Diagnostic options for gastrointestinal tuberculosis

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Tuberculosis is a major public health problem throughout the world and particularly in developing countries like India. Both human (*Mycobacterium tuberculosis*) as well as bovine (*M. bovis*) strains have been responsible for tuberculosis. In addition, atypical mycobacterium *viz. M. avium* and *M. intracellulare* can also cause tuberculosis. The disease is known to be associated with poverty, deprivation and immunodeficiency.

In recent years notification of tuberculosis cases has become stable due to improved diagnostics. Annually about 9 million new cases of tuberculosis are reported and 2 million deaths occur which is largely due to HIV acquisition. Eighty-five percent of tuberculosis cases occur in Africa and Asia (30% and 55% respectively) with India and China together bearing 35% of the load. According to World Health Organization, in India alone there are 3-4 million new cases of tuberculosis annually. In 2013, about 64% of the estimated 9 million people who developed tuberculosis were notified as newly diagnosed cases. This is estimated to have left about 3 million cases that were either not diagnosed, or diagnosed but not reported to National Tuberculosis Program.[1] With the increasing incidence of AIDS diagnosing tuberculosis is becoming a challenge to the physicians and surgeons.

Any organ system in the body can be afflicted by tuberculosis. Gastrointestinal (GI) tuberculosis can be caused by ingestion of infected material in active pulmonary tuberculosis, reactivation of a quiescent intestinal focus resulting from hematogenous dissemination of active tuberculosis, or direct spread from other organs. Tuberculosis may involve any part of the GI system, though the ileum and the colon are the most common sites, occurring in up to 90% of intestinal tuberculosis cases. GI tuberculosis is a major health problem in many underdeveloped countries. It is also a major clinical problem because, in general, it is more difficult to diagnose than pulmonary disease, often requiring invasive procedures to obtain diagnostic specimens and more sophisticated laboratory techniques than sputum microscopy. Thus GI tuberculosis is often diagnosed on the basis of clinical experience which may lead to diagnostic errors due to over-diagnosis or under-diagnosis, as it may mimic several other conditions such as lymphoma, Crohn's disease, amebiasis and adenocarcinoma. Of all clinical features, weight loss favors a diagnosis of GI tuberculosis while right lower abdominal pain on physical examination favors a diagnosis of Crohn's disease[2]. Endoscopically, localized lesion of mucosa nodularity is more typical of GI tuberculosis, whereas involvement of three or more intestinal segments favors a diagnosis of Crohn's disease.[2]

Radioimaging methods like chest radiography, barium studies, ultrasonography and computed tomographic scan are very useful in diagnosis of abdominal tuberculosis.[3] In intestinal tuberculosis the main imaging findings include symmetrical or asymmetrical parietal thickening and extrinsic compression by enlarged lymph nodes.[4] In tuberculosis of the rectum significant luminal narrowing may occur and the most common symptoms are hematochezia (88%) and constipation (37%). Nagi *et al*[5] revealed an incidence of 10.8% of colorectal involvement in a series of 684 patients affected by gastrointestinal tuberculosis along 10 years. Hepatic

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and splenic involvement of tuberculosis is rare, but in disseminated tuberculosis, the prevalence is significantly very high, *i.e.* up to 80-100%. [4,6,7]

In a study by Sinan *et al* [8] the main tomographic finding was peritonitis (38%), followed by lymph node disease (23%), involvement of the gastrointestinal tract (19%) and solid organs (10%). Diffuse pattern of lymph node commitment was most commonly observed (48%). In the gastrointestinal tract, the terminal ileum and the ileocecal region were most remarkably affected (50%). Among the solid organs, liver and spleen presented greater involvement (70%).

The microbiological techniques for laboratory diagnosis of tuberculosis are the most reliable of all techniques. Acid fast staining of clinical material for acid fast bacilli (AFB), followed by smear microscopy, remains the most frequently used microbiological test. The sensitivity of smear microscopy for detecting AFB is limited by the threshold of detection, which is 5,000-10,000 bacilli/ml of specimen. A smear negative for AFB does not eliminate the diagnosis of active tuberculosis, particularly if the clinical suspicion is high. Concentration of the clinical sample by physical methods using centrifugation or sedimentation and/or chemical methods using sodium hydroxide or bleach or ammonium sulfate may increase the sensitivity of smear examination. Traditionally, AFB are stained using the Ziehl Neelson stain or the Kinyoun acid fast stain. Microscopy has been reported to have >80 % sensitivity in diagnosis of pulmonary tuberculosis, but the sensitivity of the test has been relatively low and variable in extrapulmonary tuberculosis. Fluorescence microscopy using an acid-fast fluorochrome dye (auramine O/auramine-rhodamine), is sensitive, specific and cost effective. The *Mycobacterium* appears as brightly fluorescing rods against a dark background. The possibility of false-positive results exists because inorganic objects may incorporate fluorochrome dyes.[9]

Culture methods include the egg-based (Lowenstein-Jensen and Ogawa media) or the agar-based (Middlebrook 7H9, 7H10 and 7H11) media. Culture is the most specific of currently available tests and provides the starting point for species identification and drug sensitivity tests. However, it may require up to 6-8 weeks and in 10-20% of cases the bacillus is not successfully cultured. Faster culture of mycobacterial isolates has been achieved with manual culture systems (Septi-Chek AFB or the manual mycobacterial growth indicator tube – MGIT). Commercially available automated or semi-automated liquid culture systems have reduced the recovery time of *Mycobacteria* from culture to 2-3 weeks. Automated culture systems available are MB/BacT system, BACTEC 9000MB, BACTEC MGIT 960 and ESP Myco and AccuMed/Difco ESPII BACTEC 12B, MB/BacT Mycobacterial detection system and the ESP culture system II. Radiometric liquid culture using a broth of radiolabelled carbon has been used for many years. These systems measure changes in gas pressure, carbon dioxide production or oxygen consumption fluorimetrically or colorimetrically and allow continuous monitoring of cultures.

