Editorial

Serodiagnosis of Tubercular Infection

The diagnosis of tuberculosis (TB) continues to be surrounded by uncertainty even in teaching hospitals. Clinically, the disease remains easily misdiagnosed, underdiagnosed or paradoxically overtreated in children. Cultures are of little value since they are available after a long time and the yield is low. The genetic properties of mycobacteria being fixed, new or better culture media are unlikely to accelerate the appearance of colonies. The use of Bactec Radiometric Method certainly speeds up the isolation of all mycobacteria but it is expensive and the consumable costs are likewise expensive. Molecular methods-Polymerase Chain Reaction (PCR) at present are very expensive and in any case their reliability in cerebrospinal fluid (CSF) is always limited because of the presence of inhibitors. Under such circumstances, serology is an attractive possibility in patient management. Serology refers to detection of antibodies or antigen or immune complexes (IC) using monoclonal or polyclonal reagents. Serodiagnosis offers scope for an early diagnosis in children with TB meningitis (TBM), brain tuberculomata, TB bone, exudative ascites and pericardial effusion. Arloing developed the first seroagglutinin test for TB in 1898(1). Despite extensive work on various mycobacterial antigens over the last 100 years, no specific test is yet available for clinical use. The problem is in formulating an appropriate test with defined epitopes and not looking for new antigens.

Choice of antigen used is a major deter-minant of test specificity. Highly purified species specific epitopes of *Mycobacterium tuberculosis* (MTB) derived either from monoclonal antibody (Mo Ab) affinity chromatography or gene cloning methods have been used for serodiagnosis ef humna TB. They include 38 kDa, 19 kDa, 14 kDa, TB72, TB23, TB78 and TB 68 epitopes(2). Though the peptides had a high level of specificity, their sensitivities were low. It is therefore thought that a pool of peptides ("cocktail antigens"!) would result in a test with higher sensitivity. One such combination of antigens studied included 19 kDa antigen/ML34 and TB78 epitope. Antibody to TB68 epitope (16 kDa) gave the best rating in children with radiologically positive TB. TB72 epitope (38 kDa) appears most successful in smear negative pulmonary TB(3). A panel of Mo Ab are used since most specific epitopes evoked response only with certain individuals.

Assays for IgG reactivity to purified lipoarabinomannan (LAM) antigen were clinically useful for diagnosis of adult TBM(4). The levels of sensitivity and specificity in CSF assays with 17 kDa antigen, antibody system were useful in our study on childhood TBM. The relative value of an ELISA system for diagnosis of TBM will be determined by the preponderance of antigen, antibody or immune complex in the CSF depending on the stage of the disease, as the case may be. The result of CSF assays must therefore be interpreted in relation to the stage of the disease(5).

Antibody titers following infection and primary tuberculosis appear directed towards polysaccharide and cytoplasmic antigens unlike secreted or shed antigens in post-primary TB(6). Different antibody

specificities are stimulated in various stages or types of TB infection(7). It is known that Human Leucocyte Antigen-DR (HLA-DR8) phenotype has been associated with a general unresponsiveness to mycobactcrial antigens; this phenotype has a significant effect on the antibody levels to 38 kDa antigen(8). It is therefore not surprising that 30% of patients with even smear positive pulmonary tuberculosis did not have any detectable antibody to any reagent(9).

In children antibody levels to MTB antigens are generally lower. Such a hyporesponsiveness could be due to nature of exposure, extent of disease, immunocompetence and genetic background of the child. It must also be recognized that variations in MTB strains specific for the region may affect the performance of the tests. Serology is often positive in patients with bacteriologically or otherwise obvious disease. But results in diagnostically difficult cases are often equivocal. Nobody has ever come up with a reliable way to detect infection with a high degree of specificity. A specific test giving a low level of unequivocally positive result is arguably better than no test at all due to the inherent limitations of other diagnostics in childhood TB.

The question before a practitioner is the reliability of serodiagnostic tests for TB available at a cost of Rs. 400 - Rs. 500 per evaluation at many commercial laboratories. Serology practised with crude antigens of MTB gives many false positive results. Such tests are indeed confusing, rarely rewarding and financially burdening the parents. Global patents' search indicate availability of newer kits for TB over recent years (approximate cost US $ 10 per test). Experience with newer kits assessed at Mycobacteria Reference Laboratory abroad is disappointing at present (Watt B, *Personal communication,* 1996). The commercial kits

available in India for detecting antibody by ELISA use BCG 60 antigen /KP 90 antigen. Semipurified in nature, the antigen is nonspecific for mycobacteria; it cannot discriminate environmental exposure, BCG vaccination and infection. It met with less specific results in National Institute of Communicable Disease(10), National Tuberculosis Institute(11) and other trials in India(12-14). The overlap in antibody values between patients and controls is unacceptably high.

Kits which have not worked well in Europe(15,16) or USA(17) will never work in India simply because population in India is already vaccinated resulting in basal level of Anti-Mycobacterial Antibodies (AMA) and by repeated exposure to TB they become higher in adults. The adult sera in India have such high levels of antibodies that nearly 95% is non-specific. Whenever a specific antigen is used (most of these specific antigens also carry non-specific epitopes) this 5% reactivity is masked by the binding of AMA to their other epitopes. The only method to detect such antibodies is by SACT-SE assay (Sandwich ELISA adaptation of Solid Phase Antibody Competition Test)(18) or by use of specific peptide fragments.

A Japanese firm introduced a Tuberculo-Phosphatide Kaolin Test(19) but gave it up later because of technical errors in agglutination. Agglutination based studies are not of much use since they are good for detecting IgM antibody and in tuberculosis IgG antibody is encountered more often. But this simple test with Tuberculophosphatide had good levels of specificity for infection even in India(20). Recently, a simple slide agglutination test, with good degree of sensitivity, has been reported(21). However, field trials are required before accepting it for routine use in India.

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The sensitivity and specificity of a diagnostic test can only be properly evaluated in confirmed disease. Once the specificity of a test is established, its usefulness can then be assessed in subgroups of patients, such as those who are culture negative. The difficulties of proving tuberculous etiology in suspected study cases cannot be overemphasized. Hence the solution is to compile cases of well defined disease from multiple centers that see large number of patients and not including those with unproved disease. The WHO initiative (Memorandum RSD/7.2) in collecting a bank of TB sera with standardized clinical data may thus prove beneficial for evaluating serodiagnostic tests.

At present there is no adequate comparison to evaluate any new serodiagnostic test for MTB infection other than culture results or clinical assessment. In children, isolation of MTB is not necessary if the contact history, tuberculin test, radiographic information (with a reasonable negative evaluation for other possible etiologies) is compatible with the disease(22). It may not be possible to do any test on a routine test to prove or disprove the gold standard. Rather a new serological test should be evaluated with four other parameters: culture, PCR, clinical presentations and healing after institution of therapy. There are mathematical models available to predict then whether serodiagnosis was correct in diagnosis of TB.

Serodiagnosis requires further development and prospective evaluation. The success rate of TB serology must be compared with alternative standard diagnostic procedures in children with suspected disease adequately followed up. Awaiting such research developments, *appropriate skills* for clinical diagnosis, *diligence* in contact tracing and tuberculin testing, *perseverance* for smear microscopy and adequate quality

control of MTB culture media should be strengthened at all levels of medical care.

One thing is certain—a new antigen is not going to make diagnosis easier; rather an optimal combination of antigenic epitopes in assay is going to give us a good test. Till then reliance will have to continue to be placed on conventional methods of diagnosis.

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