Infection and Drug Resistance

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ORIGINAL RESEARCH

Diagnostic Role of Metagenomic Next-Generation Sequencing in Tubercular Orthopedic Implant-Associated Infection

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Objective: The diagnosis of tubercular orthopedic implant-associated infection (TB-IAI) is challenging. This study evaluated the value of metagenomic next-generation sequencing (mNGS) for the diagnosis of TB-IAI and developed a standardized diagnostic procedure for TB-IAI.

Methods: The records of all patients with TB-IAI diagnosed and treated at our institution between December 2018 and September 2022 were retrospectively reviewed. Patient demographic characteristics, medical history, laboratory test, microbial culture, histopathology, and mNGS results, and time to diagnosis were recorded. The diagnostic efficiency of mNGS for TB-IAI was assessed by comparing the results and diagnostic time with that of other diagnostic modalities.

Results: Ten patients were included in the analysis, including eight with prosthetic joint infections and two with fracture-related infections. The mNGS positivity rate was 100% (10/10), which was higher than that of TB-antibody (11%, 1/9), real-time quantitative polymerase chain reaction (22%, 2/9), T-SPOT.TB (25%, 2/8), purified protein derivative (50%, 4/8), microbial culture (50%, 5/10), and histopathology (20%, 2/10). mNGS shortened the time to diagnosis of TB-IAI. A standardized diagnostic procedure for TB-IAI was developed based on the findings.

Conclusion: mNGS is useful for the diagnosis of TB-IAI. mNGS is recommended in cases where it is difficult to identify a pathogen using routine diagnostic tests. The standardized diagnostic procedure might improve TB-IAI diagnosis.

Importance: TB-IAI is a rare infection, which occurs after orthopedic surgery and hard to diagnose microbiologically. mNGS is a new detection technique not yet discussed in current literature as a means for TB-IAI diagnostics. Here we describe a cohort of patients with TB-IAI diagnosed by mNGS show high efficiency of mNGS for detection of this pathology and present a clinical algorithm supplementing conventional methods for TB-IAI assessment.

Keywords: metagenomic next-generation sequencing, implant-associated infection, *Mycobacterium tuberculosis* complex, orthopedic infection, diagnosis

Introduction

Medical implants are widely used in orthopedic treatments. However, implant-associated infection (IAI) is a catastrophic complication of orthopedic surgery.[1] According to statistics, the incidence of IAI ranges from 1 to 30%.[2] Tubercular IAI (TB-IAI) is a rare infection caused by the Mycobacterium tuberculosis complex (MTBC), constituting approximately 0.14% of the cases of tuberculosis (TB).[3] Its incidence is very rare, and only a small number of cases have been reported in the literature to date. The low prevalence and lack of clinical suspicion may lead to a delay in the diagnosis or even

Received: 13 October 2023
Accepted: 8 May 2024
Published: 17 May 2024

misdiagnosis of TB-IAI, potentially resulting in multiple surgical procedures until MTBC is diagnosed.[4,5] It has been reported that the length of time until a diagnosis was made, ranged from 1 month to 3 years.[6] Mahale et al[7] reported 17 patients with implant-associated mycobacterium tuberculosis infection, of which 9 patients had delayed diagnosis (more than three months).

Pathogen diagnosis is an important link in the diagnosis of IAI; however, negative pathogenic cultures often increase the difficulty in the diagnosis and treatment of the disease. The MTBC culture method is time-consuming and cultivation takes up to 2–8 weeks to produce results with a low positivity rate, so it is not useful for clinical guidance.[8] MTBC-specific diagnostic tests, such as TB-antibody (TB-Ab), real-time quantitative polymerase chain reaction (qPCR), the tuberculin purified protein derivative (PPD) test, and T-cell spot test (T-SPOT.TB) have a very low positive rate at the diagnosis of extrapulmonary tuberculosis. It has been reported that the rate of laboratory-confirmed positivity is approximately 1%.[9] Although the acid-fast staining is quick and effective in diagnosing TB, it has a false negative rate of 30%–65%, which makes TB often missed.[8] A timely and accurate diagnosis of MTBC is urgently needed for TB-IAI to facilitate faster and more targeted treatment. Metagenomic next-generation sequencing (mNGS) is a fast, sensitive, and accurate diagnostic method for pathogens detection, which has been increasingly applied in the field of clinical infectious diseases.[10] The advantages of mNGS are reflected in identifying potential pathogens from culture-negative cases with which the positivity rate is as high as 81.9%.[11] However, to the best of our knowledge, no study has systematically evaluated the value of mNGS for the diagnosis of TB-IAI. Thus, we conducted a retrospective cohort study to evaluate the utility of mNGS for TB-IAI diagnosis.

