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Clinical Study

Application of metagenomic next-generation sequencing in the detection of pathogens in spinal infections

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Abstracts

BACKGROUND CONTEXT: The precise diagnosis and treatment of spinal infections (SI) remains challenging for spine surgeons. Identifying the pathogens of SI through metagenomic next-generation sequencing (mNGS) may be a key approach to addressing this challenge.

PURPOSE: To evaluate the accuracy and applicability of mNGS in determining the etiology of SI.

STUDY DESIGN: Diagnostic test study.

PATIENT SAMPLE: Twenty-five patients who had a clinical suspicion of SI and underwent mNGS testing.

OUTCOME MEASURES: The specificity, sensitivity, and time cost of mNGS and bacterial culture were compared. Clinical outcomes were assessed using the numeric rating scale (NRS) score, Oswestry Disability Index (ODI), and the Japanese Orthopedic Association (JOA) score. Demographic data and laboratory results (blood cell count (WBC), erythrocyte sedimentation rate (ESR), neutrophil percentage (NEUT%), and C-reactive protein level (CRP) were also evaluated.

METHODS: In this retrospective study, samples were obtained from 25 eligible patients via surgery or CT-guided puncture and subjected to histopathological examination, bacterial culture, and mNGS. The sensitivity and specificity of the bacterial cultures and mNGS were calculated with respect to the histopathological results as a reference. Postoperative antibiotics or antituberculosis drugs were administered on the basis of mNGS results, combined with clinical manifestations, imaging examination, and histopathology. The changes of clinical outcomes and laboratory results after treatment were observed.

RESULTS: Of the 25 patients, 21 had a positive pathology, of which 10 showed a tuberculous pathology, and the remaining 11 showed a nontuberculous inflammatory pathology. The sensitivity of mNGS was higher than that of the bacterial culture. However, the difference in specificity between bacterial culture and mNGS was not significant. Moreover, the time needed to perform mNGS was significantly lower than that of bacterial culture and pathology. All patients were followed up for more than three months, and CRP and NEUT% significantly decreased by three months after treatment. There was no significant difference in WBC and ESR. The ODI, NRS and JOA scores were significantly improved after treatment.

FDA device/drug status: Not applicable.

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CONCLUSION: Metagenomic next-generation sequencing technology can play an important role in the detection of pathogens in SI and should be further investigated and applied in future studies.

Keywords: Metagenomic next-generation sequencing; Pyogenic infection; Spinal infections; Spinal tuberculosis; Sensitivity; Specificity

Introduction

Spinal infections (SI) represent a group of rare conditions affecting the vertebral bodies, intervertebral discs, paraspinal soft tissues, epidural space, meninges, and spinal cord [1], accounting for 2% to 16.7% of all cases of osteomyelitis [2,3]. The incidence of SI has increased in recent years, with a worldwide prevalence of approximately 2.2/100,000 individuals per year [4−6]. SI have an insidious onset with a long disease course and are associated with a very high rate of disability, so early diagnosis and appropriate treatment are critical [7].

SI constitute a demanding diagnostic and treatment concern; in most cases, the diagnostic approach for patients with an SI should include blood workup, blood or CT-guided needle cultures and histology, and imaging evaluation with radiographs and MR imaging [4]. These methods have limitations such as low sensitivity, low positivity and long analysis time, however. In addition, the identification of pathogens in spine infections is extremely difficult and bacterial culture technique often fails to identify pathogenic microorganisms [8−10]. Delayed diagnosis or misdiagnosis of pathogens can cause disastrous consequences for patients. Back pain is the most common symptom of SI, and further progression of the condition can lead to neurological symptoms that, if not treated promptly, can lead to serious complications such as paralysis, spinal instability, and ultimately fatality [11−14]. In practice, broad-spectrum antibiotics are used empirically in the absence of an etiology, and these antibiotics may cause adverse effects, including antibiotic resistance, disruption of the human gut microbiota, and liver and kidney damage [15,16]. Therefore, early identification of the pathogens of SI is a major unmet challenge in the clinical treatment of this condition.

