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Tuberculin Skin Testing

GEORGE M. LORDI and LEE B. REICHMAN

Skin Testing

Tuberculin testing is the major method of diagnosing tuberculous infection. Although a significant degree of variability exists in reading reactions (1), the test continues to be the most reliable and available in diagnosis. It is used to diagnose tuberculous infection in contrast to tuberculous disease. Its reactivity separates the infected individual (class 2—significant skin test with negative bacteriologic and roentgenographic studies) from the exposed individual without infection (class 1—tuberculosis-exposed history with nonsignificant tuberculin skin test). The implication of a significant tuberculin test is infection with tubercle bacilli.

Infection with Mycobacterium tuberculosis results in sensitivity to antigens derived from that organism. Tuberculin is composed primarily of tuberculoprotein obtained from cultures of the tubercle bacillus. When the material is injected intracutaneously, a classic delayed hypersensitivity reaction occurs in the infected individual. The initial process of sensitization following infection takes about 6 to 8 weeks with sensitized T-lymphocytes developing in regional lymph nodes and entering the circulation. Restimulation of these lymphocytes by intracutaneous injection of tuberculin results in the indurated skin reaction of a significant test. The induration is due to cellular infiltration mediated by the sensitized lymphocytes. The reaction is maximal at 48 to 72 hours and then slowly fades, although it commonly lasts more than 96 hours. Almost all infected individuals will have a significant reaction.

Tuberculin Preparations

Two types of tuberculin preparations are in use, old tuberculin (OT) and purified protein derivative (PPD).

Old Tuberculin

Old tuberculin was first used and manufactured by Robert Koch in Germany in 1908. He originally used and touted it as therapeutic, although this classification was erroneous. It is made from heat-sterilized cultures of tubercle bacilli that are filtered and concentrated. Since it is a relatively unrefined product with extraneous material present, a significant reaction is not always diagnostic of infection. Currently OT is only available in multiple puncture tests.

Purified Protein Derivative

Purified protein derivative was originally developed by Florence Siebert in 1939 at the Phipps Institute in Philadelphia. It is a precipitate prepared from filtrates of OT with ammonium sulfate or trichloroacetic acid. The reference standard material for all tuberculins is PPD-S (Siebert's Lot 49608).

There are three dosage strengths of PPD available, 1 tuberculin unit (TU), 5 TU, and 250 TU. In 1972 the Bureau of Biologics of the Food and Drug Administration mandated that the standard test dose of all Tween containing PPD tuberculins licensed for use in humans be biologically equivalent to 5 TU of
PPD-S (2). No biologic standard is required for 1 TU and 250 TU preparations. Basically, these preparations are not useful in the diagnosis of tuberculous infection. The definition of tuberculous infection is: a significant reaction to 5 TU PPD (3). Tween-80 is added to the PPD diluent by the manufacturer to prevent material from being adsorbed by glass and plastic containers and syringes, thus preventing decreased potency of the preparation.

Purified protein derivative antigen is available in both multiple puncture tests and in the intracutaneous Mantoux test.

**Mantoux Test**

This test is performed by injecting 0.1 mL of PPD tuberculin (5 TU) into the skin of the volar or dorsal area of the forearm. The injection must be intracutaneous. A single-dose plastic syringe is used with a 26- to 27-gauge needle. The injection is done with the needle bevel upward. A wheal 6 to 10 mm in diameter should result. Proper dosage is important; the larger the dose, the larger the reaction. Weaker doses produce smaller reactions.

The test is read in 48 to 72 hours. Test significance depends on the presence or absence of induration. The presence of induration is determined by touch. The diameter of the induration is measured transversely. Erythema is not considered. The size of induration in millimeters, the antigen strength and lot number, the date of testing, and the date of reading are all recorded.

**Significance of Reactivity**

In the United States a reaction of 10 mm of induration of 5 TU of PPD with the Mantoux test after 48 hours is usually considered significant and indicative of infection with the tubercle bacillus. The 5-TU dose is used because of its specificity. But tuberculin is a biologic product, and *M tuberculosis* shares antigens with other nontuberculous mycobacteria, so the 5-TU is not completely specific. The use of large doses such as 250 TU of PPD would result in an increased number of nonspecific reactions.

The use of 10 mm of induration as a significant test is also a compromise. Figure 4.1 (4) shows a bimodal distribution of reactions to 5 TU PPD among Alaskan Eskimos. In this group a reaction size of 5 mm results in a clear separation between reactors and nonreactors. In Alaska, reactions above 5 mm also correlate well with the findings in individuals in this population group known to have tuberculous disease. There are no known cross-reacting mycobacteria in Alaska. So, for this population a reaction size greater than 5 mm of induration instead of 10 mm can be considered significant.

Figure 4.2 (5) shows the distribution of reactions to 5 TU PPD in Navy recruits from the state of...