Methods

Patient Selection

This study was approved by the local ethics committee. Patients with suspected TB-IAI who were diagnosed at our center between December 2018 and September 2022 were retrospectively enrolled in this study. Periprosthetic joint infection (PJI) was diagnosed based on the 2018 International Consensus Meeting diagnostic criteria for PJI,[12] and fracture-related infections (FRI) were diagnosed according to the consensus definition of FRI published in 2018.[13] Patient who met the following criteria was included: 1) diagnosis of IAI in accordance with the international consensus guidelines and underwent surgery at our center; 2) suspected TB-IAI according to the patients' medical history, pathogen detection (mNGS or culture), laboratory test results (TB-Ab, qPCR, PPD test, and T-SPOT.TB), and histopathological examination (intraoperative observation of typical granulomatous inflammation or Ziehl–Neelsen-positive staining); and 3) available full medical history and follow-up information. The exclusion criteria were as follows: 1) the clinical and laboratory findings were insufficient for the diagnosis of TB-IAI; 2) specimens were contaminated or suspected of contamination during sampling, transfer, or processing; and 3) presence of other inflammatory diseases or malignant tumors that might have affected laboratory test results. Demographic characteristics, medical history, physical signs, serum inflammatory indices (erythrocyte sedimentation rate and C-reactive protein), synovial fluid white blood cell count, percentage of polymorphonuclear cells in the synovial fluid, triple-phase bone scan or positron emission tomography–computed tomography (PET-CT), MTBC-specific laboratory tests (TB-Ab, qPCR, PPD test, or T-SPOT. TB), histopathological examination, conventional microbial culture, and mNGS results were recorded.

Specimen Collection and Processing

The infection site was punctured under ultrasonic guidance by the same physician before surgery. Samples were sent for white blood cell (WBC) count determination, culture, and mNGS. In patients in whom preoperative puncture failed, joint or purulent fluid (PF) was collected during surgery, and the samples were sent for WBC count determination and conventional microbiological culture. Additionally, intraoperative samples including periprosthetic tissue (PT) and prosthetic sonicated fluid (PSF) were collected. At least five PT samples were collected intraoperatively from sites with the most obvious inflammation for pathological frozen section examination, histopathological examination, and culture. We followed the implant sonication procedure and workflow described by Shen et al.[14] PSF was used for culture. All specimens were transported to the laboratory and processed within 6 h. PT was sent for mNGS if significant

granuloma lesions were observed during surgery; otherwise, PSF was sent. The specimens sent for mNGS testing were stored at −80°C to avoid contamination.

Microbiological Procedures

The PF and PSF culture methods were described by Shen et al,[14] and we followed the tissue culture procedure described by Cai et al.[15] The VITEK Compact Identification System (bioMérieux, Marcy-L'Étoile, France) was used for bacterial identification and antimicrobial susceptibility testing. ATB FUNGUS fungus identification and susceptibility test strips (bioMérieux, La Balme-les Grottes, France) were used for fungus identification and susceptibility testing. TB DNA was extracted and detected using a Diagnostic Kit for *Mycobacterium tuberculosis* DNA (PCR-Fluorescence; Daan Gene, China) and an ABI Prism 7500 Real-Time PCR System.

mNGS

mNGS based on the next-generation sequencing technology, mNGS identifies microorganisms directly from clinically extracted DNAs, followed by library preparation and bioinformatic analysis. This technology that compares the microbial nucleic acid sequences in samples with the existing sequences in the database for analysis, to identify the suspected pathogenic microorganisms in the sample. The mNGS workflow included processing the sample, nucleic acid extraction, construction of DNA libraries, sequencing, and bioinformatic analysis. Full details of the method of sequencing and the algorithm for the identification of a positive result are shown in Supplementary Material and Supplementary Figure 1.

