underline the importance of studying and understanding the epidemiology and clinical history of childhood tuberculosis in Canada.

Why is TB difficult to diagnose in children?

Both latent tuberculosis infection (LTBI) and tuberculosis (TB) disease can be difficult to diagnose in children, in large part because the disease is often paucibacillary and samples for microbiologic testing are difficult to obtain compared to adults. Clinicians have long used immune-based tests to assist in diagnosis. Here we review both the old and new immune-based TB tests available to clinicians.

What tests are available to clinicians to help diagnose TB infection?

Tuberculin skin test

The tuberculin skin test (TST) has been used for over a century to evaluate cell-mediated immunity against TB. The internationally recognized method of TST is the Mantoux test in which purified protein derivative (PPD) is injected intradermally into the inner surface of the forearm. PPD is an extract of over 200 antigens, some of which are common to *M tuberculosis*, *M bovis* and other environmental mycobacteria. Appropriate technique for the TST is essential for accurate results; step-by-step guidelines for injecting PPD and measuring the result are available in the Canadian Tuberculosis Standards.[1] Sensitization to the antigens in PPD occurs through previous

exposure to a Mycobacterium species included in the extract and results in a delayed hypersensitivity-type response that is visualized as an area of induration at the site of the PPD injection.

The result of the TST is read as mm of induration 48 to 72 hours after placement. The interpretation of the TST depends on four factors: 1) the degree of delayed hypersensitivity response, 2) the individual's risk of being infected with TB, 3) the positive predictive value of the test given the above two factors, and 4) the individual's risk factors for progression from TB infection to disease if infected. Current Canadian guidelines for interpretation of the TST are shown in the table on page 3. Practically speaking, the TST is considered positive if the size of the induration is greater than or equal to 5 mm in children who have been exposed to TB disease or have risk factors for progression to disease such as HIV infection. According to Canadian guidelines any induration ≥10 mm is considered a positive TST. American guidelines are slightly different, because they use a ≥15 mm induration cutoff if there are no risk factors for TB, and consider ≥10 mm a positive TST if the child has immigrated within the last five years from a high-prevalence country, is less than 4 years old, or has been exposed to high-risk adults.

The time to development of sensitivity following infection with *M tuberculosis* is generally three to six weeks, however, in rare cases, sensitivity may occur as long as eight weeks after initial infection.[2] Importantly, up to 10–20% of immune-competent children with proven TB disease may have a falsely negative test.[3] False-negative tests can also be caused by several factors, including the use of corticosteroids, recent viral infections (especially measles), recent live viral vaccines (again, especially measles), skin anergy (absence of immune response), young age (under 6 months), and incorrect administration of PPD.[4]

Sensitization to PPD tends to remain for life and may occur from previous infection with non-TB mycobacteria or previous receipt of the Bacille Calmette-Guérin (BCG) vaccine. However, a positive response to the TST following BCG in infants will generally wane quickly. More than 95% of children who receive BCG in the first year of life will lose reactivity by 10 years of age.[1] Older children and adults who receive BCG will maintain a positive TST response for a longer period; over 40% of older children and adults will continue to test positive after 10 years. Repeated administration of BCG can prolong the period of positive response to the TST. As BCG is most commonly given in areas with high rates of endemic TB, a history of BCG is generally ignored when administering and interpreting a TST.

Interferon-gamma release assays

Two major limitations to the TST include the need for a repeat visit to a qualified professional to read the test results and false-positive tests due to BCG or other nontuberculous mycobacteria (NTM). Immunologic-based testing measures the in vitro production of interferon-gamma from circulating T lymphocytes exposed to *M tuberculosis*-specific antigens that are absent from BCG and most NTM. Interferon-gamma release assays (IGRA) are an alternative to the TST for the diagnosis of LTBI. IGRA minimize the chance of false-positive results relative to the TST and have the potential of requiring only a single visit to a health care professional. Similar to the TST, IGRA testing cannot differentiate between latent and active disease.

There are two commercially available IGRA tests: the QuantiFERON-TB Gold test (QFT-G) (Cellestis Ltd., Carnegie, Australia) and the T-SPOT TB assay (T-SPOT) (Oxford Immunotec Ltd., Abingdon, UK). Evaluation of such tests is hampered by the lack of a gold standard for LTBI. Sensitivity and specificity are therefore based on performance in patients with suspected or proven TB disease. The sensitivity of IGRA testing in adults is similar to the TST but shows a higher degree of specificity.[5–12] In a recent meta-analysis,[11] the QFT-G had a pooled sensitivity of 79% (95% CI 75-82), the T-SPOT had a pooled sensitivity of 67% (95% CI 62–73) and the TST had a pooled sensitivity of 78% (95% CI 74–82). The specificity for IGRA tests was 97% (95% CI 96–98) for QFT-G and 98% (95% CI 96–99) for T-SPOT compared to 92% (95% CI 89–93) for TST.

Is one test better to use than the other, generally or in specific circumstances?

