

REVIEW ARTICLE

Diagnostic Value of Next-Generation Sequencing in Periprosthetic Joint Infection: A Systematic Review

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Next-generation sequencing (NGS) has developed rapidly in the last decade and is emerging as a promising diagnostic tool for periprosthetic joint infection (PJI). However, its diagnostic value for PJI is still uncertain. This systematic review aimed to explore the diagnostic value of NGS for PJI and verify its accuracy for culture-negative PJI patients. We conducted this systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. Medline, Embase, and Cochrane Library were searched to identify diagnostic technique studies evaluating the accuracy of NGS in the diagnosis of PJI. The diagnostic sensitivity, specificity, and positive and negative predictive values were estimated for each article. The detection rate of NGS for culture-negative PJI patients or PJI patients with antibiotic administration history was also calculated. Of the 87 identified citations, nine studies met the inclusion criteria. The diagnostic sensitivities and specificities of NGS ranged from 63% to 96% and 73% to 100%, respectively. The positive and negative predictive values ranged from 71% to 100% and 74% to 95%, respectively. The detection rate of NGS for culture-negative PJI patients in six studies was higher than 50% (range from 82% to 100%), while in three studies it was lower than 50% (range from 9% to 31%). Also, the detection rate of NGS for PJIs with antibiotic administration history ranged from 74.05% to 92.31%. In conclusion, this systematic review suggests that NGS may have the potential to be a new tool for the diagnosis of PJI and should be considered to be added to the portfolio of diagnostic procedures. Furthermore, NGS showed a favorable diagnostic accuracy for culture-negative PJI patients or PJI patients with antibiotic administration history. However, due to the small sample sizes of studies and substantial heterogeneity among the included studies, more research is needed to confirm or disprove these findings.

Key words: Diagnosis; Next-generation sequencing; Periprosthetic joint infection; Systematic review

Introduction

Periprosthetic joint infection (PJI) is one of the most devastating complications after total knee arthroplasty (TKA) or total hip arthroplasty (THA). Though a rare complication, PJI is associated with substantial morbidity, mortality, and high economic costs¹. Published evidence suggests the incidence of PJI is approximately 1.55% within two years after TKA and 0.45% within two to ten years after TKA²; the incidence of PJI after THA surgery is about 1%^{3,4}. PJI is also a leading cause of revision after knee and hip arthroplasty^{5,6}. Although there exist many methods for the diagnosis of PJI, final diagnosis and evaluation of PJI in clinical practice is still demanding^{7,8}. On the one hand, the detection rate of culture,

which is the primary mean to identify the pathogen involved, is relatively low. Li *et al.* demonstrated that sensitivity of periprosthetic tissue culture in blood culture bottles was 70%⁹. Gallo *et al.* showed that the positive results of joint fluid culture were only 44% PJI patients¹⁰. On the other hand, there is no one recognized approach that is able to replace the culture for identifying the pathogen. Therefore, finding a new effective detection method to improve the detection rate of pathogenic microorganisms is becoming urgently necessary.

With the continuous development and improvement of high throughput sequencing technology, an increasing number of scientists recognize its potential value for identifying the pathogen. Next-generation sequencing (NGS), a DNA

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sequencing technology that has revolutionized genomic research, has developed rapidly over the last decade¹¹. Next-generation sequencing has shown good value in identifying pathogens and diagnosing many infectious diseases^{12,13}. In the last five years, there has been emerging evidence on the potential value of NGS in diagnosing PJI^{14–20}. A few studies have explored the diagnostic value of NGS for PJI^{14–17}, but the evidence is inconsistent and uncertain. In a recently published study¹⁶, NGS had limited value in the diagnosis of PJI, a finding which was inconsistent with previous studies¹⁴. Some studies have also suggested that NGS has excellent diagnostic value for patients with negative microbial culture^{14,15,17}, which is not at par with that of Kildow *et al.*¹⁶. The most challenging issue in the diagnosis of PJI is patients with negative microbial culture^{21,22}. Whether NGS can accurately identify culture-negative PJI patients is uncertain. Besides, considering the difficulties in diagnosis for patients with antibiotic administration history, it is also worth exploring whether the NGS could play an important role in diagnosis.

