The Tuberculin Test: Revisited

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ABSTRACT

Still in many of the developing countries tuberculin test is used as a common test to diagnose tuberculosis. Main purpose of this review is to assess the reliability, sensitivity, specificity, false positive and negative factors and other regional and ethnic variation. Based on the reviews of the major article published on tuberculin test it was observed that because of availability and because of the cost factor still tuberculin test can be used as an effective method to diagnose tuberculosis. But the same time the observer should be cautious about possible technical errors, chances of false positive or false negative results and other associated factors that can modify the interpretation.

KEY WORDS: Tuberculin, Mantoux, Tuberculosis, Booster phenomenon.

Introduction

Tuberculin skin testing still enjoys the privilege of being an important investigative tool for detecting M. Tuberculosis infection. Since its initial description, this simple immunodiagnostic tool has achieved significant clinical and epidemiological relevance and hence has stood the test of time.

Background

The origin of tuberculin dates back to 1890, when an intense search for therapy for M. Tuberculosis infection had led Robert Koch to develop a filtrate of heat - killed culture of tubercle bacillus known as ‘Koch's lymph’ or ‘Koch's remedy’ as a cure for tuberculosis. This reagent, later was called “old tuberculin. Epstein and Escherich identified the diagnostic potential of skin response to tuberculin. Bujvid, a Polish researcher renamed Koch’s remedy as ‘Tuberculin’ in 1891[1]. The extensive studies of the Austrian physician, Clemens Von Pirquet, showed that ‘dermal reactivity to a small quantity of tuberculin was indeed indicative of past infection by the tubercle bacillus’.

In 1910, Mantoux stressed the value of a negative tuberculin test response - “in contrast to most clinical methods, the value of the intracutaneous test lies in its negative results. A negative test except in measles, meningitis and miliary tuberculosis and advanced cases with marked toxemia is an argument of the first order in excluding clinical tuberculosis.. “.

In 1932, D’Arey Hart reported that giving a series of four or five graded doses of tuberculin was incorrect and a 1:10 dilution of old
Tuberculin (10g per 0.1ml) is to be used as the practical upper limit for tuberculin skin testing. In 1932, Florence Seibert and Munday, isolated a low molecular weight protein from the culture filtrate of tubercle bacilli, which was referred to as Trichloroacetic acid Precipitated Tuberculin. Though it had a lower molecular weight than the MA-100 protein, it was less antigenic. As a result of her continuing efforts, Florence Seibert, in 1934, extracted a new protein, termed “purified protein derivative”(PPD), also called as Synthetic medium Old Tuberculin Trichloroacetic acid (SOTT)[2]. Seibert and Glenn produced a large batch of PPD in 1939 for use as a standard for tuberculin preparation[3]. This material, lot number 49608, was designated as PPD-S and was sent to National Institute of Health, United States of America and to the State Serum Institute, Copenhagen, Denmark for use as a reference standard and was accepted in 1952 by WHO.

Rich and Lewis explained that tuberculin reaction is a generalized allergic response to M. Tuberculosis infection that produced the local response after tuberculin injection and circulating antibodies were not necessary. The present view regarding the Tuberculin reaction is that it is T lymphocyte mediated delayed type of hypersensitivity due to previous infection with M. Tuberculosis[4].

**Immunological Basis For Tuberculin Reaction**

Tuberculin reaction is a type IV hypersensitivity and develops 2 to 10 weeks after initial infection[5]. Subsequent to intracutaneous inoculation of tuberculin, the sensitized circulating T lymphocytes get activated at the local site and lead to a series of events, which culminates as the cutaneous reaction. It is characterized histologically by the initial accumulation of neutrophils (within 1–2 hours) with sequential replacement by monocytes and lymphocytes commencing by 12 hours and peaking by 48 hours. Characteristically, clinical reaction to tuberculin begins at 5 to 6 hours, is maximal at 48 to 72 hours, and subsides over a period of days[6].

