10.5005/jp-journals-10040-1084

REVIEW ARTICLE

Diagnosis of Foot and Ankle Tuberculosis

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ABSTRACT

Osteoarticular tuberculosis (OATB) is reported to occur in 1 to 3% of tuberculosis (TB) cases. Involvement of ankle and foot is a rare entity which comprises 10% of the OATB cases. The biggest diagnostic dilemma associated with OATB comes due to the fact that it is paucibacillary in nature. Newer diagnostic techniques like light-emitting diode microscopy, mycobacterial growth indicator tube (MGIT) culture system, and nucleic acid amplification test-based techniques could aid now in a quicker and definitive diagnosis of TB. Despite numerous studies on this based on pulmonary TB, there is paucity of literature on diagnostics in OATB, especially foot and ankle TB. Significant work is needed to evaluate the efficacy of various diagnostic modalities which would help in timely management, thereby contributing to a better prognosis. The present review summarizes the modern diagnostic modalities and typing techniques that could aid in the management of foot and ankle TB.

Keywords: Diagnosis, Foot and ankle tuberculosis, Osteoarticular tuberculosis.

How to cite this article: Sharma K, Batra N, Sharma M, Goyal K, Sharma A, Dhillon MS. Diagnosis of Foot and Ankle Tuberculosis. J Foot Ankle Surg (Asia-Pacific) 2018;4(3):21-26.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Tuberculosis can virtually involve any organ of the body; of all the patients presenting with TB, 15 to 20% harbor extrapulmonary tuberculosis (EPTB).[1] The burden of EPTB is on the rise, especially in patients with suboptimal immune function. Lymph nodes, meninges, genitourinary tract, bones, and intestine are the various extrapulmonary sites involved.[2] Osteoarticular tuberculosis as a result of hematogeneous seeding is reported to occur in 1 to 3% of TB cases, with the most common sites being the spine and hip.[3] Involvement of bones of ankle and foot is a rare entity and comprises less than 10% of the OATB cases.[4] Chio et al and Hyakumachi et al reported the incidence to be around 4.1 and 8.3% respectively.[5,6] Ankle TB is somewhat common, followed by midfoot involvement. Nevertheless, the available literature is insufficient, with very few large studies, leaving many issues underreported.

Foot and ankle TB presents a significant diagnostic dilemma; because of the nonspecific clinical presentation, it is difficult to differentiate this entity from other conditions like pyogenic osteomyelitis, fungal arthritis, gouty arthritis, and inflammatory processes like amyloidosis and sarcoidosis.[6] The variability in clinical and radiological presentation can lead to diagnostic errors, and the paucibacillary nature of this disease makes techniques like microscopy and culture less sensitive.[7] All these factors hamper timely management and contribute to potentially poor prognoses. Better diagnostic techniques are thus required for timely diagnosis and prevention of further deterioration and local spread or destruction. Another upcoming dimension to this problem is the emergence of multidrug resistance; till date, there are not many studies on drug resistance in OATB. Padarya and Agashe[8] reported 31.62% drug resistance in TB of the spine, followed by foot (8%), tibia (8%), and ankle joint (8%).

The present review emphasizes the methods of confirming the diagnosis, and summarizes the various diagnostic modalities and typing techniques that can aid in the management of foot and ankle TB.

DIAGNOSTIC TECHNIQUES

Histopathology

Patients with OATB based on clinical suspicion, when presenting with chronic pain, swelling, or deformity are subjected to undergo radiological examination to differentiate it from other diseases. Being an excellent mimicker, OATB can not solely be diagnosed based on radiographic findings because of the low sensitivity and specificity. Hence, tissue evaluation is the key to diagnosis confirmation. Granulomatous inflammation with or without a typical tubercle, with caseous necrosis, is the key finding on histopathology.[5] Biopsy from the site of infection is recommended as establishment of an early diagnosis will facilitate in providing early treatment to the patient.

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Microscopy

One of the rapid and cost-effective methods of diagnosing TB is microscopy after biopsy. Conventional microscopy technique like Ziehl–Neelsen (ZN) staining is being replaced by recent advances like light-emitting diode (LED) microscopy, because of its lower sensitivity.[7] According to the World Health Organization (WHO), the LED microscopy has shown a sensitivity of 84%, which is 6% higher than the conventional method and a specificity of 98%.[9] It also offers the advantage of being more rapid (mean time per smear examination of 1.41 *vs* 2.48 minutes for ZN stain) and cost-effective.[10] Though there are many studies on pulmonary TB, there is a paucity of literature in EPTB. Shenai et al showed a lower sensitivity and specificity of 34 and 88.8% respectively, for microscopic examination in EPTB samples as compared with 78.3 and 92% respectively, in pulmonary samples.[10] Alvarez-Uria et al[11] also observed a smear positivity rate of 9.1% for EPTB samples as against 63.9% for pulmonary samples on LED microscopy. A single study, conducted by Gu et al[12] on bone and joint TB using LED microscopy, reported a sensitivity of 26%. Further studies evaluating the role of microscopy, specifically for foot and ankle TB, will help in defining its true diagnostic potential.