In this context, we conducted a systematic review of the published literature to summarize: (i) the diagnostic accuracy of NGS for PJI; (ii) the detection rate of NGS for culture-negative PJI; and (iii) the detection rate of NGS for PJI with antibiotic administration history.

Materials and Methods

Data Sources and Search Strategy

We performed this review following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines²³. The systematic literature search was performed by two independent authors, with a third author resolving disagreements where necessary. The electronic databases Medline (PubMed), Embase (OvidSP), and Cochrane Central Register of Controlled Trials were searched for relevant studies published from January 1990 to March 2021. The literature search strategies for these three databases followed medical subject headings combination with terms. The detailed literature search strategies are reported in the Table S1. Also, unpublished and gray literature was sought and retrieved in established journals of the orthopedic field (such as, *The Journal of Bone and Joint Surgery*, *The Journal of Arthroplasty*, *Clinical Orthopaedics and Related Research*, or *International Orthopaedics*) from January 1990 to March 2021.

Inclusion and Exclusion Criteria

Two authors independently assessed the search results for inclusion in this systematic review by initially scanning titles/abstracts and conducting full-text evaluation of potentially eligible studies. Any disagreements between the two authors were resolved by consensus or through discussion with a third author.

The studies which evaluated the diagnostic values of the NGS for identifying PJI in patients were included. The inclusion criteria were:

1. participants: patients suspected of having PJI;
2. interventions: not applicable;

3. comparisons: not applicable;
4. outcomes: diagnostic values of the NGS for identifying knee or hip PJI, and patients were categorized as infected or aseptic using the Musculoskeletal Infection Society (MSIS) criteria, Infectious Diseases Society of America (IDSA) PJI diagnostic criteria or International Consensus Meeting (ICM) criteria as the reference standard^{24–26};
5. study design: cohort study, cross-sectional study, or case-control study (prospective or retrospective).

The exclusion criteria are:

1. case reports, commentaries, expert opinion, and narrative reviews; and
2. non-English language publications.

A PRISMA flow diagram of the literature screening process was constructed after study selection. The detailed results are shown in Fig. 1.

Data Extraction

We imported all the retrieved articles into EndNote (version X9, Clarivate Analytics, Philadelphia, PA, USA). After identifying and excluding duplicate records, ineligible articles, and those published before 1990, the two researchers independently conducted literature screening and extracted data based on the inclusion and exclusion criteria. A third investigator resolved any disagreements.

We extracted the following information:

1. study information (author, year of publication, country, institution, journal, type of study, etc.);
2. study population baseline information (age, gender, body mass index (BMI), etc.);
3. the number of PJI and non-PJI patients diagnosed with MSIS in each study;
4. the number of NGS-positive, NGS-negative, culture-positive or culture-negative patients in both PJI and non-PJI groups;
5. the number of NGS-positive or NGS-negative in culture-negative PJI patients for each study; and
6. the number of NGS-positive or NGS-negative PJI patients who had an antibiotic administration history for each study.

Literature Quality Evaluation

The methodological quality of included studies was appraised using the QUADAS (Quality Assessment of Diagnostic Accuracy Studies)-2 tool²⁷. The QUADAS-2 tool evaluates bias based on the following four domains: patient selection, index test, reference standard, and flow and timing. The risk of bias was assessed in each domain, and concerns about applicability were assessed in the first three domains with signaling questions. These questions were answered with “yes” for a low risk of bias/concerns, “no” for a high risk of bias/concerns, or “unclear” when the relevant information was not clearly provided. Two authors independently evaluated these studies. A third author resolved any controversy to achieve a final consensus.

