An Approach to the Detection of Mycobacteria in Clinically Suspected Cases of Urinary Tract Infection in Immunocompromised Patients

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An Approach to the Detection of Mycobacteria in Clinically Suspected Cases of Urinary Tract Infection in Immunocompromised Patients

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Abstract

Urinary tract infections (UTIs) are one of the most common infections in human beings. Most of these infections are caused by bacterial agents. Mycobacterial agents causing UTIs are less frequent in immunocompetent individuals; they are more common and severe in immunocompromised individuals. The incidence of tuberculosis is rising, particularly due to Human Immunodeficiency Virus (HIV) infection. The HIV sero-prevalence among tuberculosis (TB) patients in India ranges from around 2 to 20%, with an estimated 60% of HIV infected persons breaking down with active TB disease in their lifetime. The diagnosis of TB with HIV positive is more difficult than in those without HIV infection.

Extra pulmonary tuberculosis (EPTB) represents a progressively greater proportion of new cases and the genitourinary tract (GUT) is the most common site of EPTB. The most common causative organism of kidney and urinary tract tuberculosis is the Mycobacterium tuberculosis & occasionally Mycobacterium bovis can also be responsible. Mycobacterium tuberculosis (MTB) has an important impact on kidney transplant recipients, particularly during the first year after surgery.

The sure criterion for definite diagnosis of TB is the demonstration of the presence of tubercle bacillus in clinical specimens. This is based on traditional and conventional methods Ziehl Neelsen (ZN) acid fast stain and laboratory culture of M. tuberculosis on Lowenstein Jensen (LJ) medium. However, ZN staining lacks specificity and sensitivity, while confirmation by culture requires several weeks. Rapid diagnostic methods have been developed that are based either on liquid culture techniques, such as BACTEC or molecular techniques but they are expensive, requires specialist personnel and equipment hence limited their use especially in developing countries. Apart from this, FASTPlaque TB is a phage based test, which uses the mycobacteriophage to detect the presence of M. tuberculosis directly from the specimens. It is a rapid, manual test, easy to perform and has an overall higher sensitivity when compared with sputum smear microscopy, in newly diagnosed smear positive TB patients.

Review

Introduction:

Urinary tract infections (UTIs) are one of the most common infections in human beings. Most of these infections are caused by bacterial agents. Mycobacterial agents causing UTIs are less frequent in immunocompetent individuals; they are more common and severe in immunocompromised individuals. The incidence of tuberculosis is rising, particularly due to Human Immunodeficiency Virus (HIV) infection. The HIV sero-prevalence among tuberculosis (TB) patients in India ranges from around 2 to 20%, with an estimated 60% of HIV infected persons breaking down with active TB disease in their lifetime. The diagnosis of TB with HIV positive is more difficult than in those without HIV infection.

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mycobacteriophage to detect the presence of M. tuberculosis directly from the specimens. It is a rapid, manual test, easy to perform and has an overall higher sensitivity when compared with sputum smear microscopy, in newly diagnosed smear positive TB patients.

**URINARY TRACT INFECTION (UTI):**

In healthy people, urine in the bladder is sterile. Bacteria or other organisms are absent. The channel that carries urine from the bladder out of the body (urethra) contains no bacteria or few to cause an infection. However, any part of urinary tract can become infected: an infection anywhere along the urinary tract is called a urinary tract infection (UTI). UTIs are usually classified as upper and lower according to where they occur along the urinary tract. Lower UTIs are infections of kidneys (pyelonephritis) or ureters (ureteritis). UTIs can occur in children as well as in adults.8

**Primary or Recurrent UTI:**

UTIs are classified as primary or recurrent, depending on whether they are the first infection or whether they are repeat events.

**Uncomplicated and Complicated UTI:**

UTIs are also sometimes further defined as either being uncomplicated or complicated depending on the factors that trigger the infections. Uncomplicated infections are only associated with bacterial infection, most often Escherichia coli. They affect women much more often than men. Complicated infections, which occur nearly as often in men as women, are also caused by bacteria but they occur as a result of some anatomical or structural abnormality, such as catheter use in the hospital setting, bladder and kidney dysfunction, or kidney transplant.