Diagnostic accuracy in children under 5 years of age is especially important, as diagnosis is notoriously difficult in this age group. Unfortunately, there is a lack of IGRA studies in this young age group. In a Canadian study of children, the QFT-G used in addition to the TST did not detect a significant number of extra cases of LTBI compared to TST alone. This study used close household contacts of smear positive, and thus highly infectious patients, and the agreement between the tests was good (kappa=0.67).[13] While the TST remains a good test for the detection of infection in high-risk contacts, many lower-risk contacts and those without contact history were TST-positive and QFT-negative. As well, poor correlation between tests was much more likely if patients had received BCG. In lower-risk groups, the use of an IGRA instead of a TST or as a rule-out test in TST-positive individuals would exclude many individuals from treatment of LTBI. A recent publication from Montreal suggested this is the preferred approach by clinicians and it accords with clinically important outcomes.[14] Longitudinal follow-up of TST-positive QFT-G-negative individuals who are not treated for LTBI will be needed to confirm that this is a safe approach.

What are the current Canadian recommendations for TST interpretation?

Canadian guidelines for interpreting a tuberculin skin test (TST) are as follows:

<table>
<tr><th>Factor</th><th colspan="2">Guideline</th></tr>
<tr><td>Time</td><td colspan="2">• Results should be read by a trained health professional 48 to 72 hours after administration.</td></tr>
<tr><td>Factors used to interpret a positive TST</td><td colspan="2">• Size of induration (palpable, raised, hardened area)
• Positive predictive value
• Risk of developing disease if person is truly infected</td></tr>
<tr><td rowspan="2">Size of induration</td><td>TST result</td><td>Situation in which reaction is considered positive</td></tr>
<tr><td>0–4 mm</td><td>• Generally considered negative and no treatment is indicated
• Child under 5 years of age and high risk of TB infection</td></tr>
</table>

Factor	Guideline	
	≥ 5 mm	• HIV infection • Contact with infectious TB case within the past 2 years • Presence of fibronodular disease on chest X-ray (healed TB and not previously treated) • Organ transplantation (related to immune-suppressant therapy) • TNF-alpha inhibitors • Other immunosuppressive drugs such as corticosteroids (equivalent of ≥15 mg/day of prednisone for 1 month or more; risk of TB disease increases with higher dose and longer duration) • End-stage renal disease
	≥ 10 mm	• Criterion of 10 mm has a sensitivity of 90% and specificity of >95% – recommended criterion for most clinical situations in Canada • All others, including the following specific situations: o TST conversions (within 2 years) o Diabetes, malnutrition (<90% ideal body weight), cigarette smoking, daily alcohol consumption (>3 drinks/day) o Silicosis o Hematologic malignancies (leukemia, lymphoma) and certain carcinomas (e.g., head and neck)
Positive predictive value	• Probability that a positive test result represents the true presence of TB infection.	
Risk of developing active TB	• Many medical conditions and treatment regimens can increase risk of reactivation: o HIV, diabetes, renal failure, malnutrition, certain cancers, alcohol overuse, and cigarette smoking	

Table adapted from reference 1.

Conclusion

TB continues to be an important disease in Canadian children. Clinicians should use available tests to screen children at high risk of infection, both to protect these children now and to avoid their becoming the next generation of adults with infectious TB.

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Quiz

1. Which of the following is true with reference to interferon-gamma release assays (IGRA) for tuberculosis?

a. May help differentiate between latent TB infection and active TB disease.
b. Use antigens found in BCG.
c. Are recommended for serial testing; e.g., for those who are to undergo annual testing.
d. May be more specific than the tuberculin skin test (TST) especially in BCG-immunized patients.
e. Is preferred in current Canadian Guidelines over the TST for those immunized with BCG at birth.

2. **A 9-month-old boy is tested for TB using a TST at a routine visit. He is Canadian born, has not travelled outside of Canada and has no known contact with tuberculosis. His test produces 8 mm of induration at 72 hours. Which of the following are true?**

   a. The patient should be treated with nine months of isoniazid (INH).
   b. The patient should have a chest X-ray as soon as possible to rule out TB disease.
   c. Routine tests for TB infection are not recommended as most positive tests are false-positive.
   d. The patient should be offered INH.
   e. The patient has a negative skin test.

3. **Which of the following is false in relation to the tuberculin skin test?**

   a. May be falsely negative after receipt of MMR vaccine.
   b. Is given subcutaneously.
   c. Should be administered intradermally.
   d. Should be read between 48 hours and 72 hours after administration.
   e. Should be read in mm of induration and reported as such.

4. **A 45-kg, 12-year-old child is due to undergo treatment with infliximab for refractory Crohns disease. He is on a weaning course of prednisone and is currently receiving 10 mg daily. Which of the following are true?**

   a. A TST should not be performed because it will be unreliable.
   b. An IGRA should not be performed because it will be unreliable.
   c. The patient should be tested with a TST as well as an IGRA if available and should be treated for latent TB infection if any test is positive and TB disease has been ruled out.
   d. Induration of 5 mm or greater should be regarded as a positive test for this child.
   e. False-negative results may be obtained because of the corticosteroid therapy for both the TST and IGRA.

5. **A 24-year-old woman is diagnosed with smear positive (1+) pulmonary TB. Her 2-year-old child receives a TST that produces 12 mm induration at 48 hours. Which of the following are true?**

   a. If the child has received BCG immunization, the skin test result should be attributed to the BCG.
   b. The child should be immediately treated with INH with an intended course of nine months.
   c. The child should undergo a chest X-ray and clinical evaluation to rule out TB disease.
   d. If there is no evidence of TB disease, the child should be treated with a course of treatment for latent infection based on the sensitivities of the mother's strain of *Mycobacterium tuberculosis.*

Answers: 1-d, 2-c,e, 3-b, 4-c,d,e, 5-c,d