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ORIGINAL RESEARCH

Comparison of the Diagnostic Performance of Culture, Histopathology, and mNGS for Periprosthetic Joint Infection Using Periprosthetic Tissue Samples: A Prospective Clinical Study

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Background: This study evaluated the applicability of histopathology, culture, and Metagenomic next-generation sequencing (mNGS) in diagnosing periprosthetic joint infection (PJI).

Methods: In this prospective trial, 215 consecutive patients with suspected knee PJI were enrolled. Tissue specimens were aseptically collected and processed for histopathological analysis, culture, and mNGS. PJI diagnosis was primarily based on the 2011 MSIS criteria, with reference to the 2018 ICM criteria for improved diagnostic accuracy. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR) of each diagnostic method were calculated.

Results: Among 58 patients included in the final analysis, 38 were diagnosed with PJI and 20 without PJI. The mNGS assay demonstrated a sensitivity of 63.2% (95% CI: 53.6–77.7%), specificity of 80.0% (75.7–90.1%), PPV of 85.7% (76.4–95.3%), NPV of 53.3% (44.6–61.2%), PLR of 1.84 (1.22–2.77), and NLR of 0.27 (0.10–0.40). Culture showed higher specificity at 95.0% (84.6–-99.8%) and PPV at 96.5% (88.7–99.7%), with sensitivity of 68.4% (58.2–78.9%). Histopathology exhibited 52.6% sensitivity and perfect specificity (100%). The most commonly detected pathogens by both culture and mNGS were *Staphylococcus aureus* and coagulase-negative Staphylococci, which are frequently implicated in PJI.

Conclusion: mNGS shows promise as a complementary tool for diagnosing PJI, especially in culture-negative or atypical cases. However, it did not outperform conventional methods in accuracy. Its limitations-including a high false-positive rate, interpretive challenges, and lack of susceptibility data-warrant cautious use. Further large-scale studies are needed to define its role in clinical decision-making.

Keywords: periprosthetic joint infection, metagenomic next-generation sequencing, bacterial culture, histopathology, diagnosis

Introduction

Periprosthetic joint infection (PJI) is one of the leading causes of primary joint arthroplasty failure and joint revision failure.[1–4] PJI is an ongoing issue in joint arthroplasty because the fatality rate associated with PJI-related revision is five times higher than aseptic revision.[5,6] Hence, a quick diagnosis is profoundly crucial in the early management of PJI. There are diverse diagnostic methods for PJI, such as preoperative laboratory examination, tissue cultivation, plain radiographic imaging, and joint aspiration.[7–10] Laboratory examination, however, has inadequate sensitivity and specificity, making it challenging to differentiate PJI from joint failures of other etiology (eg, aseptic loosening).[11–13] Periprosthetic histopathology is a frequently applied method for diagnosing PJI as neutrophils can be seen histologically

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as a response to the infected joint.[14] The presence of PJI can be determined by recording the neutrophil counts (PMN) per high-power field (HPF).[15] Due to the high sensitivity and specificity, The Musculoskeletal Infection Society (MSIS) has included leukocyte numbers per high-power field as a minor diagnostic criterion in their guidelines.[16] Recently, reports have demonstrated that this diagnostic method has higher accuracy than preoperative tests.[17,18] However, its samples could only be obtained intraoperatively, and therefore it cannot guide the preoperative treatment effectively. Hence recently, the emphasis has been shifted to the application of DNA sequencing techniques for diagnosing PJI and identifying organisms.[19–21]

Metagenomic next-generation sequencing (mNGS), which integrates bioinformatics analysis and high-throughput sequencing, has emerged as a revolutionary technique. After nucleic acid examination from direct sample extracts, it performs the abundance and species recognition of all the microbial samples by using the BLAST database. mNGS is capable of pathogen recognition in patients with systemic sepsis and nerve system infections.[22,23] The ability of mNGS to enhance the PJI diagnosis has also been reported formerly, achieved by tissue identification of microbial pathogens.[24,25] However, a good collection of synovial fluid is not always clinically possible. In contrast, bone or periprosthetic tissue samples are more readily obtainable during revision surgery and may better represent embedded pathogens, especially those within biofilms. PJI diagnosis based on periprosthetic tissue mNGS has been scarcely reported so far, and its feasibility to periprosthetic bone tissues remains unclear.[26] Some investigations have explored mNGS in PJI, recent meta-analyses—such as the one conducted by Hantouly et al—highlight the methodological heterogeneity across studies and emphasize the need for standardized evaluations of mNGS performance in specific sample types.[27]

To address this gap, we conducted a prospective study to assess the diagnostic performance of mNGS in periprosthetic bone tissue samples from patients undergoing revision surgery for suspected knee PJI. We hypothesized that mNGS could serve as a valuable complementary method to conventional histopathology and microbial culture, improving the accuracy of pathogen detection in PJI.