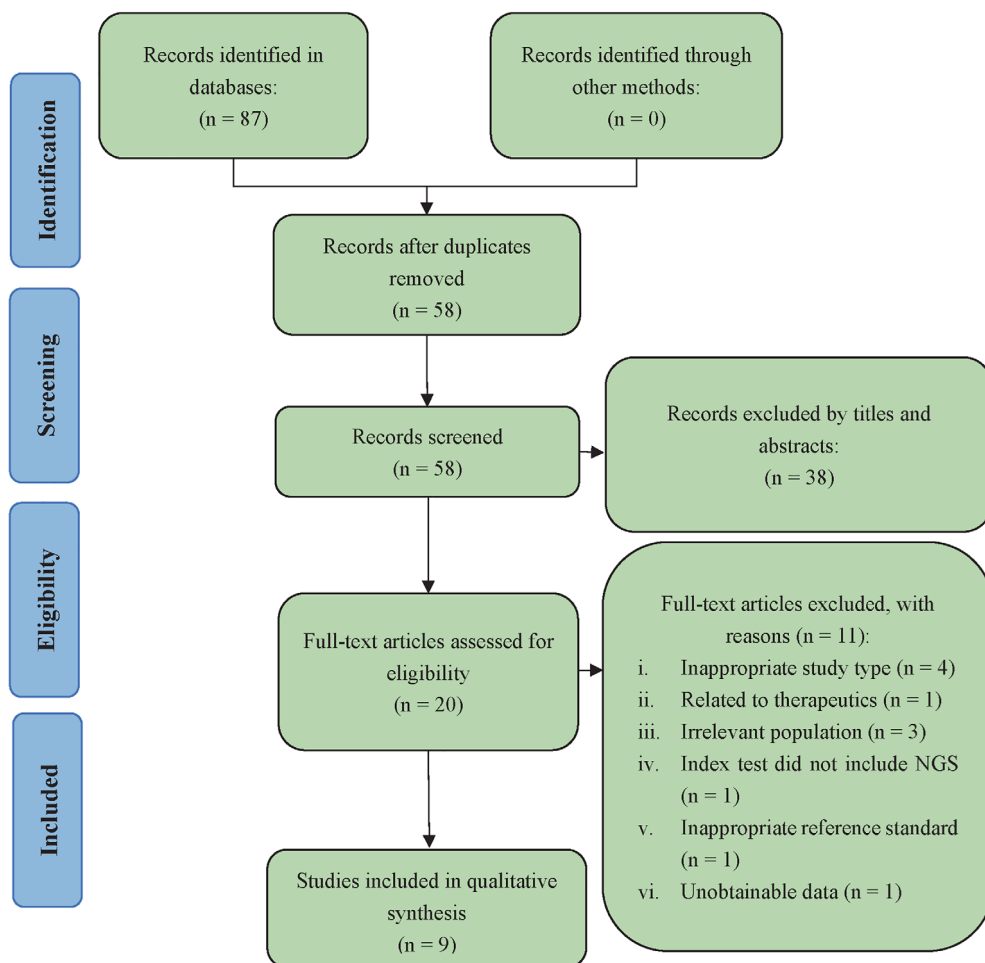


Fig. 1 Flow diagram of study selection process. NGS, next-generation sequencing.

Statistical Analyses

A standardized Microsoft Excel spreadsheet was used to track the extraction of quantitative data from each study. True positive (TP), false positive (FP), true negative (TN) and false negative (FN) results were collected and plotted in a two-by-two contingency table. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+LR) and negative likelihood ratio (−LR) were calculated for each study. Simultaneously, the detection rate of NGS for culture-negative PJI patients, which was equal to NGS-positive / culture-negative PJI patients expressed as a proportion of the total number of culture-negative PJI patients, was also calculated. Besides, the detection rate of NGS for PJI patients with antibiotic administration history would be calculated, which was equal to NGS-positive/PJI patients with antibiotic administration history. The statistical analyses were performed by two researchers independently; a third investigator resolved any disagreements.

Results

Study Selection

We retrieved a total of 87 potentially relevant citations from the four electronic databases. After excluding 29 duplicates and 38 irrelevant citations based on titles and abstracts, 20 citations remained for full-text evaluation. Following this, we further excluded 11 articles because: (i) study designs did not meet the inclusion criteria (two reviews, one case report, and one editorial)^{18,28–30}; (ii) one study was related to therapeutics³¹; (iii) the population or sample included in three studies was not relevant^{20,32,33}; (iv) the index test in one study did not include NGS³⁴; (v) one study did not use the MSIS criteria, IDSA criteria, or ICM criteria as a reference standard; and (vi) one study involved the establishment of a PJI diagnostic model in which the data relating to NGS diagnosis could not be obtained^{19,35}. Nine articles were finally eligible for the review^{14–17,36–40}. There was no disagreement between the reviewers regarding the inclusion of these studies.