Culture

The gold standard for confirmative diagnosis of TB is the isolation of tubercle bacilli. Culture not only has a higher sensitivity [10^2 colony-forming units (CFU)/mL] than microscopy (10^5 CFU/mL), but also is beneficial in species identification and drug susceptibility testing. Table 1 summarizes the studies done on extrapulmonary samples using solid Lowenstein–Jensen (LJ) culture medium, with the culture positivity rate varying between 11 and 60%.[13-16] An overall low sensitivity and turnaround time of 6 to 8 weeks needed for solid culture media has led to the introduction of better culture methods. A liquid culture-based, fully automated, continuously monitoring system BACTEC MGIT 960 has revolutionized the otherwise tedious method of "growing" Mycobacterium tuberculosis (MTB) for identification as well as drug susceptibility testing. It offers advantages of faster results and higher yield by 10% in case of EPTB.[7] A meta-analysis of 10 studies on pulmonary TB concluded that MGIT had an appreciable sensitivity and specificity of 81.5 and 99.6% respectively.[17] Unlike pulmonary TB, limited studies exist on the evaluation of MGIT in OATB. The overall sensitivity of MGIT in diagnosis of foot and joint TB has been reported to be 48% by Gu et al,[12] while Agashe et al[18] reported a higher sensitivity of 97% with a specificity of 82%. Agashe et al[18] further reported that the culture positivity rate was highest for pus samples (82%) followed by tissue biopsies (45%), and none of the synovial fluid samples was positive. The average isolation time was 14 days as compared with 36 days with LJ culture. Negi et al[19] conducted a study wherein they compared all the diagnostic techniques with BACTEC, showing a low positivity rate of 39.13%. A study done by Wang et al[20] compared LJ culture with MGIT 960 for the diagnosis of OATB and showed an overall diagnostic efficacy of 26.92 and 48.08% respectively. The positivity rate in granuloma, pus, and caseous necrosis samples was 19.61, 17.31, and 13.64% respectively, with LJ culture and 31.37, 38.46, and 31.82% respectively, with MGIT 960.[20]

Table 1: Culture positivity rate using LJ culture on OATB samples

Sample type	*Culture positivity rate*	*References*
EPTB	11.2%	Yoong et al[13]
OATB	63%	Haider[14]
OATB	19.2%	Muangchan and Nilganuwong[15]
OATB	11.1%	Sharma et al[16]

Molecular Methods

Diagnostic techniques based on nucleic acid amplification test (NAAT) have led to a marked improvement in the diagnosis of TB. Along with diagnosis, detection of resistance is an added advantage with these techniques. Considering the low diagnostic sensitivity and prolonged turnaround time of conventional techniques in EPTB, molecular techniques are the need of hour.

Polymerase Chain Reaction

The NAAT-based molecular techniques can be beneficial in varied forms of EPTB. The deoxyribonucleic acid (DNA) extracted from samples, subjected to amplification of target genes, is summarized in Table 2.[21-26] Molecular techniques offer the advantage of having a higher analytical sensitivity with detection limit of 1 to 10 organisms. A study done by Siddiqui et al[26] on 100 extrapulmonary samples yielded a positivity rate of 70, 15, and 5% with

Table 2: Various targets of PCR in studies on OATB

Gene target	*Sensitivity (%)*	*Specificity (%)*	*References*
IS6110	100	N.D.	Verettas et al[21]
	85	80	Jambhekar et al[22]
	94	63	Agashe et al[18]
	73	94	Pandey et al[23]
	80	100	Sharma et al[16]
rpoB	53	N.D.	Yun et al[24]
65 kDa	78	100	Negi et al[19]
16S rRNA gene	98	N.D.	Jain et al[25]

