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Immunologic Diagnosis of Active Tuberculosis

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Clinically evident active pulmonary or extrapulmonary tuberculosis (TB) does not pose a significant diagnostic challenge, but the diagnosis of subclinical or atypical forms of TB is much more troublesome. More accurate tests that can specifically diagnose active TB have been much awaited. Interferon-γ (IFN-γ) release assays (IGRAs) are recently developed tests recommended for the diagnosis of latent TB, together with the tuberculin skin test (TST), which had for a long time been the only diagnostic tool for this condition [1]. Although the original intent of IGRAs was for the diagnosis of latent TB, the tests have recently become attractive as a possible option in the diagnosis of active TB, as well.

IGRAs measure the *in vitro* cellular immune responses to *Mycobacterium tuberculosis*-specific antigens, including early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), which are not present in any strain of *Mycobacterium bovis* BCG as well as in many nontuberculous mycobacteria. In contrast, the TST uses a nonspecific whole culture filtrate of tubercle bacilli containing over 200 antigens. This leads to its low specificity. Two types of IGRAs are now commercially available. The first is QuantiFERON-TB Gold (QFT-G; Cellestis), an assay that uses the patient's whole blood to measure the level of IFN-γ from the stimulated supernatant. A variant method, QuantiFERON-TB Gold In-Tube (QFT-GIT), uses tubes prefilled with antigens. The second type of IGRA, T-SPOT.*TB* (Oxford Immunotec), uses the enzyme-linked immunospot (ELISPOT) assay to measure the number of IFN-γ-secreting T cells on stimulation by *M. tuberculosis*-specific antigens. A fixed number of peripheral mononuclear cells is used in this assay. Both types of IGRAs have internal positive and negative controls to guard against technical errors. The failure of the controls in the tests, defined as indeterminate test results (ITRs), means that the reliability of results cannot be guaranteed. The incubation step in IGRAs selectively amplifies replication of effector memory T cells, since central memory T cells require a longer period of in vitro incubation than effector memory T cells. Central memory T cells are major components within the indurated skin produced by the TST. Therefore, IGRAs are more likely to be positive in persons recently infected with *M. tuberculosis* [2].

Assessing the accuracy of IGRAs in diagnosing *M. tuberculosis* infection is difficult due to the absence of a gold standard to confirm a diagnosis of latent TB infection or culture-negative active tuberculosis. Thus, the performance of the tests has been measured based on the epidemiology of TB or by using cases of active TB. Studies regarding household contacts with active TB showed that the pooled sensitivity of IGRAs for the prediction of active TB after exposure was 80 to 90%, the specificity 56 to 83%, the positive predictive value (PPV) 4 to 8%, and the negative predictive value (NPV) 99 to 100%. For the TST, the sensitivity was 90 to 100%, the specificity 29 to 39%, the PPV 2.7 to 3.1%,

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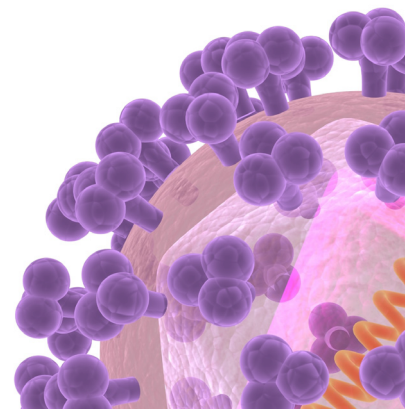
and the NPV 99 to 100% [3-6]. IGRAs seem to be more specific and less sensitive for prediction of the future development of active TB than the TST. One meta-analysis using cases of active TB estimated the pooled sensitivity to be 78% for QFT-G and 90% for T-SPOT.*TB* [7]. The pooled specificity for QFT was 99% among non-BCG-vaccinated participants and 96% among BCG-vaccinated participants. The pooled specificity of T-SPOT.*TB* was 93%. In comparison, the pooled sensitivity of the TST was 77%, and the specificity in non-BCG-vaccinated participants was consistently high, at 97%. The specificity of IGRAs was high and unaffected by BCG vaccination. TST specificity was high in non-BCG-vaccinated populations, but low and variable in BCG-vaccinated populations. The sensitivity of IGRAs and the TST was not consistent across tests and populations, but T-SPOT.*TB* appeared to be more sensitive than both QFT and the TST.

High prevalence of TB may lead to more false positives with the IGRAs, such that cases of latent TB infection could be interpreted as active TB. Moreover, the relatively high sensitivity of the tests suggests that IGRAs, especially T-SPOT.*TB*, are better at ruling out active TB than ruling it in. In this context, the magnitude of ITRs can further limit the utility of IGRAs in the diagnosis of active TB. The study by Lee and colleagues in this issue of *Infection & Chemotherapy* analyzed the level of ITRs in T-SPOT.*TB* tests from data of their previous studies regarding extrapulmonary TB, and tried to determine risk factors related to the ITRs of the tests [8]. They reported a rate of 8.7% of ITRs, but did not find any significant contributing factors. Positive results might help interpret the ITRs under specific conditions. Although the level of ITRs was relatively high, it surely added a valuable regional detail to the performance of T-SPOT.*TB* in South Korea having intermediate TB burden. The authors have conducted a series of studies about the utility of T-SPOT.*TB* in the diagnosis of active extrapulmonary TB, which encompassed meningitis, lymphadenitis, abdominal infections, osteoarticular infections, and miliary TB [8]. The tests produced a wide range of values according to the site of extrapulmonary TB; the sensitivity was 74-100%, the specificity was 46-67%, the positive predictive value was 36-98%, the negative predictive value was 25-100%, the positive likelihood ratio was 1.38-2.63, and the negative likelihood ratio was 0.19-0.56. The performance of IGRAs seems to be similar between pulmonary and extrapulmonary TB. A recent study from China, for example, showed that the sensitivities of T-SPOT.*TB* in pulmonary and extrapulmonary TB were 95.6% versus 93.3% and the specificities were 69.2% versus 88.9%, respectively [9].

Results of IGRAs vary widely in reported studies. Factors influencing the results include test interpretation criteria, preva-

lence of infection, proportion of microbiologically confirmed infections, estimates of recent and remote exposure, age, race, prior BCG vaccination, recent TST, and coexisting diseases. In addition, uncertainty exists regarding the reproducibility of IGRA results in individual patients and the clinical significance of fluctuations in measured IFN-γ responses. The fluctuations usually remain unexplained and nonspecific [1].

There has been significant progress in the research regarding the utility of IGRAs, but further studies determining the value and limitations of IGRAs in clinical care are needed. IGRAs possess many advantages over the conventional TST in the diagnosis of latent TB. However, they are not expected by themselves to play a critical role in the diagnosis of active TB, because the principle of the assays is based on immune responses of the host that are influenced by various factors and are not individually predictable. Rather, as an adjunctive they may be incorporated into a combination of optimal protocols which must be developed by future research.

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