In recent years, unbiased metagenomic next-generation sequencing (mNGS) has been applied in medical microbiology as an emerging and powerful technique due to its short detection time [17]. It overcomes the limitations of conventional diagnostic tests allowing hypothesis-free, culture-independent pathogen detection directly from biological specimens [18]. Thus, mNGS could provide a new approach for identifying pathogens identification [19]. However, to date, studies on the application of mNGS to SI remain scarce thus far.

The present study reviewed a cohort in which mNGS was applied to identify the pathogens of SI. The sensitivity, specificity, and time cost of mNGS and bacterial culture technique in the detection of pathogens of SI were compared. Our aim was to evaluate the performance of mNGS in determining the etiology of spinal infection. Furthermore, the results of the mNGS analyses were used to develop appropriate antibiotic regimens for the patients. Changes in blood test and clinical outcomes after this treatment were also investigated.

Materials and methods

Study design

This retrospective study was conducted after receiving approval from the Institutional Review Board of Xinqiao Hospital and the Affiliated Hospital of Guizhou Medical University. IRB approval number 2022-R.No.262-01. The patient data were collected from the electronic medical record system of the institution from 2020 to 2022. The enrollment criteria for this study were as follows: (1) suspicion of SI based on symptoms, blood tests, and imaging features [1], (2) no history of invasive manipulation in spine, (3) mNGS of tissues obtained by surgery and CT-guided needle biopsy. Exclusion criteria included age less than 18 years, follow-up duration less than three months. The demographic data of the patients were collected from the records at Xinqiao Hospital and the Affiliated Hospital of Guizhou Medical University.

Sample collection and processing

Tissue samples were collected through CT-guided needle biopsy or debridement surgery. Each sample was divided into three parts. One part was placed into a sterile transport container and sent to the hospital laboratory for pathogen culture. Another part was stored in a sterile container with dry ice and delivered to the laboratory of BGI Genomics (Shenzhen, China). The cost for each test was 4600 RMB. The remaining part was fixed with formalin and sent to the Department of Pathology for histopathological analysis.

mNGS

Sample processing and DNA extraction

Tissue blocks approximately the size of a soybean were collected according to the standard sample collection process and physically homogenized with 600 microliters of lysate and 250 μL of 0.5 mm glass beads. Then, 7.2 μL of Lyticase (RT410-TA, TIANGEN BIOTECH, Beijing,

China) was added for cell wall digestion. Then, 250 μL of 0.5 mm glass beads were added for further wall breaking, mixing, and shaking and 300 μL samples were extracted according to the instructions of a TIANamp Micro DNA Kit (DP316, TIANGEN BIOTECH, Beijing, China).

Construction of DNA libraries and sequencing

DNA libraries were then constructed through DNA fragmentation, end repair, adapter ligation, and PCR amplification. An Agilent 2,100 was used for quality control of the DNA libraries. The qualified libraries were pooled and DNA nanoballs (DNBs) were made and sequenced by the BGISEQ-50/MGISEQ-2000 platform [20].

Bioinformatic analysis

High-quality sequencing data were generated by removing low-quality reads, followed by computational subtraction of the human host sequences mapped to the human reference genome (hg19) using Burrows–Wheeler Alignment [21]. The remaining data were classified by removal of low-complexity reads and simultaneous alignment to the Pathogens Metagenomics Database (PMDB), consisting of bacteria, fungi, viruses, and parasites. The classification reference databases were downloaded from NCBI (ftp://ftp.ncbi.nlm.nih.gov/genomes/). RefSeq contains 4,945 whole genome sequences of viral taxa, 6,350 bacterial genomes or scaffolds, 1,064 sequences of fungi related to human infection, and 234 sequences of parasites associated with human diseases. The sample collection and inspection process were shown in Fig. 1.

Blood tests and clinical outcomes

All patient blood samples were tested in the laboratories of the hospitals before and three months after antibiotic treatment. WBC, ESR, NEUT%, and CRP levels were recorded. Clinical metrics included the numeric rating scale (NRS) score, Oswestry Disability Index (ODI), and the Japanese Orthopedic Association (JOA) score. The scores on clinical outcomes before and three months after antibiotic treatment were analyzed.