**Symptomatic and asymptomatic UTI:**

Many patients are asymptomatic. Symptoms that may occur include dysuria, urinary frequency, and incontinence onset, flank pain, and fever. Confusion and delirium are often attributed to UTI, although without high fever or sepsis, uncomplicated UTI is unlikely to cause serious central nervous dysfunction. The diversity of potential uropathogens mandates that urine cultures be obtained in all elderly persons with suspected UTI.8

Bacteriologic diagnosis of complicated, recurrent UTIs and of asymptomatic bacteriuria is usually based on the concept of clinically important bacteriuria, which for these patients is usually defined as >105 CFU/ml in a clean catch midstream urine sample after > 4 hours of bladder incubation.8

**Pyelonephritis:**

Pyelonephritis is a bacterial infection of one or both kidneys. It is more common in women than men. E. coli, which is normally found in the large intestine, causes about 90% of cases of pyelonephritis among people who live in the community. Infections usually ascend from the genital area through the urethra to the kidneys from another part of the body through the blood stream.8 The risk of pyelonephritis is increased in people with obstruction of the ureters, diabetes, in people with a weakened immune system, and in pregnant women.8

**Ureteritis:**

Ureteritis is infection of one or both ureters. The spread of an infection from the kidneys or bladder is the most common cause followed by slowing of the flow of urine because of a defective nerve supply to the ureter.8

**Cystitis:**

Cystitis is the most common UTI and is sometimes referred to as acute uncomplicated UTI. It occurs in the lower tract (the bladder and urethra).

**Urethritis:**

Urethritis is infection of the urethra. It may be caused by bacteria, fungi, or viruses. In women, the organism generally travels to the urethra from the vagina. In most women, the bacteria come from the lower intestine and reach the vagina from anus. Men are much less likely to develop urethritis because the opening of the male urethra is far removed from the anus and is not as easily injured during sexual intercourse.

**CAUSES OF UTI:**

UTIs are the most common infections of human being especially in hospital setting. Most of these infections are caused by bacterial agents. Mycobacterial agents of UTIs although less frequent in immunocompetent individuals, they are more common and more severe in immunocompromised individuals.

**IMMUNOCOMPROMISED HOST**

The immunocompromised host is generally defined as a person who has one or more defects in the normal defense mechanisms that protect host from infectious agents predisposing the individual to an increased risk of severe life threatening infections. These defects may be malignancy or acquired immunodeficiency syndrome.

**URINARY TRACT INFECTION AND MYCOBACTERIA:**

Genitourinary tuberculosis (GUTB) has been reported in 8-10% of all cases in developed countries and in 20% in third world countries. 15% to 20% of TB patients in developing countries are found with M. tuberculosis in the urine. The most common causative organism of kidney and urinary tract tuberculosis is the human tubercle bacillus (M. tuberculosis) but the bovine tubercle bacillus (M. bovis) occasionally
can be responsible. The vaccine strain, Bacillus Calmette Guérin (BCG), also has been the cause of renal lesions as a complication of intravesical instillation of BCG for the treatment of superficial cancer.4

**Kidney:**

Tuberculosis may involve the kidney as part of generalized disseminated infection or as localized genitourinary disease. The kidney is usually infected by hemogenous spread of bacilli from a focus of infection in the lungs and/or bowel. Mostly, GUTB is a reactivated of the tuberculosis from a period of dormancy. Clinically, renal tuberculosis usually presents unilaterally, but post mortem studies showed that the disease frequently was bilateral. The healing process results in fibrous tissue and calcium salts being deposited, producing the classic calcified lesion. The occurrence of renal calcification is common in TB and may require surgical excision.11

**Ureter:**

Tuberculous urethritis is always an extension of the diseases from the kidney. The site most commonly affected is the ureterovesical junction. This is invariably secondary to extensive disease of the kidney and, if not recognized early, can rapidly cause complete destruction. The whole of the ureter is involved. In such patients, the kidney shows extensive disease, is often nonfunctioning, and is calcified.13

**Bladder:**

Bladder lesions are without exception secondary to renal TB. The earliest forms of infection start around one or another ureteral orifice. If the disease continues to progress, the inflammation and fibrosis which eventually follows contracts and can either produce a stricture or become withdrawn, rigid, and dilated, assuming the classic golf-hole appearance.13

**Urethra:**

Tuberculosis of the urethra is very rare and the patients should receive chemotherapy as initial treatment.14

