Tuberculin Skin Testing

History

- 1891- Robert Koch demonstrated **Koch's Phenomenon** i.e. altered reaction caused by the injection of tubercle bacillus into the skin of a normal guinea pig from that caused by similar injection into a tuberculous guinea pig
- 1907 -Von Pirquit, Tuberculin Test- introduced as evidence of CMI resulting from prior exposure to the organism

  - gave evidence Koch's Phenomenon was immunologically mediated

History

- 1908, Moro –incorporated antigen as ointment to be rubbed on to skin as Patch Test
- 1910, Montoux introduced intradermal injection of tuberculin.

Types of Tuberculin

Old tuberculin (OT)

- Heated culture filtrate of M.tb, concentrated by evaporation and preserved in glycerol
- Crude material, not standardized and unreliable
- One IU OT = 0.0111microlit of international standard
- Each ml of standard contains 90000 IU of tuberculin

Types of Tuberculin

PPD- Purified protein derivative

- Purer
- Contains less carbohydrate than OT
- Easier to standardized and fewer nonspecific reaction
- An international standard (lot no.49608) produced by Seibert in 1939 (PPD-S) is maintained by WHO against which potency of other preparation is measured.

Types of Tuberculin

- PPD –RT23 prepared in Denmark
- PPD –Weyberg –British Standard preparation.

  PPD –stored as lyophilized powder or it can be solubilized in phosphate buffer saline.
- Tween 80 (polysorbate 80) is added to PPD to prevent its adsorption on glass & plastics.
- Vial stored in refrigerator (not frozen) and kept in dark place.

Types of Tuberculin

- PPD-RT23 rich in heat stable group-II Ag
- **Newer Tuberculin** –rich in group-IV Ag that tend to be heat labile
- E.g.. T-1327 & T-1456
- Two studies in India showed good results with these tuberculin
- Compared with PPD-RT23, all three show consistent results.

Stanford et al.Tubercle 1988;96:293-8

Stanford et al. Tubercle 1987;68:169-76

Strengths of Tuberculin

- 1IU PPD = biological activity in 0.000028 mg of PPD-S(0.00002mg of PPD)
- **1 TU = 0.00002 mg PPD-S**
- 1mg PPD =50000 TU
- 1ml OT has bioactivity = 10000 IU
- 1 TU PPD is **first** strength
- 250 TU is **second** strength
- 5TU is **intermediate** strength

Tuberculin for Non-Tubercular Mycobacteria

- PPD-A ---M.avium
- PPD-B ---M.intercellulare
- PPD-F ---M.fortuitum
- PPD-G ---M.scrofulum
- PPD-Y ---M.kansasii
- PPD-Sm --M.smegmatic

Tuberculin for Non-Tubercular Mycobacteria

- Less sensitive and specific
- Mainly used in epidemiological surveys
- Some study show animal contacts may induce sensitivity to mycobacterial sensitins.
- Reaction >4 mm larger than tuberculin reaction has been advocated as diagnostic criterion for disease caused by atypical mycobacteria.

Lind et al. Sensitivity to sensitins and tuberculin in swedish children, study of school children in an urban area. Tubercle 1991;72:29-36

Criteria for Optimal Antigen

- Biologically stable & standardized
- Not induce immediate type of hypersensitivity response
- Not alter humoral responses
- Reproducible degree of reactivity on repeat testing
- Test should not be done on flaccid poorly vascularised skin (e.g..paralyzed arm)
- Precise-- Dose & depth of the antigen injection

Howard et al. Arch Intern Med 1988;148:2457-9

Technique of tuberculin test

- Montoux test—volar aspect left forearm
- Standard Dose recommended by WHO is 1TU
- Using a disposable tuberculin syringe with 27g. needle (smaller gauge cause leakage-False-ve)
- 0.1ml intradermally injected raising wheal of 6-10 mm
- Repeat test at separate site-- If inadequate sized wheal or ecchymosis develops
- Reading done at 48-72 hrs as date of inoculation, reading, name, strength of antigen and size of induration.