Materials and Methods

Ethical Considerations

The study was approved by the ethics committee of Zhengzhou orthopaedics Hospital, and all patients gave their written informed consent before inclusion into the study. All methods were carried out in accordance with approved guidelines and relevant regulations.

Patient Enrollment

All suspected patients with PJI receiving total revision arthroplasty of hip or knee were enrolled in a single-institution prospective analysis between January 2022 and December 2024. The patients were subjected to 6-months postoperative surveillance for clinical signs of infection recurrence. A total of 215 patients were initially screened. Of these, 157 were excluded due to: (1) refusal to provide samples for mNGS analysis (n = 49); (2) history of bone tumors or autoimmune diseases such as rheumatoid arthritis (n = 38); (3) absence of histopathological data or inconclusive histological results (n = 32); (4) loss to follow-up (at least 6 months postoperatively) within the surveillance period (n = 38). A final cohort of 58 patients was included in the analysis.

To minimize the impact of antibiotic pretreatment on culture and sequencing outcomes, patients who had received antibiotics for more than 48 hours prior to sample collection were excluded. For those with less than 48 hours of empirical antibiotic use, this variable was recorded and included in subgroup analysis to assess its potential influence.

Patient Categories

The patients were divides into the PJI and non-PJI groups, following the diagnostic guidelines of MSIS. PJI diagnosis was primarily based on the 2011 MSIS criteria, with reference to the 2018 ICM criteria for improved diagnostic accuracy in borderline cases.[12,16] The specific criteria for PJI diagnosis were: (1) culture isolation of a pathogen from two or more individual samples of involved prosthetic joint fluid or tissue; (2) communication of sinus tract with the prosthesis; (3) conformance to three minor diagnostic standards, which include an increase in ESR (> 30 mm/H) and CRP (> 10 mg/L),

increased synovial leukocyte count (> 3000 cells/μL) or a positive test of LE strip, increase in PMN% (> 80%), a microorganism isolated from one periprosthetic fluid or tissue culture, the histological finding > 5 PMNs per HPF at a × 9, 400 magnification in 5 HPFs. In this study, periprosthetic tissue refers specifically to grinded bone specimens harvested during surgery, unless otherwise noted. The demographic information, clinical data, and all test findings of the patients were collected from the electronic version of medical records.

Smears and Cultures

The first step included grinding periprosthetic bone tissue samples and Petroff decontamination with 4% NaOH (sodium hydroxide). The treated sediments sample was directly stained by Ziehl-Neelsen and Gram methods and detected using a 100 × oil lens. A 7-d periprosthetic bone tissue homogenate sample (0.1 mL) was inoculated at 35°C on Columbia agar-involving selective medium, chocolate agar, and sheep blood (5%) under both oxygen-free and –dependent conditions. The 1 mL homogenate was inoculated into BD BACTEC (BD, USA) aerobic/anaerobic/fungal and acid-fast bacilli bottles and incubated for 14 days. According to the standard laboratory procedures, isolated bacteria were tested for antimicrobial susceptibility of positive culture using the Phoenix 100 (BD, USA) automated system.

Histopathology Examination

Initially, periprosthetic bone tissue samples were embedded in paraffin and subsequently sliced. Further, periprosthetic bone tissue sections were subjected to hematoxylin-eosin staining (HE) or special stains, such as acid-fast staining (for *Mycobacterium tuberculosis*), silver hexamine staining (for fungi), and observed using a microscope. Samples were obtained from multiple anatomical sites surrounding the prosthesis, including both grossly inflamed and non-inflamed periprosthetic bone tissues, to reduce sampling bias. According to the soft tissue sampling regulations, one sample was taken every 1 cm with a 2–3 mm thickness. For periprosthetic bone tissues of different sizes, about 2 to 5 pieces were collected. According to the MSIS diagnostic criteria, >5 neutrophils/HPF was used as the histological diagnostic criteria for acute infection.