TABLE 1. Characteristics of studies included in systematic review

Study	Institution	Population	Study design	Prospective or retrospective	Reference standard	Sample for NGS	Volume of synovial fluid (mL)	Patients (All PJI/ Non-PJI)	Age (year, Mean [SD])	Sex (Female [%])	BMI (kg/m ² , Mean [SD])
Cai et al. ¹⁴ 2020	First Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian, China	Revision arthroplasty	Cohort Study	Prospective	MSIS	Intraoperative (periprosthetic tissues)	\	44 (22/22)	62.50 (9.40)	38.64	NR
Fang et al. ¹⁵ 2020	First Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian, China	Revision arthroplasty	Cohort Study	Prospective	MSIS	Intraoperative and Preoperative (synovial fluid)	1	38 (25/13)	62.64 (19.68)	50	NR
Huang et al. ³⁶ 2020	First Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian, China	Revision arthroplasty	Cohort Study	Prospective	MSIS	Intraoperative (synovial fluid)	1	70 (49/21)	65.67 (13.26)	44.29	27.81 (4.78)
Ivy et al. ³⁷ 2018	Mayo Clinic, Rochester, Minnesota, USA	Aseptic failure and PJI	Case-control study	Retrospective	IDSA	Preoperative (synovial fluid)	1	168 (96/72)	67.03 (12.21)	41.67	33.05 (10.02)
Kildow et al. ¹⁶ 2021	Duke University, Durham, North Carolina, USA	Revision arthroplasty and primary arthroplasty	Cohort study	Retrospective	MSIS	Preoperative (synovial fluid)	≥2	116 (48/68)	67.80 (13.85)	51.7	31.70 (7.78)
Tarabichi et al. ¹⁷ 2018	The Rothman Institute at Thomas Jefferson University, Philadelphia, Pennsylvania, USA	Revision arthroplasty	Cohort study	Prospective	MSIS	Intraoperative (synovial fluid, deep-tissue specimens and swabs)	NR	65 (28/37)	64.10 (10.73)	35.38	31.18 (6.62)
Thoendel et al. ³⁸ 2018	Mayo Clinic, Rochester, Minnesota, USA	Aseptic failure and PJI	Case-control study	Retrospective	IDSA	Intraoperative (sonicate fluid)	\	408 (213/195)	65.31 (16.72)	50	NR
Wang et al. ³⁹ 2020	First Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian, China	Revision arthroplasty	Cohort study	Prospective	MSIS	Preoperative (synovial fluid, sonication fluid or homogenized tissue)	≥4	63 (45/18)	NR	NR	NR
Yin et al. ⁴⁰ 2021	Liaocheng People's Hospital and Liaocheng Clinical School of Taishan Medical University, Liaocheng, Shandong, China	Revision arthroplasty	Cohort study	Prospective	MSIS	Preoperative (synovial fluid)	0.6	35 (15/20)	67.80 (7.40)	40	27.20 (2.30)

BMI, body mass index; IDSA, Infectious Diseases Society of America criteria; MSIS, Musculoskeletal Infection Society criteria; NR, not reported; PJI, Periprosthetic Joint Infection.

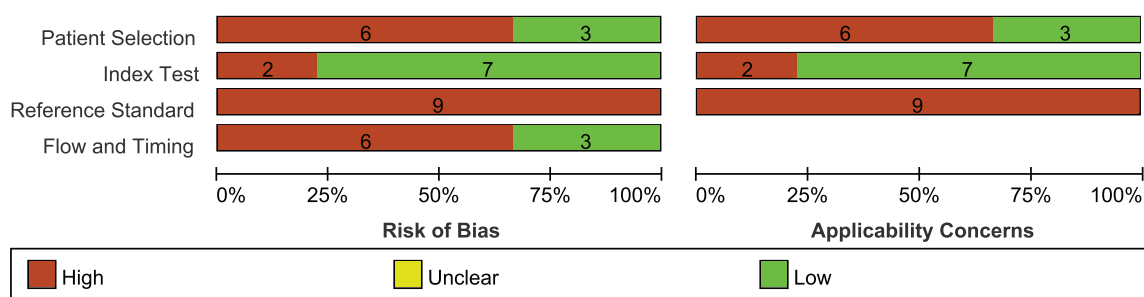


Fig. 2 Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) scores for risk of bias and concerns regarding applicability of the included studies.