**Tuberculin**

The PPD tuberculin is a more purified form, which gives less nonspecific reaction. The standard for all PPD preparations is Tuberculin PPD, lot number 49608, designated as PPD-S [7]. One IU for PPD is defined as the biological activity contained in 0.000028 mg of PPD-S, consisting of 0.000020 mg of PPD-S plus 0.000008 mg of salts. The standard is distributed as a lyophilized powder in ampoules containing 50000 IU each. 1 tuberculin unit (TU) is defined as 0.00002 mg of PPD-S.Tween 80 (polyoxyethylene sorbiton monoleate) 0.005%, a detergent is added as antiadsorbant to minimize the reduction in potency during storage. Tuberculin with Tween 80 is more potent than equal quantities of tuberculin without the detergent. There is, however, considerable variation in the potency ratios of these two reagents in different preparations, ranging from 3: 1 to 6: 1[8].

Adverse reaction to tuberculin is uncommon. Severe reactions to tuberculin like vesiculation or ulceration can occur in hypersensitive persons and do not need specific treatment. An anaphylactic or systemic reaction to any of the components of PPD or Tween 80 has not been reported.

**Morphology of Intracutaneous Tuberculin Reaction**

Tuberculin, when inoculated intradermally induces a palpable reaction in sensitized individuals, consisting of hardening of the region and spreading of the reaction. In some cases, necrosis, sloughing, ulceration and subsequent rapid and permanent healing without involvement of the local draining
lymph nodes follows. Recent clinical studies with PPD have shown morphologically different reactions occurring at specific intervals following intracutaneous injection of Tuberculin. These include an immediate wheal and flare reaction, an erythematous reaction peaking at 6 – 8 hour, a delayed reaction maximal at 24 hour and a further delayed reaction maximal at 48 – 72 hour and are possibly caused by species-specific mycobacterial antigens[9].

The qualitative difference of the delayed 72 hour Tuberculin reactions has been well described recently. There are two distinct types of tuberculin reaction – the turgid Koch type and the non-turgid Listeria type. The turgid reaction is purple colored, indurated, well demarcated and tender and is supposed to indicate delayed hypersensitivity. The non-turgid Listeria response is pink, soft, ill defined and non-tender and is postulated to be suggestive of protective immunity[10,11].

Apart from the above-mentioned reactions, certain late types of reactions have also been described. One such is “variant reactivity” defined as induration of less than 10mm at 72 hours that, when reassessed at 6 days, increases in size to 10 mm or greater. Variant reactivity has been described as a predictor of booster positivity[12]. Ramanathan et al have also described recently a late Mitsuda type of response to PPD[13].

**Administration and Reading of Mantoux Test**

In Mantoux test, 0.1 ml of 1 TU of PPD-RT 23 in Tween 80 is injected intradermally into either the volar or the dorsal surface of the forearm using a tuberculin syringe with 25-27 gauge needle. The area of the skin chosen must be free of lesions and away from veins. The tuberculin is injected along the longitudinal axis of the forearm just beneath the surface of the skin, with the needle bevel upward. When the test is performed correctly, a wheal, 6 to 10mm in diameter should be produced. The skin must not be swabbed with spirit or other antiseptic but should be washed and dried before the test is performed. If the injection is made too deep and no wheal appears, then the test should be immediately reapplied at a site at least 2 inches away.

Mantoux test is conventionally read between 48 and 72 hour after injection. The basis of reading is the size of induration, which may be determined by palpation or pen method [14,15]. The ballpoint pen technique is more reproducible when compared with the palpation Technique [16,17].

Though the conventional teaching is to read the Tuberculin at the end of 48 to 72 hours, a few studies have shown that tuberculin skin test could be read even at the end of 24 hours[18].

**Interpretation of The Tuberculin Test**

Interpretation of the Tuberculin test requires full appreciation of its idiosyncrasies. Categorization of the population as infected or non-infected is based on the central value of the valley in the bimodal graph. It is important to remember that the size of the induration depends on the amount of tuberculous protein injected, the availability of sensitized T-lymphocytes, local behavior of the skin and the number of actively multiplying tubercle bacilli in the body[19].