mNGS Protocol

The intraoperatively harvested periprosthetic bone tissue blocks were cut into small sections and transported to the laboratory for mNGS analysis. Initially, TIANamp Micro DNA Kit (DP316, TIANGEN BIOTECH, China) was used to extract the total genomic DNA from the periprosthetic bone tissue samples, followed by quantification using Qubit 2.0 (Invitrogen, USA). About 200 ng of the total genomic DNA was used for subsequent analysis, followed by DNA library construction and identification. Initially, the DNA library was established through end repair adaption and PCR amplification. Agilent 2100 Bioanalyzer (Agilent Technologies, Canada) combined with quantitative PCR was used to identify the DNA library. The library was cyclized to form a single-stranded ring structure and rolled to replicate and generate DNA nanospheres for next-generation sequencing. Further, the samples were chip-loaded and sequenced in 20M 50-bp reads (single-end) on a Illumina NextSeq CN500 platform (Illumina, USA). The superior quality sequencing data was derived by eliminating poor quality, sequence number repeats, adaptor contamination, and reads shorter than 35 bp. The human host sequence mappings to hg19 (human reference genome) were subtracted by computational means via the Burrows-Wheeler alignment tool. Concurrent categorization of the rest data was accomplished by aligning to viral, bacterial, fungal, and parasitic Genome Databases downloaded from NCBI (ftp://ftp.ncbi.nlm.nih.gov/genomes/). Whole genomic sequences of viral taxa totaling 1, 798, 6, 350 bacterial scaffolds/genomes, 1, 064 human infection-associated fungi, and 234 human disease-related parasites were covered.

To control contamination, negative controls (blank extraction controls and sterile saline) were included in each sequencing batch. No microbial reads were detected in these controls.

Statistical Methods

The sensitivity, specificity, likelihood ratio (LR), positive/negative predictive value (PPV/NPV), and AUC of the histopathology, culture, and NGS were separately analyzed on SPSS 17.0, and corresponding confidence interval (CI) at 95%. Baseline characterization was conducted based on descriptive statistics. *T*-test or Mann–Whitney

U nonparametric test was employed to compare two sets of continuous parameters. The Chi-square test was adopted for the evaluation of categorical parameters. Inter-method comparison was made using the McNemar test. Differences were considered significant when $P < 0.05$.

Results

Overall Cohort

This study recruited 215 clinically suspected patients with PJI. After eliminating 157 patients following the exclusion criteria, periprosthetic bone tissue samples (58 patients) were collected from a total of 215 patients for assessment. Among these patients, 38 (65.5%) were confirmed with PJI, and 20 (34.5%) were non-PJI cases with the MSIS criteria as the reference standard (Figure 1). Of these, 33 (57.4%) were male, and 25 (42.6%) were female (male: female ratio 1.3) with the mean age of 66.4 ±6.7 and 67.3 ±8.2 years for the PJI and non-PJI groups, respectively. ESR levels were higher in the PJI group (41.5 ±18.2 mm/H) than in the non-PJI group (14.4 ±6.1 mm/H) ($P = 0.001$). The difference in CRP levels between the groups was insignificant (22.1 ±53.1 mg/L vs 13.8 ±2.5 mg/L, $P = 0.08$). The levels of PCT were 1.3 ±0.1 ng/mL in the infection group, which was higher than in the non-infection group (0.2 ±0.1 ng/mL, $P = 0.00$). The inter-group difference in D-dimer levels (1.6 ±0.2 mg/L vs 1.4 ±0.1 mg/L) was insignificant ($P = 0.41$). Figures 1, 2 and Table 1 display the demographic information and clinical features of the cohort, respectively.

Diagnostic Value of CRP, ESR, PCT, and D-Dimer

The AUC of CRP and D-dimer was 0.531 and 0.579, respectively ($P > 0.05$). The AUC of ESR and PCT was 0.930 and 0.795, respectively ($P < 0.05$) (Table 1 and Supplement Figure 1). The corresponding clinical diagnostic cut-off values were 17.9 mg/L for CRP, 24.5 mm/H for ESR, 0.56 ng/mL for PCT and 2.13 mg/L for D-dimer. According to this critical point, the sensitivity of CRP, ESR, PCT and D-dimer was 57.9%, 78.9%, 60.5%, 34.2%, respectively. In addition, the specificity was 80.0%, 90.0%, 85.0%, 100%, respectively (Supplement Table 1).