**CLINICAL FEATURES OF URINARY TRACT TUBERCULOSIS:**

Tuberculosis of the urinary tract is easily overlooked. Symptoms that sometimes occur include back, flank and suprapubic pain, hematuria, frequency, and nocturia. These might also suggest conventional bacterial urinary tract infection. Symptoms such as fever, weight loss, and night sweats also are unusual.1

**Renal Tuberculosis:**

Renal tuberculosis is usually the sequela of a primary pulmonary infection that had occurred as long as 10-15 years. Tubercle bacilli lodge in the corticomedullary junction and form cortical granulomas. These granulomas remain stable for many years, but if reactivation occurs, the organisms spread into the medulla causing papillitis. As disease progresses, extensive papillary necrosis may develop with the formation of frank cavities destroying the renal parenchyma and may extend into the collecting system. Advanced disease leads to cortical scarring, infundibular and pelvic strictures. The end result of diffuse disease is destruction, loss of function, and calcification of the entire kidney.1 A tuberculous kidney may become calcified and nonfunctioning. If the gross anatomic distortion is advanced and bilateral, the glomerular filtration rate will fall and, in some patients, there is progression to end-stage renal failure.15

**Tuberculosis and glomerular disease:**

Chronic tuberculosis sometimes is complicated by amyloidosis, which, in India is an important cause of renal disease. There are cases reports of tuberculosis associated with various forms of glomerulonephritis, but no firm associated have been established.16

**Tuberculosis in patients on regular hemodialysis:**

The patients manifest fever, anorexia, weight loss and usually either is known to have had pulmonary or other forms of tuberculosis or is a member of a high-risk ethnic or social group. Disease caused by environmental mycobacteria also occurs in hemodialysis patients; it usually becomes apparent as pulmonary or disseminated disease or sometimes as skin lesions. Some infections have occurred as the result of contaminated of the dialysis machine by environmental Mycobacteria.17

**Tuberculosis in Renal Transplant Recipients:**

Tuberculosis is a serious complicating factor in renal and other forms of transplantation, with an incidence, depending on geographic region, of 0.35 to 15.0%.18 Immunosuppression can obscure the diagnosis by producing false-negative tuberculin test.

**DIAGNOSIS:**

In urinary tuberculosis, voiding problems and chronic urgency non-responding to antibacterial drug regimens are indicative of GUTB. Symptoms that sometimes occur include back, flank, and supra-pubic pain, hematuria, frequency, and nocturia. Renal colic is uncommon, occurring in fewer than 10% of patients, and constitutional symptoms such as fever, weight loss, and night sweats are unusual.19

**TUBERCULIN TEST:**

The first intracutaneous immunodiagnostic test used in humans was the tuberculin skin test introduced by Von-Pirquet in 1907. The tuberculin test is accomplished by intradermal (ID) injection of a purified protein derivative (PPD) of tuberculin. An inflammatory reaction develops at the site and reaches a maximum between 48 and 72 hours after injection. This reaction
consists of a central indurated zone surrounded by an area of erythema; it is assessed by measuring the diameter of the induration area. A person’s ability to respond to the local concentration of the injection may be decreased by malignancy, nutritional deficiencies, steroid therapy, irradiation, and AIDS.20
Basic approaches for diagnosis of tuberculosis.2
Direct approach- includes detection of Mycobacteria or its products.
Indirect approach- includes measurements of humoral & cellular responses of the host against tuberculosis.

DIRECT APPROACH

MICROSCOPY:
Microscopy is the simplest and most rapid procedure currently available to detect acid- fast bacilli (AFB) in clinical specimens by Ziehl-Neelsen staining method. Fluorescent staining method offers the advantages of screening the smear under low power where large number of slides is screened in less time.21 Detection of acid- fast bacilli from urine samples by microscopy (Ziehl-Neelsen acid fast stain) is not reliable, because of the possible presence of M. smegmatis, which are acid fast bacilli too. Bacillary load, extend of disease severity and therefore the appropriate treatment. Renal tuberculosis is accompanied by manifestations of urinary syndrome in 70.4% of cases and by the presence of M. tuberculosis in 100%.21