ND: No data

polymerase chain reaction (PCR), culture, and ZN staining respectively. There are very limited studies conducted on foot and ankle TB. The in-house nested IS6110 PCR developed by Agashe et al[18] demonstrated positive result in 94% of the confirmed cases, 88% of probable cases, and 32% of the possible cases. An overall sensitivity and specificity of 93 and 63% respectively, were seen when compared with culture.[18] IS6110, although the most commonly targeted region, suffers from a drawback of being absent in 10 to 40% of Indian isolates.[10] Hence, targeting more number of genes leads to an increase in sensitivity. Recently, Sharma et al[27] developed a multiplex PCR targeting IS6110 and MPB-64 protein genes on pus and synovial fluid samples from 80 cases of OATB. A high sensitivity of 100 and 81.8% was reported among the confirmed and suspected cases respectively, with a specificity of 100%.[28] Colmenero et al[28] recently developed multiplex real-time PCR targeting 31 kDa of *Brucella abortus* and Sen X3-Reg X3 (intergenic region of MTB) in differentiating tubercular vertebral osteomyelitis and brucellar vertebral osteomyelitis, with an overall sensitivity and specificity of 93.3 and 90% respectively. Limitations of molecular techniques are that they are expensive, show false-positivity, need technical expertise, and cannot differentiate viable and dead bacteria.

Loop-mediated Isothermal Amplification

Loop-mediated isothermal amplification (LAMP), a novel molecular technique having shown high sensitivity and specificity in pulmonary TB, is now emerging as a promising technique for rapid diagnosis of EPTB. The LAMP assays are performed under isothermal conditions using an ordinary water bath or heating block. Generation of large amounts of DNA using *Bacillus stearothermophilus* DNA polymerase in less than an hour makes LAMP a favored method for rapid diagnosis. The ease of result interpretation, by noting the increase in turbidity either by naked eye or by adding fluorescent dyes like SYBR Green and visualizing under ultraviolet light, makes LAMP technically less demanding than conventional PCR.[24] Not only this, the three primer sets used in LAMP make it more sensitive and specific than a single set used in PCR against the same target gene. Although not many studies have been conducted, a study done by Sharma et al[27] on OATB using IS6110 and MPB64 as gene targets showed an overall sensitivity and specificity of 90 and 100% respectively. The overall sensitivity using IS6110 PCR, IS6110 LAMP, and MPB64 LAMP was 80, 100, and 100% respectively, for confirmed cases and 72.5, 81.75, and 86.25% respectively, for probable cases.[24] Thus, this technique can aid in a reliable and early diagnosis.

GenXpert

Based on the principle of hemi-nested real-time PCR, the Foundation for Innovative New Diagnostics has developed a novel molecular-based diagnostic technique. The major advantage of this cartridge-based technique is that it requires minimal technical expertise, enabling diagnosis of TB along with rifampicin resistance detection (MTB/RIF assay) in a time period of 2 hours.[29] It was initially developed and standardized for pulmonary TB but is now being assessed for EPTB. The reported sensitivity generally exceeds 50%, varying from 25 to 95.1% in cases of EPTB.[7] In a study done by Vadwai et al,[30] the sensitivity and specificity of GenXpert was 81 and 99.6% respectively, when compared with culture (both solid and liquid). In another comparison done by Alvarez-Uria et al[11] between LED microscopy and GenXpert testing, positivity rate of EPTB was 9.1 and 29.2% respectively. There are very few studies on foot and ankle TB. Gu et al[12] in their study identified 75.68% of smear negative and 65.38% of culture negative specimens, with an overall sensitivity and specificity of 82 and 100% respectively. Also the sensitivity for detecting rifampicin resistance was observed to be 100%.[12] Table 3 summarizes the performance of limited number of studies done on Xpert MTB/RIF on foot and joint TB and EPTB.[31,32] Currently under development are GeneXpert Omni and GeneXpert Ultra, which are being proposed as rapid point of care tests.

Molecular Line Probe Assays

Line probe assay (LPA) is a reverse probe-based hybridization technique used for diagnosis along with rifampicin and isoniazid resistance. It was endorsed by the WHO in 2008. Previously two companies INNO-LiPA Rif. Tuberculosis test (Innogenetics NV, Gent, Belgium) and the Geno Type MTBDR*plus* test (Hain Lifescience GmbH, Nehren, Germany) were available.[10] Geno Type MTBDR