Results

Demographic Characteristics

The demographic characteristics of the patients are shown in Table 1. A total of 12 patients were included, of which 2 patients were excluded due to insufficient laboratory data, resulting in 10 patients. Among these, eight patients had PJI and two had FRI. Only two patients (20%) had a history of TB, including one case of pulmonary TB and one case of joint TB. In the eight patients with PJI, the primary surgical procedures were total knee arthroplasty (n = 4), total hip arthroplasty (n = 2), artificial femoral head replacement (n = 1), and unicondylar knee arthroplasty (n = 1). The two patients with FRI developed postoperative internal fixation infections. Of the seven patients for whom WBC and polymorphonuclear leukocyte (PMN, %) counts were evaluated in the synovial fluid, all results indicated the presence of infection. Triple-phase bone scanning is not recommended when the time interval from prosthesis implantation to bone scanning is less than a year. Nuclear medicine examinations were performed on six patients (five patients underwent triple-phase bone scanning, one patient underwent PET-CT), with a positivity rate of five out of six (83%).

Laboratory Tests, Microbial Culture, Histopathological Examination, and mNGS Results

The results of the laboratory tests, microbial culture, histopathological examination, and mNGS for each patient are shown in Tables 2 and 3. The positivity rates of laboratory tests for MTBC were 11% (1/9) for TB-Ab, 22% (2/9) for qPCR, 25% (2/8) for TSPOT.TB, and 50% (4/8) for the PPD test (Table 2). The mNGS results in all 10 patients (5 PT samples, 4 PSF samples, and 1 PF sample) were positive (Table 3). Two patients were diagnosed with mixed pathogen infection by mNGS, one with a negative culture result and the other with a positive culture for *S. aureus*. The overall positive microbial culture rate was 50% (5/10), of which five cases had a positive culture from PT and only one case had a positive culture from both PSF and PT. Histopathological examination of the PT revealed chronic granulomatous inflammation in six cases, whereas only two out of ten (20%) samples were positive according to Ziehl–Neelsen staining.

Time to Pathogen Detection

The time required for MTBC detection in each patient is shown in Figure 1. Three patients (Patients #1, #4, and #10) had MTBC infections diagnosed only by mNGS, with a time to diagnosis of 2 days. MTBC infection in Patient #3 was determined by mNGS before surgery with a time to diagnosis of 2 days, whereas the PT sample was reported to be

Table 1 Demographic Information of the 10 Patients with Tuberculous Orthopedic Implant Infections

Patient ID	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Type	PJI	PJI	PJI	PJI	PJI	PJI	PJI	PJI	FRI	FRI
Sex/Age	F/64	M/69	M/54	M/58	M/59	M/45	F/36	M/85	M/68	M/55
Underlying disease	Arrhythmias	None	None	DM	HT	None	None	None	HT	HT
History of Tuberculosis	No	Hip joint TB	No	No	No	No	No	PTB	No	No
Primary diagnosis	OA	OA	OA	OA	OA	OA	TFHN	OA	Tibia fracture	Tibia and fibula fracture
Primary surgery	TKA	AFHR	UKA	THA	TKA	TKA	THA	TKA	ORIF	ORIF
Time to infection after surgery	7 y	11 y	2 y	8 m	5 m	2 y	1 y	2 y	20y	1y
Duration of Symptoms	6w	10d	3m	4m	14d	10w	8w	3m	5m	20d
Clinical manifestation	Pain, pus	Pain, pus	Pain, pus	Pain	Pain	Pain, swelling	Pain, swelling, pus	Pain, swelling	Pain, swelling, pus	Pain
ESR (mm/h)	76	12	75	34	35	120	39	42	39	20
CRP (mg/L)	13.3	2.31	29.6	29.71	39.7	81.2	0.5	21.4	7.09	15.93
SF-WBC (× 10^6/L)	32,580	36,145	2400	7471	8034	38,400	/	30,040	/	/
PMN (%)	87	60	82	92	96	90	/	92	/	/
Triple-Phase Bone Scanning	Positive	Negative	Positive	/	/	Positive	Positive	/	/	Positive (PET-CT)