CULTURE:
Isolation of mycobacteria from clinical samples by culture still represents the corner stone on which definitive diagnosis of tuberculosis and other mycobacteroroses relies. Mycobacterial culture can be performed on conventional egg based solid medium such as Lowenstein-Jensen medium and agar based ones, such as Middlebrook 7H10 or 7H11 and liquid media such as Middlebrook 7H9 broth. Although a combination of solid and liquid media is currently the gold standard for primary isolation of mycobacteria, a few modern, rapid methods are also available. These include micro colony detection on solid media (including the rapid slide culture technique), septi-check AFB method, and microscopic observation in broth culture. Lowenstein-Jensen Media:
The processed specimen inoculated into slanted L-J medium and incubated at 35°C in the incubator in the atmosphere of 5% to 10% carbon dioxide (Co2). Cultures are examined weekly for growth of Mycobacterium. Most isolates appear between 3 and 6 weeks; few isolates appear after 7 or 8 weeks of incubation. Radiometric BACTEC 460 TB methods:
This technique is specific for mycobacterial growth, where in C14 labeled palmitic acid in 7H12 medium used. This system detects the presence of mycobacteria based on their metabolism rather than visible growth. When the C14 labeled substrate present in the medium is metabolized, 14Co2 produced measured by the BACTEC system and reported in terms of growth index (GI) value. The BACTEC system is also useful in the identification of M. tuberculosis using specific inhibitor, para-nitro-α-acetylaminob-β-hydroxypropiophenone (NAP). Mycobacteria in clinically samples can be detected in half the time compared to conventional culture methods. Micro colony detection on Solid media:
In this method, plate poured with thin layer of middle brook 7H11 agar medium are incubated and examined microscopically on alternate days for the first 2 days and less frequently thereafter. In less than 7 days, micro colonies of slow growing mycobacteria such as M. tuberculosis can be detected. Since M. tuberculosis grows more rapidly in liquid medium forming strings and tangles, which can be observed under the inverted light microscope with 40X magnification, this method is a better alternative for culturing tubercle bacilli.

Microscopic observation of broth cultures:
This is rapid and relatively inexpensive method, which compares very well with other well-established systems in terms of both sensitivity as well as specificity, and also to some extend with speed when compared to solid media. Although this technique may be appropriate for disease endemic high-burden countries, it requires P2 bio-safety cabinets, relatively expensive Middlebrook 7H9 broth, and oleic acid dextrose catalase (OADC) anti-microbial supplements and a relatively high technical skill.

Septi-Chek AFB method:
Uniform format of Septi-Chek AFB system consists of a capped bottle containing 30.0 ml of middle-brook 7H9 broth under enhanced (5-8%) C02, a paddle with agar media enclosed in a plastic tube, and enrichment broth containing glucose, glycerine, oleic acid, pyridoxal, catalase, albumin, pyloxythylene 40 stearate, azlocillin, nalidixic acid, trimethoprim, polymixin B and amphotericin B. One side of the paddle is covered with non-selective middle 7H11 agar with NAP for differentiation of M. tuberculosis from other mycobacteria, the other section contains chocolate agar for detection of contaminants. The non-radiometric approach has the potential to expedite processing, obiate C02 incubation requirements and facilities early detection of positive cultures. The method requires about 3 weeks of incubation. MGIT 960 Mycobacteria detection system:
It is an automated system for the growth and detection of mycobacteria with a capacity to incubate and continuously monitor 960 mycobacteria growth indicator tube (MGIT) every 60 minutes for increase in fluorescence. Growth detection is based on the AFB metabolic O2 utilization and subsequent intensification of an O2 quenched fluorescent dye contained in a tube of modified MGIT. In an early comparison of this technology with the BACTEC 460 and LJ medium using 2,567 clinical specimens. Tortoli and associates found that the MGIT 960 had the shortest mean time to positively at 13.3 days, compared with 14.8 days for the BACTEC 460 system and 25.6 days for LJ medium.

MB/BacT System:
This is a non-radiometric continuous monitoring system with a computerized database management. The system is based on colorimetric detection of CO2 generated. The multicenter field trials sponsored by the manufacture indicate that the MB/BacT system recovered a higher percentage of mycobacteria with less time to detection when compared with conventional methods and compared favorably in parallel cultures with the BACTEC 460 TB system.

ESP culture system II:
This is a fully automated continuous monitoring based on the detection of pressure changes within the headspace above the broth culture medium in sealed bottle, i.e. either gas production or gas consumption due to microbial growth. The system was evaluated in clinical samples for the detection of mycobacteria against BACTEC 460 and 7H11 agar solid medium. ESP II is used in combination with a solid medium, not as a stand-alone system.