Study Characteristics and Quality

The nine eligible studies, which evaluated the diagnostic value of the NGS, were published between 2018 and 2021. Four studies^{14,15,36,39} were conducted in the same institution, and another two studies^{37,38} were also completed at the same institution. All included studies recruited a total of 1007 patients (541 PJI patients and 466 non-PJI patients). Six studies enrolled patients who underwent revision arthroplasty^{14,15,17,36,39,40}; two studies included the patients who were PJI or aseptic implant failures^{37,38}; one study enrolled patients who underwent revision arthroplasty or primary arthroplasty¹⁶. The mean ages ranged from 62.5 to 67.8 years. The percentage of women ranged from 35.4% to 51.7%. Five studies reported on patients' mean BMI, which ranged from 27.2 to 33.05 kg/m²^{16,17,36,37,40}. Seven included studies were cohort studies^{14–17,36,39,40}, and two studies were case-control studies^{37,38}. Meanwhile, six studies were prospective research^{14,15,17,36,39,40} and three studies were retrospective research^{16,37,38}. MSIS criteria were selected as the reference standard in seven studies^{14–17,36,39,40}, while two studies employed IDSA as PJI diagnostic criteria^{37,38}. The nine studies employed different sampling methods. Only synovial fluid was sampled in five studies^{15,16,36,37,40}; only

periprosthetic tissues were sampled in one study¹⁴; only sonicate fluid was sampled in one study³⁸; synovial fluid, deep-tissue specimens, and swabs were sampled in one study¹⁷; and synovial fluid, sonication fluid, or homogenized tissue were sampled in one study³⁹. For the studies which used synovial fluid for the NGS test, the minimal volume of synovial fluid was 0.6 mL, while the maximum volume was greater than 4 mL. Table 1 presents details of study characteristics.

The risk of bias was assessed for all included studies using the QUADAS-2 tool²⁷. All eligible studies were at high risk of bias. The most common reason for a high risk of bias was the reference standard, where MSIS criteria or IDSA criteria were used as the reference standard. Studies were at high risk for the “patient selection” domain because patients were not enrolled consecutively^{14–16,37,38,40}. The high risk of bias for the “index test” was because the results of the reference test were definitive before the index test^{37,38}. Also, the high risk of bias for “flowing and timing” was because not all patients were included in the analysis in three studies^{16,37,38}; one study enrolled acute PJI patients, which led to an inappropriate interval between index test and reference standard¹⁶; there was a long interval between index test and reference standard in two studies^{37,38}. The results are shown in Fig. 2.

TABLE 2 Sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio of next-generation sequencing for periprosthetic joint infection

Study	Sampling	TP	FP	FN	TN	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	+LR	–LR
Cai <i>et al.</i> 2020 ¹⁴	Intraoperative	21	2	1	20	95	91	91	95	10.50	0.05
Fang <i>et al.</i> 2020 ¹⁵	Intraoperative	24	0	1	13	96	100	100	93	N.S	0.04
	Preoperative	23	1	2	12	92	92	96	86	11.96	0.09
Huang <i>et al.</i> 2020 ³⁶	Intraoperative	47	1	2	20	96	95	98	91	20.56	0.02
Ivy <i>et al.</i> 2018 ³⁷	Preoperative	73	5	23	67	76	93	94	74	10.95	0.26
Kildow <i>et al.</i> 2021 ¹⁶	Preoperative	30	7	18	61	63	90	81	77	6.07	0.42
Tarabichi <i>et al.</i> 2018 ¹⁸	Intraoperative	25	10	3	27	89	73	71	90	3.30	0.15
Thoendel <i>et al.</i> 2018 ³⁸	Intraoperative	157	7	56	188	74	96	96	77	20.53	0.27
Wang <i>et al.</i> 2020 ³⁹	Preoperative	43	1	2	17	96	94	98	90	17.20	0.05
Yin <i>et al.</i> 2021 ⁴⁰	Preoperative	14	2	1	18	93	90	88	95	9.33	0.07

+LR, positive likelihood ratio; –LR, negative likelihood ratio; FN, false negative; FP, false positive; NPV, negative predictive value; N.S, nonsense; PPV, positive predictive value; TN, true negative; TP, true positive.