Abbreviations: PJI, prosthetic joint infection; FRI, fracture-related infection; F, female; M, male; DM, diabetes; HT, hypertension; TB, tuberculosis; PTB, pulmonary tuberculosis; OA, osteoarthritis; TFHN, traumatic femoral head necrosis; TKA, total knee arthroplasty; AFHR, artificial femoral head replacement; UKA, unicompartmental knee arthroplasty; THA, total hip arthroplasty; ORIF, open reduction and internal fixation; y, year; m, month; SF, synovial fluid; WBC, white blood cell; PMN, polymorphonuclear leukocyte.

Table 2 Laboratory Tests, Microbial Culture, and Histopathological Examination Results of 10 Patients

Patient ID	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Laboratory tests										
TB-Ab	Negative	Negative	Negative	Negative	Negative	Negative	IgG (+)	/	Negative	Negative
QPCR	Negative	Positive	Negative	Negative	Positive	Negative	Negative	/	Negative	Negative
T-SPOT.TB	Negative	Negative	/	Negative	Negative	Negative	Positive	/	Positive	Negative
PPD	Negative	++	/	Negative	Negative	++++	+++	/	+	Negative
Microbial culture										
PF	Negative	Negative	Negative	Negative	Negative	*S. aureus*	Negative	Negative	Negative	Negative
PSF	Negative	Negative	Acid-fast bacteria	Negative	Negative	*S. aureus*	Negative	Negative	/	/
PT	Negative	Acid-fast bacteria	Acid-fast bacteria	Negative	Acid-fast bacteria	Negative	Acid-fast bacteria	Acid-fast bacteria	Negative	Negative
Histopathological examination										
Intraoperative observation	Chronic inflammation	Chronic granulomatous inflammation	Chronic granulomatous inflammation	Chronic granulomatous inflammation	Chronic granulomatous inflammation	Chronic inflammation	Chronic inflammation	Chronic inflammation	Chronic granulomatous inflammation	Chronic granulomatous inflammation
Ziehl–Neelsen stain	Negative	Negative	Positive	Negative	Negative	Negative	Positive	Negative	Negative	Negative

Abbreviations: TB, tuberculosis; Ab, antibody; QPCR, quantitative Real-time PCR; PF, purulent fluid; PSF, prosthetic sonicate fluid; PT, Periprosthetic tissue;*S.aureus, Staphylococcus aureus.*

https://doi.org/10.2147/IDR.S441940

Table 3 Metagenomic Next-Generation Sequencing Results of 10 Patients

Patient ID	Metagenomic Results		
	Sample	Species	Reads
P1	PSF	Afipia birgiae	159
		Mycobacterium gordonae	13
		MTBC	1
P2	PT	MTBC	32
P3	PF	MTBC	15
P4	PT	MTBC	3
P5	PT	MTBC	7
P6	PSF	*S. aureus*	2027
		Prevotella melaninogenica	6
		MTBC	27
P7	PSF	MTBC	32
P8	PSF	MTBC	99
P9	PT	MTBC	23
P10	PT	MTBC	169

Abbreviations: MTBC, Mycobacterium tuberculosis complex; PF, purulent fluid; PSF, prosthetic sonicate fluid; PT, Periprosthetic tissue; *S. aureus*, *Staphylococcus aureus*.