Comparison of Positive Rates and Diagnostic Value from Different Tests Using MSIS Criteria

In the PJI group, 24/38 (63.2%) patients presented positive results via mNGS, 26/38 (68.4%) showed positive bacterial culture results, and 20/38 (52.6%) showed positive histology results using the MSIS criteria as the reference standard to diagnose PJI. In contrast, in the non-infection group, 4/20 (20.0%) patients revealed positive results via mNGS, 1/20 (5.0%) showed bacterial culture results, and 20/20 (100%) showed negative histopathology results. The positive rate of the culture was significantly higher than that for NGS and Histopathology ($P < 0.05$) (Table 2). Based on the MSIS

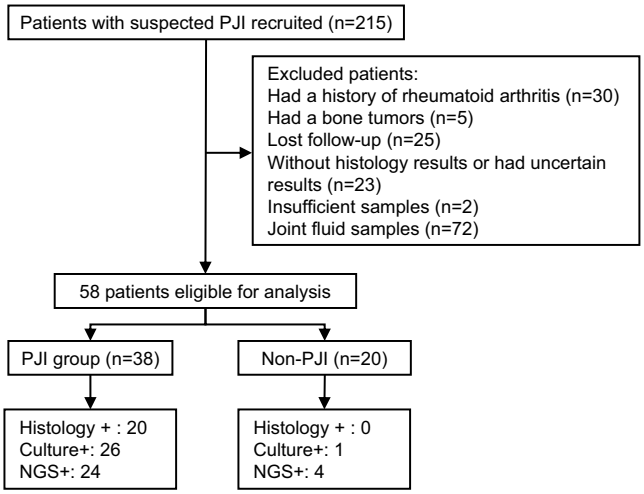

Figure 1 Enrollment of the study patients.

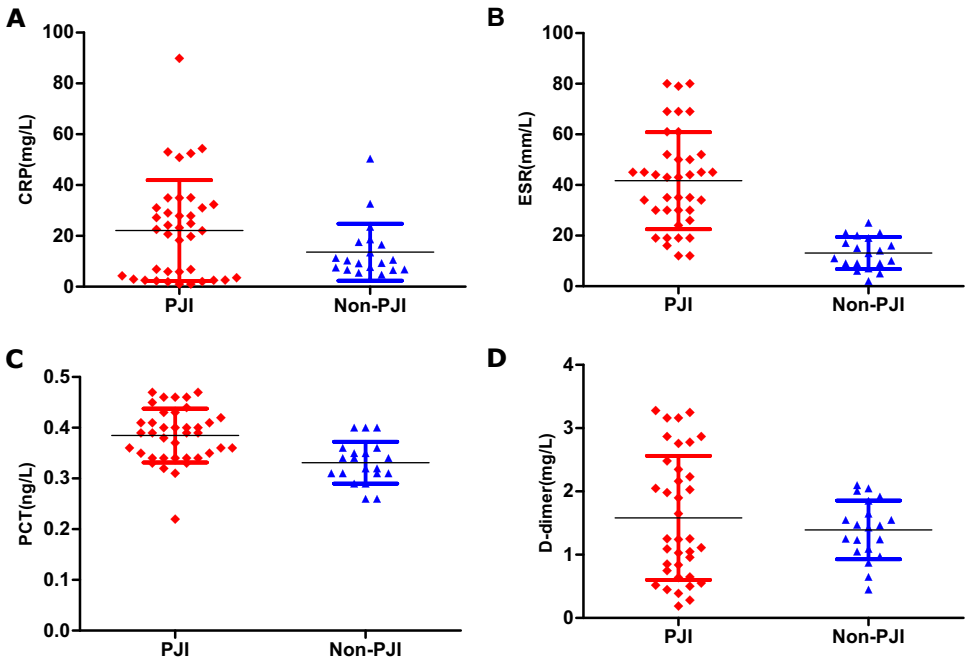

Figure 2 Comparison of different serum biomarkers levels between PJI group and non-PJI group. (**A**) There was not significantly different of CRP value between PJI group and non-PJI group (P = 0.082); (**B**) The ESR value of the PJI group was significantly higher than that of the non-PCT group (P = 0.014); (**C**) There was significantly different of PCT level between PJI group and non-PJI group (P = 0.004); (**D**) There was not significantly different of D-dimer value between PJI group and non-PJI group (P = 0.417).