The criterion for a positive tuberculin test is taken as ≤ 5 mm for HIV infected and other immuno-suppressed patients and those with abnormal chest radiograph in a low prevalence region. In people from high prevalence countries, residents of prisons, missing homes, institutions and other locally identified high risk populations, patients with medical risk factors for tuberculosis such as silicosis,
diabetes, health care workers in high risk settings and users of intravenous street drugs, a cut of value of \( \leq 10 \) mm is employed to identify a positive test\[20,21\].

The major problem in the interpretation of tuberculin test is in misinterpreting a hypersensitivity reaction to mycobacteria other than \( \text{M. Tuberculosis} \) as a positive response. These reactions tend to be smaller than reactions caused by tuberculosis infection.

It is fundamental to recognize that a negative reaction to tuberculin alone does not exclude the diagnosis of tuberculosis. Failure to elicit a hypersensitivity reaction in tuberculin skin testing may be due to many reasons (Table 2). Of the various causes of false negative tuberculin reaction, one, which is very relevant to developing countries like India, is the effect of malnutrition on tuberculin reactivity. Malnutrition is a well documented, but poorly understood cause of cutaneous anergy\[22\] . The degree of suppression is proportional to the severity of the degree of malnutrition\[23-27\].

**Sensitivity and Specificity of Tuberculin Testing**

The sensitivity and specificity of the tuberculin skin test depends on the cut off value used. If a smaller reaction is defined as indicating infection, the sensitivity of the test is increased. For example, in a study done in Navy recruits in United States, the sensitivity decreased from 94.2% to 74.9% when the cutoff was increased from 10 mm to 14 mm\[28\] . In Indian population, only 60–70% of all children with tuberculosis show tuberculin positivity\[29\]. Mantoux test could be negative in a small percentage (5%) of sputum positive patients and in a large percentage (15 to 20%) of X-ray positive, sputum negative tuberculosis patients\[30\]. Nearly 20% of newly diagnosed sputum positive tuberculosis patients can have negative tuberculin test\[31,32\]. The specificity of the tuberculin test is also variable and depends on the prevalence of atypical mycobacterial infection. But studies have indicated that a significant tuberculin reaction in any population is highly indicative of infection with \( \text{M. Tuberculosis} \) rather than atypical mycobacteria.

**Utility of The Tuberculin Skin Test**

The utility of the tuberculin skin test depends on the prevalence of \( \text{M. Tuberculosis} \) infection and the relative prevalence of non-tuberculous mycobacteria. In a population where the prevalence of infection is low (5-10%), the positive predictive value of the tuberculin test would be low if a cutoff point of > 10mm is used to define a positive test. In contrast, in population where the prevalence of tuberculous infection is high, a cut off > 10mm is more likely to indicate infection with \( \text{M. Tuberculosis} \).

Mantoux test is useful both in the diagnosis of infection and disease due to \( \text{M. Tuberculosis} \). It can be especially valuable when repeated periodically in the surveillance of tuberculin negative persons likely to be exposed to tuberculosis. A ‘converter’ is defined as a person whose tuberculin reaction has increased by at least 6mm (to accommodate variability in reaction and measurement\[33\] ) from less than 10mm in diameter to 10 or more mm in diameter, within 24 months (the time period during which the newly infected has the greatest risk of developing disease\[34\] ). In general, there is a tendency for persons who have larger tuberculin reactions to be at greater risk of developing tuberculosis\[35\].

**Persons for whom tuberculin testing is indicated are given below:**

1. Persons with signs and/or symptoms suggestive of tuberculosis.
2. Recent contacts with known tuberculosis cases.
3. Persons with abnormal chest roentgenograms compatible with past tuberculosis.

4. Persons with medical conditions that increase the risk of tuberculosis (silicosis, gastrectomy, diabetes, primary or secondary acquired immunodeficiency states, etc).

5. Groups at high-risk recent infection with M. Tuberculosis, such as immigrants from endemic to nonendemic area, residents of mental institutions, prisons etc.