positive according to Ziehl–Neelsen staining 8 days after the operation. In the same patients, the culture results of PSF and PT samples were reported to be positive after 25 and 28 days, respectively. Preoperative laboratory examination findings of Patients #2, #5, #6, #7, and #9 suggested a diagnosis of MTBC infection; however, patients #6 and #9 were

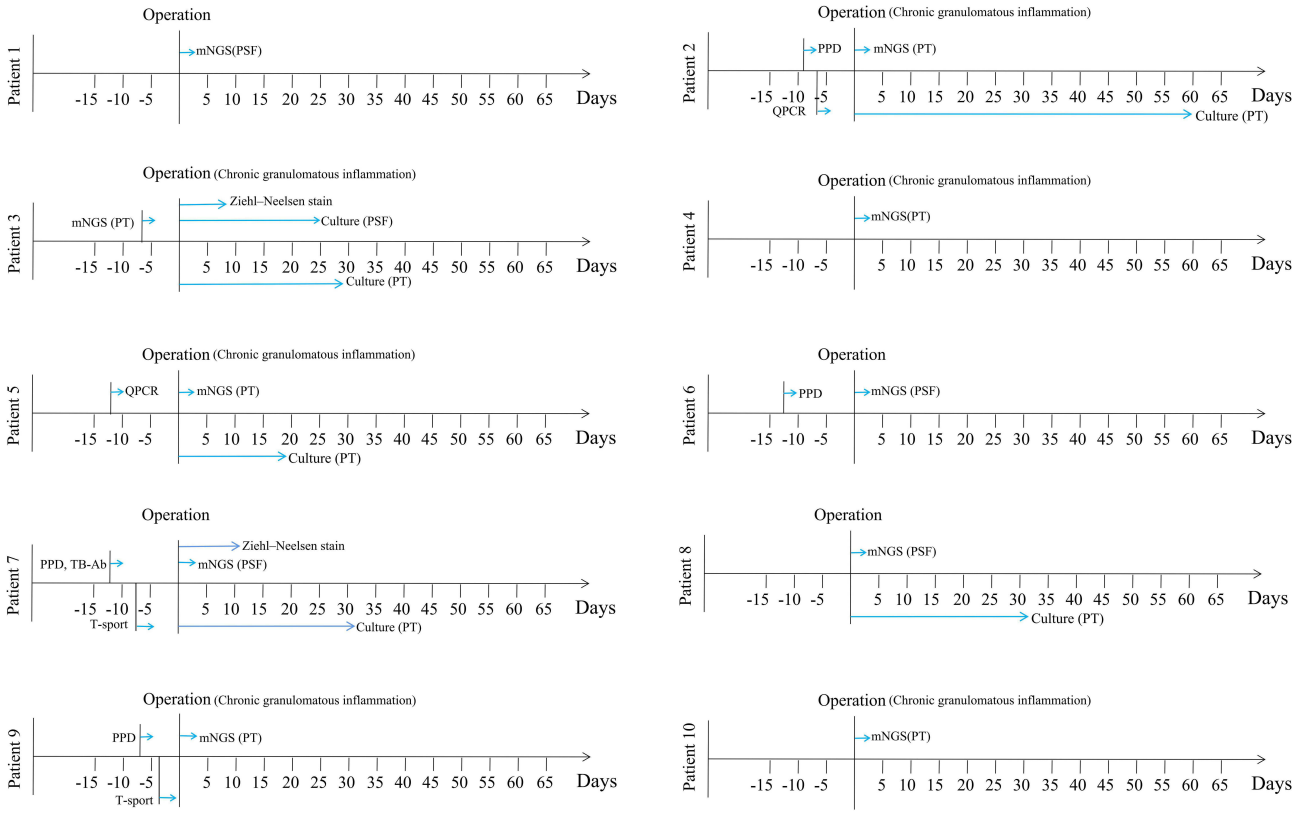

Figure 1 The performance of laboratory tests, culture, mNGS and histopathology for preoperative and intraoperative diagnosis of MTBC. Time and results of pathogen detection using laboratory tests, culture, mNGS and histopathological examination of each individual patient are respectively presented.

identified as having MTBC infection by mNGS alone 2 days after surgery. The PT samples of Patients #2 and #5 were positive according to both mNGS and culture assays postoperatively (2 days vs 60 days and 2 days vs 18 days, respectively). The PSF sample of Patient #7 was determined to be MTBC infection by mNGS (2 days) after surgery, with the PT sample reported to be positive for Ziehl–Neelsen staining and culture at 11 days and 31 days after surgery, respectively.