Statistical analysis

Specificity, sensitivity, positive and negative predictive values (PPV/NPV), and the corresponding 95% confidence intervals (CIs) of mNGS and the culture results were calculated from cross-tabulation entries with respect to the histopathologic results as the reference standard [22]. The analysis was performed in two groups: (1) patients with nontuberculous inflammatory pathology (non-TB group) and (2) patients with tuberculous granuloma (TB group). In the quantitative data analysis, descriptive data are presented as the mean (standard deviation, SD) and median (interquartile range, IQR). Between-group differences were assessed using the chi-square test or Fisher's exact test for categorical variables and Wilcoxon rank-sum test or the Mann–Whitney *U* test for continuous variables. $p<.05$ indicates statistical significance. Statistical analyses were performed with IBM SPSS statistics 22 software.

Results

Patient classification and characteristics

During the course of the study, 26 patients with suspected SI were referred to the participating centers. Of these, one was excluded from the study because the follow-up time was less than three months (Fig. 2); 20 of the remaining patients underwent debridement surgery; and the other five patients underwent CT-guided needle biopsy. The specimens of all patients were sent for pathological analysis and mNGS. Two of the 25 patients had no bacterial cultures (specimens lost during the preparation). According to the pathological results, 11 patients were classified as the non-TB group. Ten patients were classified as the TB group. The detailed information of each case was shown in Table 1. The pathological examinations of the remaining four patients were negative. The mNGS results of three of

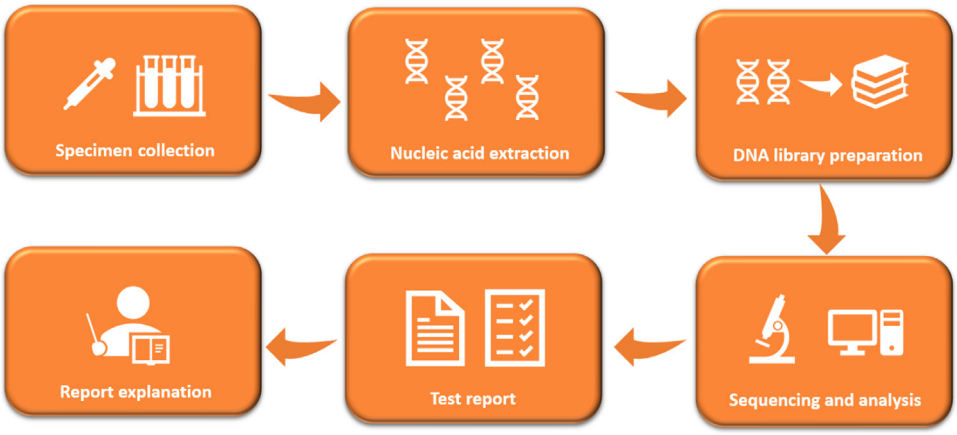

Fig. 1. Flow chart of mNGS.

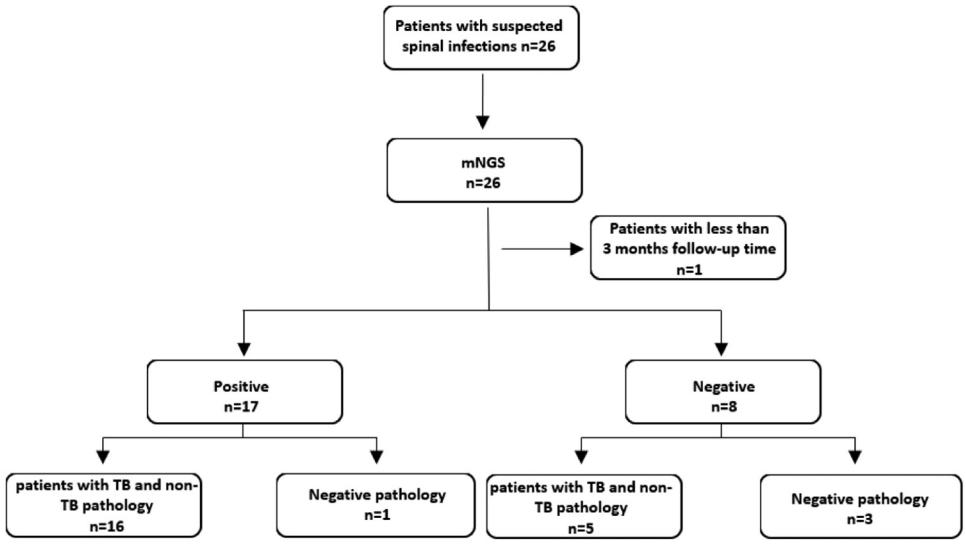

Fig. 2. Flow diagram of this diagnostic accuracy study. Non-TB pathology refers to nontuberculous inflammatory pathology. TB pathology refers to inflammatory pathology with tuberculous granuloma.