Tuberculin positivity is different in various countries, and even in the same country, there is sometimes a significant difference in the tuberculin positivity in different regions. In India, the overall prevalence of tuberculin reactivity is about 30% males: 35% and females 25% as shown in the survey conducted by the National Tuberculosis Institute, Bangalore in 1974[36].

**Controversies In Tuberculin Testing**

**BCG Vaccination and Tuberculin testing**

Prior BCG immunization can result in increased reactivity to tuberculin[37,38]. Less than 50% of infants given BCG develop a reactive tuberculin skin test at 9 to 12 month of age, the great majority will have a nonreactive skin test by 5 years of age[39]. BCG vaccination of older children and adults produce a greater percentage of reactive skin test that persists for a long duration, but by 10 to 15 years post vaccination, most individuals lose their tuberculin skin test reactivity[40]. Repeated tuberculin skin tests in a person sensitized previously by BCG vaccine or atypical mycobacterial infection may increase the reaction to subsequent tuberculin skin tests[41,42].

The probability that a positive tuberculin test reaction results from recent infection with M. Tuberculosis rather than from BCG vaccination increases (1) as the size of the reaction increases. (2) when the patient is a contact of a person with tuberculosis. (3) when the patient originates from a high prevalence zone for tuberculosis and (4) as the interval between vaccination and tuberculin testing increases[43]. As tuberculin sensitivity and immunity to tuberculous infection after BCG vaccination is highly variable, there is no reliable method of distinguishing tuberculin reactions caused by vaccination with BCG from those caused by natural mycobacterial infections. Hence all significant reactions in BCG–vaccinated persons are taken as indicative of infection with M. Tuberculosis[44], the reasons being (1) conversion rates after BCG administration is less than 100%, (2) the mean reaction size among vaccines is often less than 10 mm, and (3) tuberculin sensitivity tends to wane considerably a few years after administration.

**Booster Phenomenon**

An enhanced skin response is noted on repeat tuberculin testing at the site of a previous skin test, sometimes resulting in conversion from negative to positive reaction. This is called as booster phenomenon. Booster phenomenon can result frequently from atypical mycobacteria infection or BCG vaccination, but is rare following tuberculin testing. The booster phenomenon may be seen when the second test is given 1 week to 1 year after the first test. Booster phenomenon increases with age and is highest among persons >55 years old.

When routine periodic tuberculin testing is done, a two–stage testing should be used to minimize the likelihood of interpreting a boosted reaction as a conversion. If the reaction to the first test is <10mm induration, a second test should be given one week later at a site far away from the previous site. If this
too is ≤ 10 mm induration, then the individual is considered uninfected. A subsequent conversion during periodic testing is likely to represent the occurrence of infection with M. Tuberculosis in the preceding interval. If the second of the initial two tests is significant, this probably represents a boosted reaction[45].

**Tuberculin Testing of Infants**

There is no medical contra indication to tuberculin skin testing of infants. However their immune systems are immature, many infants < 6 weeks of age who are infected with M. tuberculosis do not react to tuberculin tests.

**Tuberculin Test in Adults**

As performed in children Tuberculin test in adult is done with 0.1 ml of 1 TU of PPD-RT 23 in Tween 80 is injected intradermally into either the volar or the dorsal surface of the forearm. The reaction should be read after 48 hrs to the maximum 72 hrs. The reaction to tuberculin begins 5 to 6 h after injection, peaks at 48 to 72 hrs, decreases after that. Occasionally, the reaction may not peak till 72 hours[51]. Immediate hypersensitivity reactions to tuberculin can occur and subsides by 24 h, and this should not be read as positive. Only induration defined as elevated, reddened and hardened area and not the erythema alone should be measured. The criteria for positive skin test is given in table 3.

Causes for false positive test include infection with nontuberculosis mycobacteria, previous BCG vaccination and incorrect method of TST administration or interpretation. Cause of false negative results includes cutaneous anergy, recent TB infection, very old TB infection, very young, recent live-virus vaccination, overwhelming TB disease, some viral illnesses and incorrect method of TST administration.