criteria, the sensitivity and specificity of mNGS of the 38 patients diagnosed with PJI were 63.2% and 80.0%, respectively. Histology had a low sensitivity (52.6%) but a specificity of 100%, while culture yielded 68.4% sensitivity and 95.0% specificity (Table 2). The mNGS positive results of the PJI group included 9/24 (37.5%) cases of CNS, 9/24

Table 1 Baseline Characteristics of Study Cases

Characteristics	PJI (n=38)	Non-PJI (n=20)	P Value
Age, years (mean ±SD)	66.4 ±6.7	67.3 ±8.2	0.43
Sex n (%)			0.00
Men	24	11	
Women	18	8	
Body mass index, kg/m^2	26.9 ±3.6	27.9 ±2.8	0.56
Comorbidities n (%)			
Hypertension	16	9	0.76
Diabetes	12	3	0.00
Smoking	15	10	0.83
Alcoholism	12	4	0.01
Ethnic group, n (%)			0.00
Han	36	17	
Hui	2	3	
Other	0	0	

(*Continued*)

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Table 1 (Continued).

Characteristics	PJI (n=38)	Non-PJI (n=20)	P Value
Laboratory findings			
ESR (mm/h, mean ±SD)	41.5 ±18.2	14.4 ±6.1	0.00
CRP (mg/L, mean ±SD)	22.1 ±53.1	13.8 ±2.5	0.08
PCT (ng/L, mean ±SD)	1.3 ±0.1	0.2 ±0.1	0.00
D-dimer (mg/L, mean ±SD)	1.6 ±0.2	1.4 ±0.1	0.41

Abbreviations: PJI, Prosthetic joint infection; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PCT, procalcitonin; SD, standard deviation.

Table 2 Performance of NGS, Culture and Histopathology Compared to the MSIS Criteria

Gold Standard	Diagnostic Test	Sensitivity% (95% CI)	Specificity% (95% CI)	PPV% (95% CI)	NPV% (95% CI)	PLR (95% CI)	NLR (95% CI)
MSIS criteria	Culture	68.4 (58.2–78.9)	95.0 (84.6–99.8)	96.5 (88.7–99.7)	61.3 (50.4–71.7)	2.488 (1.688–3.898)	0.067 (0.011–0.203)
	Histopathology	52.6 (46.1–68.7)	100 (100.0–100.0)	100 (100.0–100.0)	52.6 (46.1–68.7)	Max	0.411 (0.321–0.558)
	mNGS	63.2 (53.6–77.7)	80.0 (75.7–90.1)	85.7 (76.4–95.3)	53.3 (44.6–61.2)	1.837 (1.217–2.771)	0.268 (0.102–0.403)

(37.5%) cases of *Staphylococcus aureus*, 2/24 (8.3%) cases of NTM, 2/24 (8.3%) cases of Gram-negative bacteria, 1/24 (4.1%) cases of MTB, and 1 (4.1%) case of *Clostridium perfringens*. The culture-positive results of the PJI group contained 10/26 (38.5%) cases of *Staphylococcus aureus*, 8/26 (30.8%) cases of CNS, 4/26 (15.4%) cases of Gram-negative bacteria, 2/26 (7.7%) cases of *Candida albicans*, 1/26 (3.8%) cases of MTB and 1 case of *Brucella*. In the non-PJI group, 4 out of 20 patients (20.0%) showed positive mNGS results. The organisms identified included *Candida glabrata, Corynebacterium ureicelerivorans, Haemophilus influenzae, and Clostridium perfringens* (Table 3). These organisms are either part of the skin/mucosal flora or environmental bacteria, and none were supported by intraoperative

Table 3 mNGS, Histology and Culture Results of PJI Group and Non-PJI Group

Number	Group	mNGS	Culture	Histopathology
1	PJI	CNS	*Candida albicans*	–
2	PJI	CNS	CNS	+
3	PJI	*Escherichia coli*	*Escherichia coli*	–
4	PJI	–	*Brucella*	+
5	PJI	CNS	CNS	+
6	PJI	*Enterococcus faecalis, Aspergillus*	*Staphylococcus aureus*	+
7	PJI	CNS/*Staphylococcus aureus*	*Staphylococcus aureus*	–
8	PJI	*Staphylococcus aureus*	*Staphylococcus aureus*	+
9	PJI	*Cutabacterium acnes*	–	+
10	PJI	CNS	CNS	–
11	PJI	*Staphylococcus aureus*	*Staphylococcus aureus*	+

(*Continued*)

Table 3 (Continued).