Detection and identification of mycobacteria directly from clinical samples:
Both genotypic (molecular) and phenotypic methods are available with newer modification for the diagnostic of tuberculosis as an alternative for smear microscopy. The most common target used in the PCR is IS6110. This sequence is specific for M. tuberculosis complex and is present up to 20 times in genome, thus offering multiple targets for amplification, PCR. Detection of IS6110 in sputum (PTB) and peripheral blood (extra-PTB), when compared to culture has sensitivity, specificity and positive predictability of 83.5, 99 & 94.2% respectively. A variety of PCR methods have been described in the search for a sensitive and reliable screening test for tuberculosis in clinical specimens. Species specific and genus PCR methods are being used with various target and medication of PCR. The following are some of the methods used for identification of M. tuberculosis and Non-tuberculous Mycobacterium (NTM).

a. Transcription Mediated Amplification (TMA) and Nucleic Acid Amplification (NAA):

This approach identifies the presence of genetic information unique to M. tuberculosis complex directly from pre-processed clinical specimens. The NAA technique uses chemical, rather than biological amplification to produce nucleic acid, so that within few hours these tests distinguished between M. tuberculosis complex and NTM in an AFB-positive specimen. It is currently used only respiratory specimens; use for non-respiratory specimens is likely in near future. A positive direct amplified test in conjunction with AFB positive smear is highly predictive of TB diseases. A negative NAA with an AFB positive smear indicates that AFB is probably NTM. The M. tuberculosis direct test (MDT) and amplified mycobacterial direct test (AMDT) are highly sensitive (96%) and specific (100%).

b. Ligase Chain Reaction:
It is a variant of PCR, in which pair of oligonucleotides is made to bind to one of the DNA target strands, so that they are adjacent to each other. A second pair of oligonucleotides is designated to hybridize to the same regions on the complementary DNA. The action of DNA polymerase and ligase in the presence of nucleotides results in the gap between adjacent primers being filled with the appropriate nucleotides and ligation of the primers. Other, modification of PCR include the strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), branched DN (b-DNA) and line probe assay (LiPA).

c. Phenotypic Methods:

FASTPlaque TB:
This is an original phage based test, which uses the mycobacteriophage to detect the presence of M. tuberculosis directly from the specimens. It is a rapid, manual test, easy to perform and has an overall higher sensitivity when compared with sputum smear microscopy, in newly diagnosed smear positive TB patients. The test has a specificity of 98.7-99.0% and sensitivity of 70.3-75.2% when compared with smear microscopy, which has a specificity of 97.3-97.4% and a sensitivity of 61.3-63.4%.

Serological Diagnosis of Tuberculosis:
Most of the serological tests have low turnaround time, high negative predictive value and are useful as screening tests. The limitation of these tests is low sensitivity in smear negative patients, HIV positive cases, and disease endemic countries with a high infection rate.
Capture ELISA:
A qualitative test to detect lipoarabinomannan (LAM) has been developed for the detection of TB in urine specimens. Another test being used in the field trial is the dipstick method (semi-quantitive) for detection of LAM in both pulmonary and extra-pulmonary specimens. Preliminary reports have shown a sensitivity and specificity of 93 and 95% respectively. The methods used for antigen detection are: the sandwich ELISA, inhibition ELISA, latex agglutination and reverse passive haemaggutination tests.

INDIRECT APPROACH:
Detection of Antibodies for Diagnosis of TB:
Antibodies to mycobacterial antigens in sera of patients are detected either by using monoclonal or polyclonal antibodies. Cross-reactions by environmental mycobacteria are likely to produce false-positive result.

TB STAT- PAK:
It is a rapid in-vitro assay for the detection of antibody in active TB disease using whole blood or serum. The test employs an antibody binding protein conjugated to a colloidal gold particle and a unique combination of TB antigens immobilized on the membrane.

INSTA Test TB:
It is a rapid in-vitro assay for the detection of antibody in active TB disease using whole blood or serum. The test employs an antibody binding protein conjugated to a colloidal gold particle and a unique combination of Tb antigens immobilized on the membrane.

References

8. The Merck Manual of diagnosis and therapy, Section 17, Chapter 227, urinary Tract Infection.
Illustrations

Illustration 1

CAUSES OF UTI

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<tr>
<th>Causes of UTIs</th>
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