Repeated tuberculin test is done in high risk population (eg. health care worker). The size of the reaction may increase on repeated testing and more increase should not be taken as positive unless otherwise it meets the criteria. This phenomenon known as boosting is maximal between one and five weeks and subsides by 60 days[52-54]. The risk of future development of tuberculosis among those demonstrating the booster phenomenon appears to be lower than for individuals from the same population with a positive initial tuberculin test[55].

<table>
<thead>
<tr>
<th>Name</th>
<th>Mode of administration</th>
<th>Tuberculin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mantoux test</td>
<td>Intradermal injection using a syringe and needle</td>
<td>PPD or OT</td>
</tr>
<tr>
<td>Jet injection</td>
<td>Intradermal injection with jet gun under high pressure using a special intradermal nozzle</td>
<td>PPD or OT</td>
</tr>
<tr>
<td>Tine test</td>
<td>Skin puncture with an applicator coated with dried tuberculin.</td>
<td>OT</td>
</tr>
<tr>
<td>Heaf test</td>
<td>Penetration of the skin through a drop of tuberculin solution by a number of needles released by spring mechanism</td>
<td>PPD or OT</td>
</tr>
<tr>
<td>Appli test</td>
<td>Skin puncture with an applicator coated with dried tuberculin.</td>
<td>PPD-S</td>
</tr>
<tr>
<td>Scarification test</td>
<td>Scratching of the skin through a film of the tuberculin reagent</td>
<td>PPD</td>
</tr>
<tr>
<td>Mono – Vacc test</td>
<td>Application of 9 plastic points coated with tuberculin, mounted on a plastic ring that fits onto the thumb</td>
<td>Liquid OT</td>
</tr>
<tr>
<td>Vollmer patch test</td>
<td>Applied in the paravertebral or presternal region and removed at 48 hours</td>
<td>PPD or OT</td>
</tr>
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**Table 1: Types of Tuberculin Testing [46,47]**
Host related Factors

Associated infections – Bacterial – typhoid fever, brucellosis, typhus, leprosy, pertussis,
Overwhelming tuberculosis, Viral (measles, mumps, chicken pox, HIV)
Recent vaccination [47]  Protein energy malnutrition – severe forms
Primary and acquired immunosuppressive states [48,49]
Chronic renal failure, Malignancy, Dehydration and high fever

Reagent related factors

Adsorption to container/ syringe
Denaturation due to improper storage

Technique related factors

Inadequate dose of tuberculin, Improper technique of administration
Inter/ intraobserver variation in recording the size of induration

### Table 2: Etiology of false negative reactions

<table>
<thead>
<tr>
<th>An induration of 5 or more millimeters is considered positive in</th>
<th>An induration of 10 or more millimeters is considered positive in</th>
<th>An induration of 15 or more millimeters is considered positive in</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. HIV-infected persons</td>
<td>1. Recent immigrants (&lt; 5 years) from high-prevalence countries</td>
<td>Persons with no risk factors for TB</td>
</tr>
<tr>
<td>2. A recent contact of a person with TB disease</td>
<td>2. Injection drug users</td>
<td></td>
</tr>
<tr>
<td>3. Persons with fibrotic changes on chest radiograph consistent with prior TB</td>
<td>3. Residents and employees of high-risk congregate settings</td>
<td></td>
</tr>
<tr>
<td>4. Patients with organ transplants</td>
<td>4. Mycobacteriology laboratory personnel</td>
<td></td>
</tr>
<tr>
<td>5. Persons who are immunosuppressed.</td>
<td>5. Persons with the following clinical conditions that place them at high risk: silicosis, diabetes melitus, chronic renal failure, some hematologic disorders (e.g., leukemias and lymphomas), other specific malignacies (e.g., carcinoma of the head or neck and lung), weight loss of 10% of ideal body weight, gastrectomy, and jejunoileal bypass</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. Criteria for positive Tuberculin test [58]

**Conclusion**

Though lots of tests are available now to detect tuberculosis, because of availability and because of the cost factor still tuberculin test can be used as an effective method to diagnose tuberculosis. But at the same time the observer should be cautious about the possible technical errors, chances of false positive or false negative results and other associated factors that can modify the interpretation.

### References