TABLE 3 Detection rate of NGS in culture-negative PJI patients

Study	Sampling of NGS	Culture-negative PJI	NGS-positive in culture-negative PJI	Detection rate (%)
Cai <i>et al.</i> 2020 ¹⁴	Intraoperative	6	5	83
Fang <i>et al.</i> 2020 ¹⁵	Intraoperative	7	6	86
	Preoperative	12	10	83
Huang <i>et al.</i> 2020 ³⁶	Intraoperative	10	10	100
Ivy <i>et al.</i> 2018 ³⁷	Preoperative	16	4	25
Kildow <i>et al.</i> 2021 ¹⁶	Preoperative	11	1	9
Tarabichi <i>et al.</i> 2018 ¹⁸	Intraoperative	11	9	82
Thoendel <i>et al.</i> 2018 ³⁸	Intraoperative	67	21	31
Wang <i>et al.</i> 2020 ³⁹	Preoperative	10	10	100
Yin <i>et al.</i> 2021 ⁴⁰	Preoperative	8	7	88

NGS, next-generation sequencing; PJI, Periprosthetic Joint Infection.

The Diagnostic Value of Next-Generation Sequencing for PJI

All included studies reported the diagnostic performance of NGS for PJI. The diagnostic sensitivities and specificities ranged from 63% to 96% and 73% to 100%, respectively. The positive and negative predictive values ranged from 71% to 100% and 74% to 95%, respectively. The positive and negative likelihood ratios ranged from 3.30 to 20.56 and 0.02 to 0.42. The detailed results are reported in Table 2.

The Detection Rate of Next-Generation Sequencing for Culture-Negative PJI

All eligible studies reported the number of culture-negative PJI and NGS-positive/culture-negative PJI patients. The detection rate of NGS for culture-negative PJI patients in six studies was higher than 50% (ranged from 82% to 100%), while in three studies it was lower than 50% (ranged from 9% to 31%). The results are shown in Table 3.

The Detection Rate of Next-Generation Sequencing for PJIs with Antibiotic Administration History

Four eligible studies reported the number of culture-negative PJI and NGS-positive/culture-negative PJI patients^{14,15,37,38}. Two studies did not mention the antibiotic administration history of patients^{16,40}, two studies withheld the antibiotics before surgical procedure until samples were collected^{17,39}, and one study did not report the NGS results in patients with antibiotic administration history³⁶. Finally, the detection rate of NGS for PJIs with antibiotic administration history

ranged from 74.05% to 92.31%. The detailed results are shown in Table 4.

Discussion

The diagnosis of PJI has always been a challenging issue in the field of orthopedics¹. Though NGS has developed rapidly over the last decade, its diagnostic value for PJI has been uncertain and results of previous studies also conflicting^{14–17}. Using a systematic review, we have summarized the existing literature and shown that NGS may be a promising tool for the diagnosis of PJI, especially PJI patients with a negative culture result or antibiotic administration history. Because of the low quality of the reports in the literature and the limited number of available studies, further investigation is needed in future studies.

A total of 87 relevant records were retrieved from three databases and nine eligible studies were eventually included^{14–17,36–40}. It suggests that the application of NGS in PJI is still in its infancy, with only very few published studies related to the diagnosis of PJI. Although the literature on NGS has been in existence since the early 2000s^{41,42}, its application for clinical diagnosis only started after the early 2010s^{43,44}. Concerning the quality of the included studies, all nine studies were at high risk of bias. First, the most common reason for assigning a high risk of bias was the improper reference standard. The MSIS criteria were initially proposed in 2011²⁴ and the IDSA criteria were proposed in 2013²⁶, but after nearly a decade of development, many scholars and experts have challenged this standard and