The Standardized Diagnosis Procedure for TB-IAI

Based on the experience of these 10 cases, we introduced a standardized diagnostic procedure for TB-IAI (Figure 2). Patients are suspected of having TB-IAI if they have a previous history of TB, if they are from a region in which TB is endemic, or if they have multiple negative cultures prior to undergoing surgery. TB-antibody, T-SPOT.TB, and PPD tests are performed on admission. Wound swabs are assessed by qPCR for TB DNA. Next, an ultrasound-guided biopsy is taken of the infectious site under sterile conditions. Joint or PF is sent for culture, qPCR, and mNGS. TB-IAI is highly

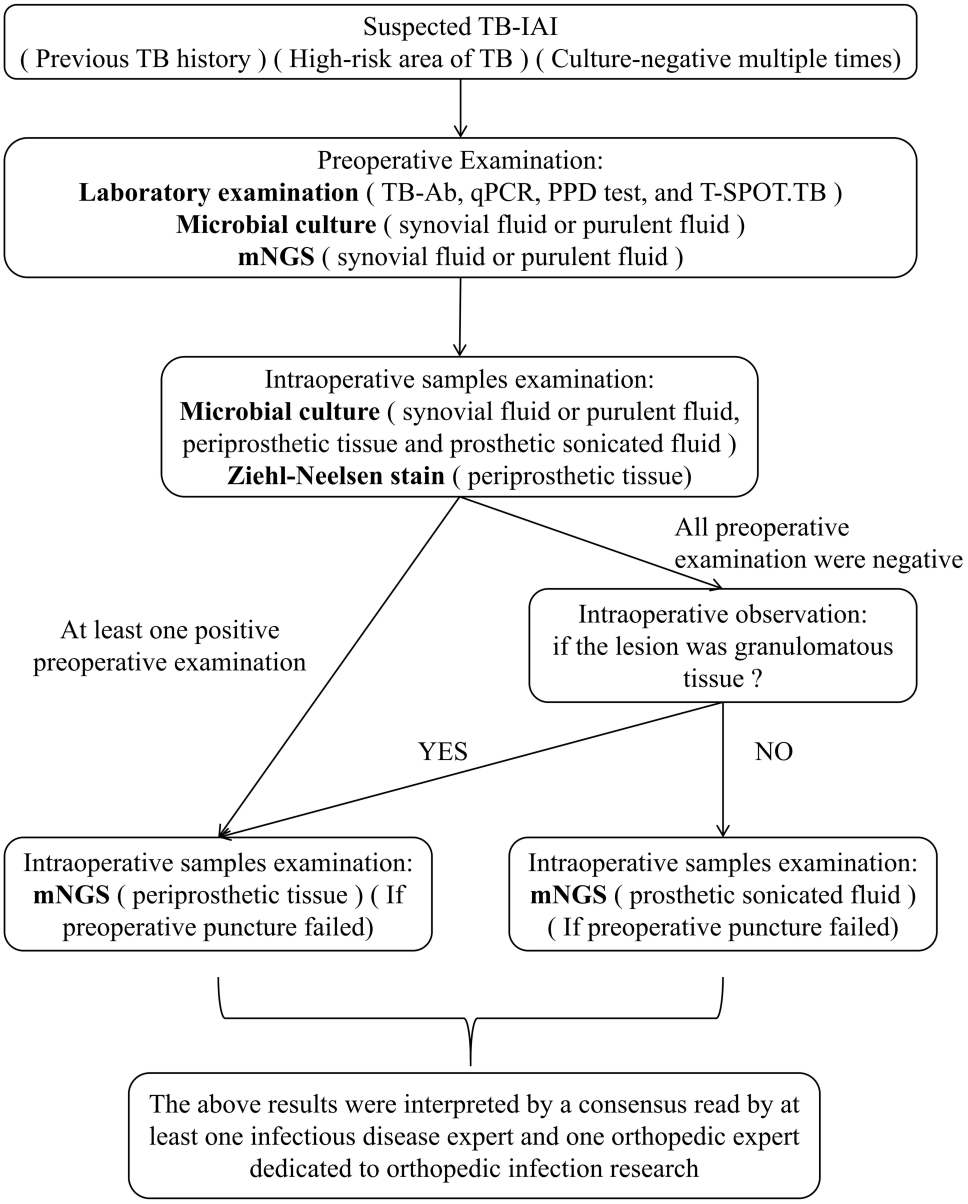

Figure 2 The standardized diagnosis procedure for TB-IAI.

suspected in patients with at least one positive test result. Joint or PF, PT, and PSF are collected during surgery and sent for conventional microbiological culture. Furthermore, PT is sent for mNGS if preoperative puncture fails. In patients with negative results, PT is sent for mNGS if typical granulomatous lesions are detected during surgery, and PSF is sent for mNGS if non-specific inflammation is found. The process of microbial culture is the same as that described above. PT samples are sent for Ziehl–Neelsen acid-fast staining. The results of these tests are interpreted by at least one infectious disease specialist and one orthopedic surgeon with experience in IAI research.

Discussion

Pulmonary and extrapulmonary TB, caused by M. tuberculosis, is an ancient disease with an enormous global impact. Despite the availability of potent anti-TB drugs and routine BCG vaccination, the global TB epidemic remains serious.[16] Rapid and accurate diagnosis and adequate treatment are key issues in TB management, particularly in the case of latent TB infection. TB-IAI is rarely observed in cases with extrapulmonary TB, and guidelines for treating TB-IAI have not yet been established. The duration of TB-IAI diagnosis ranges from 1 month to 3 years owing to an unusual clinical presentation. In cases of mixed infections, often only common bacteria are diagnosed and MTBC may be overlooked.[6] Moreover, previous studies have reported that only 33–45% of the patients with TB-IAI have a history of TB, which adds to the difficulty in diagnosis.[17] The mNGS is a new pathogenic diagnostic technology, and many successful cases and studies have proven its great potential in IAI pathogen diagnostics.[18] However, few studies have investigated the value of mNGS for the etiological detection of TB-IAI. We could only enroll a small series of 10 patients in this study because of the low rate of TB-IAI, although this is the largest cohort of patients with TB-IAI diagnosed by mNGS described to date.