A few non-specific tests are available for the diagnosis of tuberculosis. Adenosine deaminase is increased in tuberculous ascitic fluid due to the stimulation of T-cells by mycobacterial antigens. High interferon (IFN-) levels have been reported to be useful for laboratory diagnosis in tubercular ascites. Quantiferon-tuberculosis Gold is an *in vitro* test which detects the release of IFN- after stimulation of white blood cells by *M. tuberculosis* antigen ESAT-6 (early secretory antigenic target 6) and CFP-10 (culture filtrate protein 10) in a blood sample. The corresponding burst in cytokines is examined by an enzyme immunoassay. The test may have a possible role in follow up of patients on anti-tuberculosis treatment and in the diagnostic dilemma of Crohn's disease *versus* tuberculosis. Mantoux test is a delayed hypersensitivity response to the tuberculin antigen. It is of limited value as a diagnostic tool because it cannot differentiate between active tuberculosis and previous sensitization.

Molecular methods include several DNA probes developed for rapid and specific identification of *M. tuberculosis* and other *Mycobacteria*. When used along with newer methods of detection of early growth (*viz*. BACTEC, Septi-Chek, MGIT) these are of great help in rapidly confirming the diagnosis, as identity can be

established within 1 or 2 days with gene probes. For direct confirmation of diagnosis from the clinical specimens, these methods are not very sensitive and need >10,000 organisms in the specimen for positivity. Ribosomal RNA probes target rRNA, ribosomal DNA, spacer and flanking sequences. The rRNA targeting probes are 10-100 fold more sensitive than DNA targeting and may be used to confirm the diagnosis directly in the clinical specimens. The lowest detection limit is around 100 organisms. At present, these are mainly useful for rapid identification of isolates in tuberculosis.

For the diagnosis of tuberculosis, several techniques based on PCR and isothermal amplification assay have been developed. In Loop Mediated Isothermal Amplification Technique, different enzymes other than Taq polymerase are used and various steps of amplification are completed at one temperature only. Different investigators have used separate gene targets like MPB 64, repetitive sequences, GC repeats, dev R, 38kD, TRC 4, and IS-1081. The tuberculosis PCR assay is based on augmenting oligonucleotides found in chromosomes of *M. tuberculosis* that are highly specific for the organism. Sensitivity of this test is modest and there is no correlation between PCR positivity and histological lesions such as caseation or granuloma. Multiplex PCR developed in India, is useful in diagnosis and detection of drug resistance, including extensively drug-resistant tuberculosis. Line probe assay technology is one of the newer nucleic acid amplification tests available for diagnosis of tuberculosis as well as multidrug resistant tuberculosis. PCR amplification of the resistance-determining region of the gene is performed using biotinylated primers. Labeled PCR products are hybridized with specific oligonucleotide probes immobilized on a strip and detected by colorimetric development. Commercially available line-probe assays include the INNO-LiPA Rif. tuberculosis kit (Innogenetics, Belgium) and the GenoType MTBDRplus assay (Hain Lifescience, Germany).

The GeneXpert microfluidic and molecular testing platform attempts to overcome known limitations by combining 3 separate steps for specimen processing and nucleic acid extraction, nucleic acid amplification, and detection of amplified products into a single, automated process. Xpert MTB/RIF test uses molecular beacons and 6-color florescence detection for real time identification of both *M. tuberculosis* and rifampin resistance in <120 minutes.

Laparoscopic findings is an excellent but sparingly used diagnostic technique. Visual appearance may be more helpful (85-95% accurate) than histology, culture or guinea pig inoculation. Endoscopic examination for GI tuberculosis like colonoscopy, double balloon enteroscopy and capsule endoscopy are also very useful in diagnosis of abdominal tuberculosis. Identification of pathological features is an important aspect of laboratory diagnosis of tuberculosis. On gross pathologic examination, GI tuberculosis may appear as an ulcerative form (60%), a hypertrophic form (10%) or an ulcerohypertrophic form (30%) in patients. An important feature in GI tuberculosis is the coexistence of different types of granulomas.[10] Caseating granulomas may be found in 85-90% of the biopsies. Tuberculous granulomas and histopathological demonstration of AFB through a combination of histology and culture of the biopsy material can be diagnostic in over 60% of cases. Histopathology is considered as the gold standard for diagnosis of extrapulmonary tuberculosis.

There are several new diagnostics for tuberculosis available in the market but each has some limitations. The biggest concern continues to be the lack of a rapid, simple, inexpensive, point-of-care test for active tuberculosis. The need of the hour is an easy to use, inexpensive diagnostic that can deliver results within minutes. Adoption and implementation of new diagnostics is also a policy matter which requires immediate attention. The WHO has developed a post-2015 framework strategy to end global tuberculosis epidemic which has been approved by all Member States at the May 2014 World Health Assembly.[1] It aims at a 95% reduction in tuberculosis deaths and a 90% reduction in tuberculosis incidence by 2035 and a target of zero catastrophic costs for tuberculosis affected families by 2020. However all this is achievable by a good diagnostic facility for tuberculosis and reducing the burden of HIV-associated tuberculosis by HIV testing for tuberculosis patients.

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