Technique of tuberculin test

- A record of +ve /-ve is inappropriate
- Induration is measured as greatest transverse diameter to the long axis of the arm by palpation method

  --error of 2% in the measurement decreases accuracy by 25% & 5% error decreases by accuracy by 50%

- Pen method of Sokal , in a study smaller degree of induration was found than palpation method

Howard et al. Arch Intern Med 1988;148:2457-9

Immunological Basis

- Classical example of Delayed type of hypersensitivity.
- T cells sensitised by prior infection recruited to skin site—release lymphokines
- Induce erythema by local vasodilatation
- Induce indurtion by local edema, fibrin deposition & recruitment of other inflammatory cells
- reaction begins 5-6 hrs after injection, maximum induration at 48-72 hrs & subside over few days

Physiologic changes in Tuberculin Reaction

- Inc. cutaneous blood flow (10 times)---- erythema and hyperemia
- Inc. thickness of skin (200-300%)- induration peaks a day later than erythema
- Dec. -- local pH & pO_2 (hypoxia)
- Inc. -- local pCO_2
- Activated lymphocytes grow more rapidly in the presence of lactate

Tuberculin Testing

- +ve reaction –previous exposure to M.tuberculosis.
- –ve response in child, generally rules out active TB.
- Lower cut off value—increases sensitivity but lowers specificity (False +ve due to NTM included) in individuals at high risk of infection
- high cutoff– Increase specificity

Response to Tuberculin Skin Test

- Shows well defined circumscribed area of induration in +ve response
- >10 mm with PPD-RT23-- +ve
- <5 mm with PPD-RT23-- -ve
- 5-9 mm with PPD-RT23-- doubtful
- >20 mm with PPD-RT23– strongly +ve

ATS. Diagnostic standards and classification of tuberculosis. Am Rev Resp Dis 1990;142:725-35

Guidelines for +ve TST

>5 mm with PPD-RT23—+ve

- Recent contacts with infectious TB patient
- with chest X ray consistent with old healed TB
- Those with HIV infection or risk factors for HIV infection
- Patients with organ transplants & with immunosuppressed state (receiving >15mg/day of prednisolone for > 1 mth)

ATS. Diagnostic Standards and classification of Tuberculosis. Am Rev Respir Dis 1990;142:725-35.

Guidelines for +ve TST

- **≥ 10 mm**
- Recent arrival (<5yrs) from high prevalence countries
- Mycobacterial Laboratory Personals
- Clinical conditions like silicosis, DM,CRF, malignancies,
- Weight loss of >10% ideal body weight
- <4 yrs age/ infants adequately exposed to adults in high risk category

Guidelines for +ve TST

- **≥ 15 mm ---**Persons with no risk of TB
- Interpretation of results in persons with previous BCG vaccination is frequent dilemma– tuberculin reaction varies between 0 to 90%
- Factors affecting tuberculin reactivity in such persons—
- Injecting dose of BCG
- Route of administration
- Age at vaccination
- Interval between vaccination and tuberculin test

ATS. Diagnostic Standards and classification of Tuberculosis. Am Rev Respir Dis 1990;142:725-35.

Utility of Tuberculin skin test

- Prevalence of infection influences the predictive value of tuberculin test (higher prevalence high predictive value)
- TST has specificity of 99% in population with no previous exposure or BCG vaccination
- specificity reduces to 95% in population where cross reactivity with other mycobacteria is common
- In India, where prevalence is high- TST is useful diagnostic test.

Chakraborty et al. Tuberculosis infection in a rural population of south india :23 year trend. Tuber Lung Dis 1992:73:213-8

False –ve tuberculin test

Patients related factors

- **Infections- measles,mumps, influenza**
- **Recent live virus vaccination –MMR, OPV**
- **Malnutrition, CRF, CLD**
- **HIV infection & AIDS**
- **Malignancy-leukemia, lymphoma**
- **Immunosuppressive drugs**
- **Sarciodosis**
- **Neonate, elderly**

Factors related to conduct of tuberculin test

- Exposure of tuberculin to heat & light
- Improper dilution
- Contamination
- Improper technique
- Improper reading

False +ve tuberculin test

- Prior BCG vaccination
- Wrong technique
- Over dosage of tuberculin
- Contamination
- Atypical mycobacterial infection

Use of BCG for TST

- Use of BCG vaccine for tuberculin testing has been supported by various studies.
- Choudhary et.al (1977) estimated that BCG was equivalent to 5 to 10 TU of tuberculin with Tween 80.
- Induration is read on the 5^{th} day
- Induration of $\geq$14mm appears to be the best criteria for infected persons.

Use of BCG for TST

- Some workers have recommended use of BCG in tuberculin testing of children so that if they are not already infected, they will be immunized by BCG.
- However its routine use is restricted—**Low** specificity of BCG test

  --BCG vaccine as antigen of bovine origin

  -- Inappropriate antigen per dose (quality & weight)

  --Higher cost, low shelf life

Previous vaccination with BCG

- No reliable method to distinguish reactions from those caused by natural infection.
- Reasons for assumption that reaction is not due to BCG vaccination--

  1. Conversion rate after vaccination is <100%
  2. Mean size who have received BCG is often <10mm.
  3. Tuberculin sensitivity tends to wane after vaccination

Previous vaccination with BCG

- Prudent to consider +ve reaction to **5 TU** of PPD in vaccinated persons as indicating infection with M.tuberculosis especially in countries of high prevalence.