In this study, we observed that compared with conventional culture, laboratory examination (TB-Ab, qPCR, PPD test, and T-SPOT.TB), and histopathological examination, mNGS is a rapid and accurate method for MTBC identification and TB-IAI diagnosis. Unlike NTM, MTBC is not common in clinical settings or air environments.[19] A shortcoming of the high sensitivity of mNGS is that it can increase false-positive errors due to contamination, unbiased nucleic acid amplification, and human-colonizing bacteria.[20] However, this shortcoming was not applicable to the results of MTBC. Therefore, even though the sequencing reads were very low, they also have great significance in the diagnosis of TB-IAI. In the study of Liu et al,[8] there were no significant differences in the positive rate of mNGS and T-SPOT for pulmonary TB (100% vs 95%), whereas for extrapulmonary TB, the difference between the detection rate of mNGS and T-SPOT was statistically significant (100% vs 27.3%). Further, Xpert MTB/RIF is also a new rapid molecular diagnostic method using an automated real-time PCR test,[21] which can directly detect *M. tuberculosis* DNA and rifampicin resistance in clinical specimens within approximately 2 h.[22] It has been endorsed by the World Health Organization for TB diagnosis.[23] In a study by Li et al,[20] there was no significant difference in the sensitivity of T-SPOT.TB, mNGS, and Xpert for the diagnosis of spinal TB; however, T-SPOT.TB had a significantly lower specificity than mNGS and Xpert because of the high latent infection rate of TB. Xpert has the same sensitivity as mNGS for the diagnosis of spinal TB, but the sensitivity of both methods was significantly higher than those of culture and histopathological examination. This is consistent with the conclusions of this study. In addition, although the diagnostic performances of mNGS and Xpert are similar, a smaller sample volume is required for mNGS testing.[24] Hence, mNGS might be more suitable for small-volume samples, such as preoperative synovial fluid. Moreover, Xpert MTB/RIF Ultra (Ultra) is a new version of Xpert that has been introduced to increase the sensitivity for *M. tuberculosis* DNA detection and the specificity for the detection of rpoB mutations.[22] Further research is needed to compare the diagnostic performance of mNGS, Xpert, and Ultra in patients with TB-IAI.