TABLE 4 Detection rate of NGS in PJIs with antibiotic administration history

Study	Sampling	PJIs with antibiotic administration history	PJIs with NGS-positive	Detection rate (%)
Cai <i>et al.</i> 2020 ¹⁴	Intraoperative	4	3	75.00
Fang <i>et al.</i> 2020 ¹⁵	Preoperative	13	11	84.62
	Intraoperative	13	12	92.31
Ivy <i>et al.</i> 2018 ³⁷	Preoperative	42	35	83.33
Thoendel <i>et al.</i> 2018 ³⁸	Intraoperative	131	97	74.05

proposed new diagnostic standards²⁵. Compared with the new diagnostic criteria²⁵, the MSIS standard does not define acute and chronic PJI, which are now considered to have significant differences with regard to disease development, clinical manifestation, diagnosis threshold, and other aspects^{45,46}. Also, because many patients with PJI are culture-negative, the use of culture alone in diagnosing PJI is challenging⁴⁷. The new diagnostic tool proposed by Parvizi *et al.* has high diagnostic sensitivity⁴⁸, which is much higher than that of the MSIS or IDSA. Therefore, the MSIS criteria or IDSA criteria^{24,26}, which were used as the reference standard in the included studies, cannot accurately distinguish between PJI and non-PJI patients. Second, inappropriate patient selection was also one of the reasons for assigning a high risk of bias. Six out of all nine studies did not enroll patients consecutively^{14–16,37,38,40}, which could have introduced selection bias and affected the accuracy of the findings. Finally, in terms of flow and time, one study included patients with acute PJI¹⁶. As mentioned above, disease progression in patients with acute PJI is different from that of chronic PJI, which may lead to inappropriate time intervals between index test and reference standard.

In terms of the diagnostic value of NGS, there were significant differences between studies. Three studies reported that the sensitivity of NGS was lower than 80% (ranged from 63% to 76%)^{16,37,38}, which was lower than those of other studies (ranged from 89% to 96%)^{14,15,17,36,39,40}. We propose some potential reasons: First, the three included studies were retrospective research^{16,37,38}, and patients were not enrolled consecutively, which might have affected the accuracy of results. Second, two studies that reported a lower sensitivity of NGS were case-control studies^{37,38}, where the diagnosis was before the NGS test, and there was a long interval between index test and reference standard, which was also a potential reason for the lower sensitivity. Third, one study by Kildow *et al.* enrolled patients who underwent primary arthroplasty¹⁶, and it was uncertain whether there was any difference in the value of NGS between the patients who underwent revision arthroplasty and primary arthroplasty. Therefore, it is hard to say whether the diagnosis value reported in these three studies could represent the actual potency of the NGS. Moreover, for the specificity of NGS, the study by Tarabichi *et al.* showed a 73% specificity¹⁷, which was lower than other studies (ranging from 90% to 100%). Except for the small sample size, we also noticed the differences in sampling materials among included studies. Just like microbial cultures⁴⁹, NGS might exhibit different diagnostic performances with different sampling materials. Since there is no literature directly comparing the diagnostic efficacy of sampling materials for NGS, further exploration and research are needed to determine an optimal sampling method. Besides, it is worth mentioning that the setting of the threshold for the percentage of bacteria belonging to a single species might be an essential influence factor for diagnostic accuracy. One included study also reported that a higher threshold would increase the

specificity but decrease the sensitivity¹⁷. Therefore, no setting or setting a lower threshold might lead to an “over-sensitivity” state and possibly detect part of the normal flora. However, the results seem inconclusive whether the threshold should be set or what is the optimum threshold. Apart from the threshold setting, we also noted the different sample sizes for synovial fluid in different studies, which we considered that limited sample size of synovial fluid might influence the diagnostic accuracy before. However, our results unexpectedly suggested that four studies that used a ≤ 1 mL sample size of synovial fluid also had an excellent diagnostic value^{15,36,37,40}. Therefore, the NGS might be a potential advantage for the smaller sample size of synovial fluid used, suggesting that the NGS might not conflict with the microbial culture process.