Table 3: Performance of Xpert MTB/RIF in studies on OATB and EPTB

Manufacturer	*Diagnosis*	*Sensitivity (%)*	*Specificity (%)*	*References*
Xpert MTB/RIF assay (Xpert)	Bone and joint TB	82	100	Gu et al[12]
Xpert MTB/RIF assay (Xpert)	Spinal TB	95.6	96.2	Held et al[31]
Xpert MTB/RIF assay (Xpert)	EPTB	77.3	98.2	Hillemann et al[32]
Xpert MTB/RIF assay (Xpert)	EPTB	52.1	100	Zeka et al[33]
Xpert MTB/RIF assay (Xpert)	EPTB	81.3	99.8	Lawn and Zumla[34]

test was released in 2009, which can detect extremely drug resistant (XDR) TB, i.e., resistance to the second line of drugs which includes fluoroquinolones, ethambutol, and aminoglycosides. Studies show high sensitivity and specificity of 97 and 99% respectively, for detection of MDR TB.[35] The LPA has been used mainly on pulmonary TB samples which are smear positive. There are very few studies on EPTB for the performance of LPA. A study conducted by Farooqi et al[36] showed sensitivity of 71.4% and specificity of 92.8% using Geno Type MTBDR plus 2.0 Haine Lifescience. Another study by Gu et al[12] done on bone and joint TB using Geno Type MTBDR identified 62.16% of smear negative and 57.69% of culture negative specimens with a sensitivity and specificity of 72 and 100% respectively. The sensitivities for detection of rifampicin and isoniazid resistance were 83.3 and 85.7% respectively. More studies need to be conducted to validate these findings. Major advantages of LPA over other techniques are rapid detection along with direct detection of rifampicin and isoniazid resistance. The disadvantage is the requirement of proper technical expertise.

Typing Techniques

Evolution of mycobacterial strain typing techniques is paving the way to assess the spread of MTB and its correlation with the drug resistance strains. Outbreak investigation, differentiation between exogenous reinfection and endogenous reactivation, and diagnosis of mixed infections are some of the advantages offered by these techniques. The various typing techniques include IS6110-based restriction fragment length polymorphism (RFLP) analysis, IS6110-based PCR fingerprinting, spoligotyping, double repetitive element typing, MTB-specific multiple locus (MIRU)-variable number of tandem repeats (VNTR), repetitive sequence-based (rep) PCR, random amplified polymorphic DNA analysis, amplified fragment length polymorphism, multilocus sequence typing, single-nucleotide polymorphism (SNP) typing, and deletion mapping and deligotyping.[37] An ideal typing technique should be easy, cost-effective, and reproducible. Every method has its own advantage and shortcoming. The choice of typing technique depends on the sample under investigation. Earlier, IS6110-RFLP was considered the gold standard for genotyping. But because of its limitation of being a time-consuming procedure, now 24 locus MIRU-VNTR combined with spoligotyping has taken over.[38] These techniques help in studying the different lineages of MTB circulating among the patient population. This not only allows an insight into the molecular epidemiology of the strains but also highlights specific clinical correlations attributable to a particular genotype. For instance, among the various lineages of MTB, multidrug resistance has been found with the Beijing genotype, and early identification of such strains may guide in targeted therapy, thus improving patient management.[39]

Whole Genome Sequencing

There is a shift in drug resistance from MDR to XDR TB strains. This makes it worrisome and elimination of TB a more challenging task. Drug susceptibility testing by phenotypic methods is time consuming, and molecular diagnostic techniques have been mainly standardized to identify the common resistance mechanisms. Thus, with the upcoming sequencing techniques, it becomes possible to identify all sorts of common and rare mutations. The rate of mutation has been found to be constant at the rate of 0.5 SNPs per genome per year.[40] Compared with other typing techniques, whole genome sequencing (WGS) has shown a higher resolution when compared with MIRU-VNTR for typing of MTB strains.[41] The WGS has also been used in studying the various genetic lineages of MTB and is also being studied to predict drug susceptibility and resistance. The data generated could be incorporated and used for routine diagnostic approach, thus helping in an earlier detection of resistance.

CONCLUSION

The availability of rapid and accurate diagnostic techniques is enhancing the physicians' ability in diagnosing TB in extra-articular sites. Diagnostic techniques like LED microscopy, MGIT culture system, and NAAT-based techniques are the recent advances in diagnosis of TB. Molecular techniques offer advantages of higher sensitivity, faster turnaround time, and simultaneous drug resistance detection and thus are becoming favorable diagnostic modalities. Though there are a lot of studies on pulmonary TB, there is limited literature on EPTB, especially foot and ankle TB. Hence, more studies need to be conducted to assess the performance of these diagnostic techniques in this unique situation, where biopsy may be difficult and the disease is paucibacillary. Additional research focused on this area would further enhance the sensitivity of specific methods in this era of rapid advancement in typing techniques and WGS.

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