We sought the literature for studies on mNGS and TB-IAI found seven previously reported cases of TB-IAI diagnosed using mNGS in five studies.[15,25–28] All seven cases were associated with PJI. MTBC was not cultured in any of the seven cases, whereas mNGS indicated MTBC infection. This is in accordance with previous observations, and the diagnostic accuracy and detection time of mNGS were superior to those of culture. In this study, we found that the positivity rate of PT culture was the highest. Previous studies have reported that sonication of explanted prostheses followed by incubation detected more pathogens than conventional cultures owing to biofilm removal.[14] However, MTBC has poor biofilm-forming ability.[4,29] Hence, when patients are highly suspicious of having TB-IAI based on their preoperative medical history and laboratory test results, PT should be sent for both mNGS and culture, which may

improve diagnostic efficiency. Based on previous reports and the experience of the 10 cases, we proposed a standardized diagnostic procedure for TB-IAI. As far as we know, this study is the first to propose a diagnostic procedure for TB-IAI, which can also provide reference value for the diagnosis of IAI caused by other rare pathogens.

In this study, mNGS has greatly improved our ability in diagnosing TB-IAI. However, it might not properly guide clinical management due to the lack of information on antibiotic resistance. It has been reported that mNGS could sequence the antimicrobial resistance genes (ARG); there was a high consistency between these ARGs and drug sensitivity tests.[30,31] Predicting resistance genes by mNGS provides us with a new strategy to infer antimicrobial resistance, but has not been reported in the field of orthopedic IAI. Further research is needed to investigate clinical application value in orthopedic infection, which might provide a basis for guiding clinical medication.

This study has several limitations. First, the sample size was relatively small owing to the rare incidence of TB-IAI and the study was confined to a single center, which may limit the generalization of the findings. A larger sample size and multicenter validation are necessary for further investigation to verify the value of mNGS. Second, there might have been other cases of TB-IAI that existed during the study period but were misdiagnosed due to the absence of mNGS tests or negative culture results. Third, mNGS provides limited information on the drug sensitivity of MTBC and cannot provide guidance for the treatment of multidrug-resistant and extensively drug-resistant TB. Finally, our study did not compare the sensitivity and specificity of Xpert, Ultra, and mNGS in the detection of TB-IAI; further studies are required.

In conclusion, this study showed that mNGS can improve the diagnosis of TB-IAI. We recommend the use of mNGS for routine examinations when treating IAI caused by suspected MTBC.

Ethical Approval Statement

This study was approved by the ethics committee of Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China and complied with the requirements of the Declaration of Helsinki. All patients consented to the surgical and diagnostic procedures. Due to the retrospective nature of this study, the ethics committee of Shanghai Sixth People's Hospital waived the requirement for informed consent for inclusion in the analysis.

Acknowledgments

We are grateful to the Department of Clinical Laboratory of Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine for providing us with the pathogen information of the patients. We thank BGI Genomics, China, for performing the mNGS testing and the bioinformatics analysis.

Funding

This work received no specific funding.

Disclosure

The authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work.

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