Blood tests for TB—ready for prime time?

The standard test for latent tuberculous infection is the tuberculin skin test (TST) using purified protein derivative (PPD). It is among the oldest diagnostic tests in medicine, introduced in 1908 by Robert Koch, who erroneously thought tuberculin had therapeutic powers. Unfortunately, the TST has many limitations, including problems caused by errors related to administration and reading, false-positive reactions related to previous bacille Calmette-Guérin (BCG) vaccination, and cross-reactivity with environmental mycobacteria. False-negative reactions occur in the presence of immunosuppression or overwhelming disease, and there may be false-negative reactions in 15% to 20% of otherwise uncomplicated culture-proven cases.

There has been considerable interest for many decades in developing a blood test for tuberculous infection, but such tests lacked sufficient sensitivity and specificity to be useful clinically. Perhaps this is about to change.

In 2001, the FDA approved an in vitro diagnostic aid for detecting latent infection with *Mycobacterium tuberculosis*. The test, known as the QuantiFERON-TB test, is based on the quantification of interferon-gamma (IFN-γ) released from sensitized lymphocytes when whole blood containing peripheral blood monocytes was incubated overnight with tuberculin, an avian antigen, and controls using an enzyme-linked immunosorbent assay (ELISA). The results measure the amount of interferon-gamma released in response to tuberculin compared with the other antigens. The test has been modified since its introduction and the antigens now include the early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), encoded by genes located within the RD1 segment of the *M. tuberculosis* genome, proteins absent in both *M. avium* and the *Mycobacterium bovis* strain used in the preparation of BCG vaccine. This should considerably enhance its specificity. A competing test, the T SPOT-TB assay, which also relies on these antigens, is in use in Europe although it has not yet been approved by the FDA. It involves incubating peripheral blood mononuclear cells with antigens and measuring interferon-gamma-producing cells using an enzyme-linked immunospot (ELISPOT) assay. Neither test is currently approved in Canada.

What are the advantages of these tests? Only a single visit is required and the response to multiple antigens can be assessed simultaneously. They are less subject to observer bias and can eliminate the "boosting phenomenon"—the increase in the size of the reaction to PPD with repeated testing. As well, these tests may ensure that positive reactions resulting from BCG vaccination can be distinguished from true latent infection. The manufacturers claim that the test will detect infection despite immunosuppression, including HIV infection.

What are the disadvantages? Whole blood must be processed within 12 hours and the initial laboratory costs are higher. There is as yet limited clinical and laboratory experience with the tests and their ability to predict risk of developing TB remains unproven. Neither the QuantiFERON nor the T SPOT test measures the same component of the immunological response as the TST, and the tests may not be interchangeable.

The reported sensitivity of the tests in active tuberculosis ranges from 57% to 88%, which is inferior to the conventional tuberculin skin test sensitivity of 86% to 95%. The sensitivity of the TST exceeds that of the interferon-gamma assays in previously treated patients. However, the value of the blood tests may not lie in their use to diagnose active disease but rather to diagnose true latent TB infection. Studies in Europe have shown excellent specificity of 97% in low-risk, previously vaccinated patients.

There is no gold standard for latent tuberculosis infection—even the TST is not considered to provide this. Nevertheless, when the conventional skin test has been compared with the interferon-gamma tests in subjects whose probability of latent infection has been defined clinically, there is substantial discordance in both high- and low-risk populations. Discordance might be expected for PPD-positive and interferon-gamma-negative results explainable by previous BCG vaccination or sensitivity to atypical mycobacteria. More problematic is the documented discordance between interferon-gamma-positive but PPD-negative subjects. This raises the concern that interferon-gamma assays may actually increase the number of false-positive reactions in low-prevalence populations.

Two studies undertaken by the manufacturers showed high reproducibility. However, a multicentre study showed substantial variability among centres in the degree of discordance between TST and the interferon-gamma test. A large group of PPD-positive subjects undergoing serial serological testing showed unexplained conversion and reversions.

Ready for prime time? Not yet, but perhaps with refinement coming to a lab near you soon.

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