Number	Group	mNGS	Culture	Histopathology
12	PJI	–	*Candida albicans*	–
13	PJI	*Streptococcus species*	*Streptococcus species*	–
14	PJI	MTB	MTB/CNS	–
15	PJI	CNS	CNS	+
16	PJI	*Staphylococcus aureus*	*Staphylococcus aureus*	+
17	PJI	*Staphylococcus aureus*	*Staphylococcus aureus*	+
18	PJI	CNS/NTM	CNS	+
19	PJI	–	*Staphylococcus aureus*	+
20	PJI	*Staphylococcus aureus*	*Staphylococcus aureus*	+
21	PJI	NTM	–	–
22	PJI	*Staphylococcus aureus*	*Staphylococcus aureus*	+
23	PJI	CNS	CNS	+
24	PJI	*Acinetobacter baumannii, Pseudomonas aeruginosa*	*Acinetobacter baumannii*	–
25	PJI	–	*K. pneumoniae*	–
26	PJI	*Enterobacter cloacae*	*Enterobacter cloacae*	–
27	PJI	*Staphylococcus aureus*	*Staphylococcus aureus*	+
28	PJI	CNS	CNS	–
29	Non-PJI	*Candida glabrata*	*Candida glabrata*	–
30	Non-PJI	*Corynebacterium ureicelerivorans*	–	–
31	Non-PJI	*Haemophilus influenzae*	–	–
32	Non-PJI	*Clostridium perfringens*	–	–

Abbreviations: MTB, *tuberculosis mycobacterium*; NTM, Non-*tuberculosis mycobacterium*; CNS, *Coagulase-negative staphylococci*.

findings, histopathology, or clinical symptoms. Notably, Candida glabrata was also isolated by culture in the same patient (Patient 29), whereas the remaining three organisms (*Corynebacterium ureicelerivorans, Haemophilus influenzae, and Clostridium perfringens*) were detected only by mNGS. These cases likely represent background contamination or colonization rather than true infection. In addition, the culture-positive results of the infection group contained 1 case of *Candida glabrata* (Table 3). The four patients in the non-PJI group with positive mNGS results were followed for at least 6 months postoperatively. None developed signs of infection or received antimicrobial therapy during the follow-up period, supporting their classification as aseptic cases.

Diagnostic Effectiveness of the mNGS Assay Under Different Reference Standards

Using culture as the reference standard, the mNGS assay had sensitivity, specificity, positive PV (PPV), negative PV (NPV), positive LR (PLR) and negative LR (NLR) of 88.5% (68.7–96.9%), 84.2% (70.6–95.8%), 88.5% (68.7–96.9%), 84.2% (70.6–95.8%), 5.603 (3.965–7.972), and 0.137 (0.046–0.304), respectively (Table 4). Using histopathology as the reference standard, the mNGS assay had sensitivity, specificity, PPV, NPV, PLR and NLR of 70.0% (65.7–87.2%), 80.0% (75.7–93.3%), 77.78% (61.9–92.6%), 72.7% (59.9–88.4%), 2.851 (1.381–4.089), and 0.306 (0.226–0.749), respectively

Table 4 Diagnostic Efficacy of the mNGS Assay Using Culture, Histopathology and Musculoskeletal Infection Society (MSIS) Criteria as the Reference Standard

	Sensitivity% (95% CI)	Specificity% (95% CI)	PPV% (95% CI)	NPV% (95% CI)	PLR (95% CI)	NLR (95% CI)
Culture	88.5 (68.7–96.9)	84.2 (70.6–95.8)	88.5 (68.7–96.9)	84.2 (70.6–95.8)	5.603 (3.965–7.972)	0.137 (0.046–0.304)
Histopathology	70.0 (65.7–87.2)	80.0 (75.7–93.3)	77.78 (61.9–92.6)	72.72 (59.9–88.4)	2.851 (1.381–4.089)	0.306 (0.226–0.749)
MSIS criteria	65.5 (56.0–77.7)	80.0 (75.7–93.3)	85.7 (76.4–95.3)	53.3 (44.6–61.2)	1.837 (1.217–2.771)	0.268 (0.102–0.403)

(Table 4). Using MSIS criteria as the reference standard, the mNGS assay had sensitivity, specificity, PPV, NPV, PLR, and NLR of 65.5% (56.0–77.7%), 80.0% (75.7–93.3), 85.7% (76.4–95.3), 53.3% (44.6–61.2), 1.837 (1.217–2.771), and 0.268 (0.102–0.403), respectively (Table 4). In addition, a total of 2 NTM cases were detected with mNGS, but culture was both negative. Therefore, mNGS may have high sensitivity and specificity for the diagnosis of NTM PJI. The sequence number of mNGS detection was 3 and 2, respectively (Supplement Table 2).