Diagnosis of culture-negative PJI patients has always been challenging. According to the published literature, about 5% to 45% of patients with PJI have a negative-culture result^{50–54}. Culture-negative patients may be seen in cases of PJI that are caused by low virulence organisms, use of antibiotics, unsuitable culture medium, and low immunity among other reasons^{25,55–58}. Detection of pathogenic microorganisms for PJI is related to the diagnosis of the disease and affects treatment decisions, which is one of the advantages of microbial culture and NGS. However, unlike microbial culture, NGS cannot be used for microbial drug susceptibility testing, which is a major disadvantage. Combining microbial culture and NGS to comprehensively assess patients' disease status and carry out precise treatments may become the mainstay of PJI management in the future. In this systematic review, the detection rate of NGS for culture-negative PJI patients in six studies was higher than 50% (ranged from 82% to 100%)^{14,15,17,36,39,40}, while in three studies it was lower than 50% (ranged from 9% to 31%)^{16,37,38}. Except for the quality of studies, the causes which led to the negative culture results might also affect the results of the NGS test. As mentioned above, many reasons would lead to negative culture results, like low virulence organisms, use of antibiotics, and unsuitable culture medium. However, further in-depth studies are needed to elucidate this point and explore whether the NGS could be used for all culture-negative PJIs. Also, the question of whether NGS has a lower diagnostic value for patients undergoing primary arthroplasty than patients receiving revision arthroplasty remains to be further evaluated. Our study suggested that NGS has the potential to be a diagnostic tool for culture-negative PJI patients. However, due to the limited number of articles, sample sizes of studies, and inclusion of patients undergoing revision surgery, the results should be interpreted with caution. Large-scale multicenter studies are needed in the future to further explore the value of NGS in diagnosing PJI.

Another challenge for diagnosing PJI is the use of antibiotics⁵⁹. As mentioned above, antibiotics are an essential reason for negative culture results^{55–58}, which may lead to a wrong diagnosis and delay the treatment. Simultaneously, the irregular use of antibiotics is common in clinical practice.

However, this research showed the NGS had a high detection rate for PJIs with antibiotic administration history. Therefore, NGS may be beneficial for patients whose condition is difficult to diagnose with antibiotic administration history. Also, we tried to explore the value of NGS for polymicrobial PJI by comparing the NGS results and the condition of actual infections. Unfortunately, on the one hand, we could not judge the condition of actual infections in most studies included^{15–17,36–40}, on the other hand, because of the presence of false positives, it was hard to say whether the results of NGS could reflect the condition of actual infections. However, NGS does have the ability to detect various organisms and is also not limited by culture conditions. Therefore, it is necessary to further evaluate the consistency between the NGS results and actual infections. Moreover, the evidence available suggested the NGS was a potential method to diagnose polymicrobial PJI.

Limitations

Our systematic review has some limitations:

1. Because of the short application time of NGS in the diagnosis of PJI, the research is usually at the initial stage and hence the limited number of studies with small sample sizes;
2. As mentioned above, the quality of the literature included in the study was relatively low and the risk of bias was high, so the conclusions deserve cautious interpretation;
3. Four studies^{14,15,36,39} were conducted at the same institution, and another two studies^{37,38} were also completed at the same institution, which may suggest the inclusion of duplicate patients, which may affect the accuracy of results;
4. Since the search strategy was restricted to only English language studies, there is a minimal chance that other relevant literature may have been omitted.

Conclusion

This systematic review suggests that NGS may have the potential to be a new tool for the diagnosis of PJI and should be considered to be added to the portfolio of diagnostic procedures. Furthermore, NGS showed a favorable diagnostic accuracy for culture-negative PJI patients or PJI patients with antibiotic administration history. However, due to the small sample sizes of studies and substantial heterogeneity among the included studies, more research is needed to confirm or disprove these findings.

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Author Contributions

Yuchen Tang and Dacheng Zhao contributed equally to this article. Yuchen Tang and Dacheng Zhao contributed the central idea, analyzed most of the data. Yuchen Tang wrote the initial draft of the paper. The remaining authors contributed to refining the ideas, carrying out additional analyses, and finalizing this paper.

Supporting Information

Additional Supporting Information may be found in the online version of this article on the publisher's web-site:

Table S1. The literature search strategies for PubMed, Embase, and the Cochrane Library.

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