

INTRODUCTION

Extrapulmonary tuberculosis (EPTB) is a significant public health problem that poses diagnostic dilemma. The changing epidemiology of tuberculosis (TB) due to acquired immunodeficiency syndrome (AIDS) has brought extra pulmonary tuberculosis (EPTB) into focus. The diagnosis of EPTB is challenging and more so when it involves deeply located inaccessible area. The protean non-specific presentations, paucibacillary nature, difficulty in procuring appropriate and adequate sample, lack of awareness among clinicians, and poor performance of conventional microbiological techniques in EPTB are all contributory to challenges in diagnosis of EPTB leading to delayed diagnosis, missed diagnosis and over diagnosis and hence increased mortality. EPTB constitutes 15 to 20% of all case of tuberculosis in immune competent patients and for more than 50% of cases in Human Immunodeficiency Virus (HIV) positive individuals. Literature regarding the relative contribution of EPTB to the total number of cases of tuberculosis is lacking and hence there is no reliable epidemiological data. Due to the stigma associated with and the reluctance to perform invasive procedures especially in HIV positive patients, the notified estimates of EPTB under the Revised National Tuberculosis Programme (RNTCP) are often based on presumptive diagnosis and are an over estimate of the problem.

AWARENESS REGARDING EPTB

EPTB is often not even considered as a potential differential diagnosis. Lack of sufficient knowledge and not maintaining a high index of suspicion often leads to diagnostic delays. Raising awareness among non-pulmonary physicians is the need of the hour. If the suspicion of TB is high or the patient is very ill, anti-tubercular treatment may be considered as soon as diagnostic specimens are obtained. Absence of PTB should not deter one from looking for EPTB.

LACK OF UNIFORM GUIDELINES

Lack of an efficient sample processing technique universally applicable to all type of extra pulmonary (EP) samples and non-uniform distribution of microorganism due to apportioning of sample for various diagnostic tests is another obstacle in rapid diagnosis. EPTB guidelines are not well documented and poorly understood.

ATYPICAL AND VARIABLE PRESENTATION

EPTB can affect virtually any site of the body. The clinical

presentation is atypical, variable, vague, non-specific and mimics symptoms of other disease. There is disparity between recent and older clinical series of clinical presentation as well as among series from different countries. EPTB patients may manifest constitutional symptoms such as fever, anorexia, weight loss, malaise and fatigue. Pyrexia of unknown origin maybe the only diagnostic clue. It is uncommon to have patients manifesting symptoms and signs related to the organ system involved.

LOW LEVELS OF BACTERIOLOGICAL CONFIRMATION

Factors responsible for it are paucibacillary nature and difficulty in obtaining appropriate and sufficient sample, invasive and repeated procedures required, faulty sample collection and varying sensitivity and specificity of the available tests. Mycobacterium tuberculosis (MTB) is in abundance in lesions showing rapid caseation.

Sample Collection

First few millilitres of urine should be allowed to flush the external urethra; thereafter clean voided total volume of the 1st early morning urine should be collected on three consecutive days and transported as soon as possible. 5 to 10 ml of Cerebro spinal fluid is usually collected. Repeated sampling (upto 3 lumbar punctures on different days) & cisternal or ventricular CSF maximises chances of isolation. Largest possible volume of pleural, pericardial, synovial and ascitic fluid and sufficient quantity of tissue should be collected. Direct smear examination of gastric lavage is misleading as AFB maybe present in food and water.

Smear microscopy

Direct demonstration of MTB by staining techniques is of great importance as the time required, in comparison to culture, is much lesser. Zeihl-Neelsen (ZN) procedure is most commonly used. However, visual fatigue leads to deterioration of reading. When more than 50 smears are examined daily, fluorescence microscopy is recommended. Its sensitivity and specificity is comparable to ZN technique but it allows examination of a much larger area per unit of time as lower magnification is used. Concentrating the bacillary content of clinical specimens, increases the sensitivity. Urine, CSF and body fluids are centrifuged and the deposit is stained for microscopy. However benefit of smear microscopy is limited in EP samples due to their paucibacillary nature. Sediment and clot of CSF should be used for culture and it is desirable that two independent readers examine the smear. Pus and

thick aspirates should be made thin before direct smear examination.