Discussion

PJI, a serious complication secondary to artificial joint arthroplasty, is extremely difficult to diagnose and treat due to the insignificant clinical manifestations and signs and the presence of culture-negative PJIs. The present study investigated the value of mNGS for diagnosing PJI compared to other diagnostic criteria. In our cohort, mNGS achieved a sensitivity of 63.2% and specificity of 80.0% for PJI diagnosis. Compared with mNGS, conventional bacterial culture had slightly higher sensitivity (68.4%) and notably higher specificity (95.0%), while histopathology showed 52.6% sensitivity and 100% specificity. These findings suggest that mNGS, while promising, did not outperform traditional methods in terms of diagnostic accuracy in this setting. The commencement of the "NGS revolution" was marked by the genome-wide sequencing in 2005 conducted by Margulies et al on *Mycoplasma genitalium* and *Streptococcus pneumoniae*.[28] Later, Tarabichi et al were the first to report the application of the NGS technique for PJI diagnosis in 2018.[29] Among 17 primary and 65 revision arthroplasties assessed, one sole (5.9%) sample was negative on NGS, whereas positive on culture compared to the MSIS standards. Yin reported a 93.3% rate of positive diagnosis by mNGS for infected cases and 5.3% for culture-negative and non-infected cases.[30] According to our findings, the mNGS assay is not as sensitive as formerly described.[29,30] 14 cases met the MSIS criteria but were negative by mNGS. Possible explanations include low pathogen DNA burden due to deep-tissue localization, inefficient DNA extraction from periprosthetic bone tissue homogenates, or primer-template mismatch during library amplification. These findings highlight the importance of optimizing sample preparation and sequencing protocols in orthopedic infections.

Conversely, mNGS demonstrated a relatively high false-positive rate of 20% (4/20) among non-PJI cases, exceeding rates reported in prior studies (eg, 15.6% by Tarabichi et al).[29] Upon detailed review, three of these detections (*Corynebacterium ureicelerivorans, Haemophilus influenzae, Clostridium perfringens*) were not supported by clinical, histopathological, or microbiological findings. These are considered likely contaminants or low-virulence organisms, potentially introduced during sample handling or present as colonizers. Although classified as non-PJI based on MSIS criteria, all four patients had an uneventful 6-months postoperative follow-up with no signs of infection or need for revision surgery, which supports the interpretation of these findings as false positives. Nevertheless, their possible role in early subclinical infection or biofilm formation cannot be ruled out, highlighting the need for clinical correlation and cautious interpretation of mNGS data.[31] One potential strength of mNGS is its relatively rapid turnaround time, with results often available within 48–72 hours. This may offer clinical value compared to bacterial culture (typically 5–7 days) and histopathology (often >7 days). However, we did not prospectively compare diagnostic turnaround times in this study. Therefore, any advantage in timeliness remains speculative and should be verified in future real-world studies.

In this study, the sensitivity and specificity of mNGS in the diagnosis of 2 cases of PJI due to NTM reached 100%. 2 cases of NTM, all of which were *mycobacterium avium tuberculosis* and belongs to slow-growing NTM. The routine culture of slow-growing NTM generally lasted for 2–12 weeks. While, the culture of clinical samples in the laboratory was usually stopped after 3–5 days of culture with negative results according to the type of specimen, which made the

positive rate of NTM culture even lower. mNGS detection are usually obtained within 2 days and can be directly identified to the strain level, which plays a sentinel role in clinical diagnosis and treatment. However, there has been no specific study of mNGS on PJI due to NTM, and this study included too few cases. Therefore, the diagnostic efficiency of mNGS on non-tuberculous mycobacterium PJI, as well as the comparison with other commonly used detection methods, still needs in-depth and objective evaluation in large sample clinical studies.