ATS. Diagnostic Standards and classification of Tuberculosis.

Am Rev Respir Dis 1990;142:725-35.

-ve TST & Active Disease

- Older age and fewer s/s than +ve TST
- Advanced bilateral disease,critically ill, excrete large mycobacteria in sputum
- Anergy is usually transient & become TST +ve during treatment
- DTH depressed due to circulating immune complexes containing mycobacterial polysaccharide

-ve TST & Active Disease

- Compartmentalization of antigen specific circulating T-lymphocytes to the site of lesion so that few are available to participate in dermal reaction.
- Production of immunosuppressive IL-10, TGF-B, PGE-2 from circulating monocytes and lymphocytes.

TST & HIV Infection

- It has been reported that tuberculin skin reaction is reduced.
- Though few studies, report preservation of reactivity in HIV +ve with history of tuberculosis or BCG vaccination

Selwyn et al. A prospective study of risk of tuberculosis among intravenous drug users with HIV infection.
N Engl J Med 1989;320:545-50

- Reaction of ≥5mm in HIV+ve patients is investigated for active tuberculosis

TST & HIV Infection

- In order to reduce the measured prevalence of anergy and increase the proportion of nonreactors who are '**truly PPD negative'**

  CDC recommends additional use of at least two DTH antigens (mumps plus candida antigen or tetanus toxoid)
- Thus, tuberculin –ve persons in high prevalence areas without anergy may be spared chemo- prophylaxis

TST in Children

- Not contraindicated in infants however infants below 12 weeks show –ve response.
- Since, young children are at increased risk for active tuberculosis once infected, skin testing and evaluation for preventive therapy is recommended if they are exposed to a person with active tuberculosis.

TST & Pregnancy

- Well designed study has indicated that pregnancy does not measurably affect the response to TST
- No evidence of adverse effect on women or their babies
- Few earlier studies suggested suppression of CMI in pregnancy, however these were not properly designed.

Boosting, Conversion & Reversion

- **Reversion**– loss of tuberculin reactivity over time.(declines with age)
- Estimated at rate of 5%/year in a healthy population (specific waning of CMI for tuberculin antigen rather than generalized anergy)
- This can be restored progressively by repeated administration of tuberculin antigen ---------**Booster phenomenon**

Thompson et al.The Booster phenomenon in serial tuberculin testing. Am Rev Resp Dis 1979;119:1172-6

Boosting, Conversion & Reversion

- **Booster phenomenon—**

  -- increase in induration of at least 6 mm and an increase form <10mm to > 10mm on second test of two step tuberculin testing (1-3 week duration).

  -- has lower specificity than that of single test.

- Routine periodic test should be used to minimize the likelihood of interpreting a booster reaction as **conversion.**

Thompson et al.The Booster phenomenon in serial tuberculin testing. Am Rev Resp Dis 1979;119:1172-6

Boosting, Conversion & Reversion

- Recent TST conversion –

  Increase of **at least 10mm** with in a 2 year period for those < 35 years

  Increase of **at least 15mm** with in a 2 year period for those > 35 years

  All infants and children < 4 yrs with **≥10 mm** are also included.

Havlir et al. A 19 year followup of tuberculin reactors. Assessment of skin test reactivity and in vitro lymphocytes responses.

Chest 1991;99:1172-6

Allergic Reaction to Tuberculin

- Immediate wheal-flare reaction with erythema & induration at 6 hrs and subsides with in 24 hrs (IgE mediated response to 7% polysaccharide in PPD)
- Fever
- Vesiculation
- Ulceration
- Regional lymphadenopathy & proximal lymphangitis
- Phlycten
- Rarely shock

Limitations of Tuberculin Test in Developing Countries

- Immunization of BCG at birth may produce false positive tuberculin reaction later in life
- High prevalence of malnutrition & parasitic and infective illness including HIV infection --- immunosuppressive states limits its use.

Limitations of Tuberculin Test in Developing Countries

- Frequency of Tuberculin positivity in adults with tuberculosis and HIV ranges between 0 to 70 % depending on degree of immune suppression
- +ve TST in malnourished or immune-suppressed non-vaccinated person highly suggestive of probable TB
  - ***Reider HL et al. Tuberculosis and AIDS-Florida. Arch Intern Med 1989;149:1268-73***

Conclusion

- Despite various limitations, it is the only tool for measuring prevalence of tuberculosis infection in the community.
- *‘It has been aptly said that Tuberculin Test should be approached with respect, administered with care, read with deliberation and interpreted with caution.’*

All the best..