CULTURE

Case finding in EPTB is increased by 30 to 50% by culture as compared to smear microscopy. It is the definite diagnosis but unfortunately the result is not so promising in EPTB. Time required for growth in L J Medium, which is the gold standard, is 6 to 8 weeks and maybe even longer in extra pulmonary samples. In order to overcome this, rapid culture methods were developed. BACTEC radiometric system for mycobacteria based on the principle of measuring radioactive CO₂ liberated during decarboxylation of ¹⁴C labelled substrates has led to automated detection of bacterial metabolism. It is a standard process for isolation of mycobacteria. However if there is too much blood then the medium becomes turbid and there is a high background reading. Other rapid liquid tuberculosis culture known as Mycobacteria Growth Indicator Tube (MGIT) and mycobacteriophage based detection tests have led to higher and quicker isolation, positive tests within week and two days respectively. But they are technically complex and have high rate of contamination and so are difficult to perform and interpret.

Histopathological findings

Even when representative tissue or body fluid is accessible and adequate, the histopathological finding maybe suggestive of Granulomatous infection which has a wide range of differential diagnoses apart from tuberculosis.

Body fluid analysis

Exudative fluid with high protein, low Serum ascitic fluid albumin gradient (SAAG), lymphocytic pleocytosis and high Adenosine deaminase (ADA) are suggestive of tubercular aetiology. Similar CSF picture maybe seen in other chronic meningitis and partially treated pyogenic meningitis. Early in tubercular pleural effusion neutrophils may predominate but on serial thoracenteses lymphocytosis becomes evident. Measurement of ADA activity is one of the most studied and widely used biomarkers in body fluids for the diagnosis of EPTB and was proposed to be a useful surrogate marker, but variable and conflicting results as well as considerable overlap levels between cases of tuberculosis, bacterial and viral adds very little additional information and hence not recommended.

RADIOLOGICAL AND ENDOSCOPY FINDINGS

They are often confusing. For instance, encysted effusion maybe confused with a mass lesion of the pleura, mediastinum, chest wall and lungs. In TBM there may be no or nonspecific findings in the initial stage, meningeal enhancement may initially worsen on treatment prior to showing signs of resolution, Neuroimaging findings and clinical picture might not correlate etc. Endoscopically diffuse colitis of tubercular aetiology is similar to ulcerative colitis. Presence of septae in ascitic fluid is suggestive of tuberculosis but is also seen in malignancy.

IMMUNODIAGNOSIS

Tuberculin skin test (TST) and IFN- γ releasing assay (IGRA) are supportive methods for diagnosing EPTB, but have limited diagnostic value. Interpretation of TST reactivity can be complicated by cross-reactivity with previous bacilli Calmette-Guerin vaccination or latent TB infection in countries where TB is prevalent. Several antigen and antibody (against 38KDa, 30KDa, A60) detection tests have been evaluated. These assays are inherently more sensitive than specific as presence of low level circulating cross reactive antibodies compromise the result. They cannot distinguish between latent and active infection and negative results cannot rule out the disease. Lipoarabinomannan is a component of tubercular bacterial cell wall which is detectable in urine. Polyclonal antibodies can be detected in body fluids by using flow through filter devices.

MOLECULAR ASSAYS

Newer molecular assays have aided rapid diagnosis of EPTB but they also have many pitfalls. Expensive, requirement of uninterrupted electric supply, infrastructural & technical expertise, cross reactivity, varying sensitivity and specificity in different studies, presence of inhibitors etc. are few of the constraints of these assays. Sensitivity of Cartridge based nucleic acid amplification test (CBNAAT) is high in FNAC & biopsy specimen of lymph nodes, solid tissues and CSF but lower in pericardial, ascitic and synovial fluid sample and still lower in pleural fluid. Presence of inhibitor substances in high concentrations in clinical specimens, specially biopsies and proteinaceous pleural effusions which could include blood, eukaryotic DNA, host proteins etc may undermine the amplification. Polymerase chain reaction and Ligase chain reaction have high sensitivity so even minutest amount of contaminating DNA can be amplified leading to false positives.

CONCLUSION

Whenever EPTB is suspected as a possible diagnosis, all efforts should be made to procure representative and relevant tissue body fluid to establish microbiological confirmation and for subjecting to various diagnostic tests. The various non-conventional diagnostic tests which are relied upon as concrete evidence to initiate or withhold anti-tuberculosis treatment fail to stand the criteria of 'gold standard'. Lack of reproducibility of these tests renders the information generated by these tests as inconclusive. Rapid and accurate diagnosis is critical for proper initiation of treatment and control of drug resistance TB. Need to develop novel diagnostic technique is urgent. The pipeline for diagnostics, though abundant in new technology, offers little hope of a true point-of-care test in the near future.

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