Further, mNGS is unbiased sampling, generating the identification of known and rare organisms, such as *Parvimonas micra, Fusobacterium nucleatum*, and *Benacostia*. While rare pathogen detection is a theoretical advantage of mNGS, its clinical utility is limited unless corroborated by clinical and microbiological findings. The main inherent disadvantage of mNGS is that it is ineffective in distinguishing between pathogenic and background bacteria. mNGS test results are a list of pathogens, which may present multiple positive results simultaneously. It is difficult to effectively distinguish contaminant bacteria, colonization bacteria, or the real pathogenic microorganisms. Another potential challenge is that metagenes can detect pathogenic bacteria and cannot be simultaneously used for drug sensitivity tests. In addition, the price of mNGS is higher than traditional technology (mNGS vs culture vs histopathology: USD 357 vs 25.6 vs 21.5). Therefore, mNGS is only used in patients whose etiology cannot be determined by routine examination, making up for the low positive rate and prolonged time of routine bacteriological tests such as histopathology and bacterial culture.

According to the MSIS diagnostic criteria, the specificity of histopathological diagnosis is 100%, but the sensitivity is only 52.6%, which may be related to the sampling. Moreover, the surgical specimen may be insufficient, or the sampling site may be inaccurate. Therefore, it was suggested that sampling from multiple points, sites, and ranges should be done to avoid false-negative results caused by inadequate sampling. Additionally, the number of standard pathological samples is not standardized. Thus, we suggest that at least five samples should be collected from synovial and soft tissue, and the site highly suspected of infection should be the first choice. In general, different areas of the lesion should be roughly included. We hypothesized that following the above criteria might reduce the false-negative rate of infection diagnosis. The prosthesis of neutrophils in the surrounding periprosthetic bone tissue for the diagnosis of infection can be understood if we imagine the future can reduce the number of neutrophils meter or reduce the number of count field of vision. Thus, the sensitivity of histopathologic diagnosis of infection can improve irrespective of the frozen and paraffin sections. However, to guarantee its specific degrees, the threshold value of neutrophils still needs to be defined by a larger sample size and more studies.

The present study also compared the diagnostic value of frequently applied serological biomarkers to detect PJI. Serological markers are the most commonly used method for clinically diagnosing PJI.[32] According to our results, the sensitivity of ESR to diagnose PJI was 78.9%, higher than that reported by previous research (75.1%),[33] and primarily attributed to > 24.5 mm/H ESR diagnostic cut-offs based on the Youden index computation in the present work, a value lower than the MSIS recommendation (> 30 mm/H). Besides, a decreased cut-off can result in sensitivity enhancement. Our study revealed that the AUC for CRP was 0.531, while D-dimer was 0.579, with no statistical significance. D-dimer for the PJI diagnosis has been recently used as a coagulation-associated index, despite its unknown reliability. According to a meta-analysis, its PJI diagnosing ability is limited.[34] Our results are consistent with the literature findings. Our study showed that PCT could be used as diagnostic biomarkers to support clinicians in differentiating PJI from aseptic loosening non-PJI. The PJI group had significantly higher PCT values than the non-PJI group. With a standard cut-off level of 0.56 ng/mL, PCT had an AUC of 0.795, a 60.5% sensitivity, and 85.0% specificity, consistent with previously reported in the literature.[35–37]

However, the present study still has several limitations. First, this study is a prospective case series with a single center and lower level of evidence than randomized controlled or prospective cohort studies. Second, sample size was relatively small, and possible grouping deviations resulted from the adoption of MSIS guidelines as the gold diagnostic standard for PJI. Third, although mNGS showed promising sensitivity for PJI diagnosis, it has several inherent limitations: It does not provide information on antimicrobial susceptibility. There is a risk of false-positive results due to environmental or reagent contamination. It can be challenging to distinguish between true infection and microbial colonization. Its sensitivity may be reduced in cases with low pathogen burden.

To summarize, mNGS is a promising adjunct in the diagnostic toolkit for PJI, particularly in cases with negative culture or rare organisms. However, it did not demonstrate superior sensitivity or specificity over conventional methods

in our study. Its high false-positive rate, interpretive complexity, and high cost currently limit its routine application. While MSIS 2011 was our operational standard, future studies should consider using the more recent EBJIS or ICM-2018 criteria as the reference standard. Future large-scale studies should focus on establishing standardized interpretation thresholds, real-world cost-effectiveness, and clinical integration strategies for mNGS in orthopedic infections.

Ethics Approval and Consent to Participate

The study protocol was approved by the Institutional Review Board of Zhengzhou Orthopaedics Hospital (202201). This study was conducted in accordance with the declaration of Helsinki and patient data were kept confidential.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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