these pathology-negative patients were also negative remarkably, mNGS identified human herpesvirus, and *Aerococcus viridans* in the remaining pathology-negative patient (patient 1 [PT-1], Table 1). Considering that these two micro-organisms exist in the natural environment and on the surfaces of human skin, we believed that this result was a false-positive due to contamination from sample collection or preparation. mNGS identified pathogens in eight patients of the non-TB group, but the bacterial cultures were positive in only four of these patients. Three of these four culture-positive patients showed consistent results with mNGS. For the TB group, mNGS identified *Mycobacterium tuberculosis* (*M. tuberculosis*) in eight patients, however, only one patient showed a positive culture result. Noticeably, the pathological result of PT-9 was spondylodiscitis without tuberculous granuloma, while mNGS identified *M. tuberculosis*. In addition to *M. tuberculosis*, mNGS identified *Aerococcus viridans, Streptococcus intermedius, Staphylococcus aureus, Candida albicans, Staphylococcus epidermidis*, and *Proteus mirabilis* (*P. mirabilis*) (Table 1). In addition, some microorganisms were also identified by mNGS including *Cutibacterium acnes* (*C. acnes*), *Candida glabrata, Moraxella osloensis*, Torque teno virus, etc. However, they were considered as the background microorganisms due to their relatively low abundance (Supplementary Table 1).

Sensitivity, specificity, and time cost of mNGS and culture

The metrics of diagnostic efficacy for mNGS and culture in the non-TB group are presented in Table 2. The sensitivity of mNGS (72.7%) was higher than that of culture (36.4%), while the PPV and NPV of mNGS were 100% (95% CI, 62.8%−100%) and 57.1% (95% CI, 25.0% −84.3%), respectively. However, there was no significant difference in specificity between culture and mNGS (100% vs. 100%, respectively, p>.05) (Table 2).

For the TB group, mNGS showed a significantly greater sensitivity (80.0%) than culture (11.1%), and the PPV and NPV of mNGS were 88.9% (54.3%−100%) and 87.5% (62.7%−97.8%), respectively. There was no significant difference in specificity between culture and mNGS (100% vs. 93.3%, p>.05) for the TB group (Table 3). Furthermore, the time cost to obtain the results for mNGS (2.16±0.69 days) was significantly lower than that for bacterial culture (4.74±1.71 days) and histopathological analysis (3.04± 1.06 days) (Fig. 3).

Blood tests and clinical outcomes

The infection status of the patients in this study was decided based on multiple criteria, including clinical presentation, radiological findings, mNGS, pathology, bacterial culture, and therapeutic response. The patients' antibiotic regimens were prescribed based on a comprehensive analysis of mNGS, culture and histopathology results. The TB group and PT-9 were treated with "triple" or "quadruple" therapy (rifampicin+pyrazinamide+ethambutol/soniazid+rifampicin+pyrazinamide/isoniazid+rifampicin+pyrazinamide+ethambutol/rifampicin+pyrazinamide +ethambutol+moxifloxacin) for at least 12 months. Four patients with Staphylococcal infection were treated with vancomycin or linezolid and meropenem and one patient with fungal infection was treated with itraconazole and moxifloxacin. The patient with *Staphylococcus intermadius* infection was treated with moxifloxacin and the patient with the *P. mirabilis* infection was treated with levofloxacin. All antibiotic courses were continued for at least six weeks. The detailed medication regimens of the patients are shown in Table 1; all patients were followed up three months after treatment. The results of the blood tests are

Table 1
Detailed information of each case

Patient ID	Age, y/gender	Pathology type	Culture	mNGS	Sampling method	Therapeutic regimen
PT-1	41/M	Negative	Negative	Human herpesvirus; *Aerococcus viridans*	Surgical	None
PT-2	49/M	Positive (TB)	Negative	*Mycobacterium tuberculosis*	Surgical	Isoniazid+rifampicin+pyrazinamide+ethambutol
PT-3	43/F	Positive (TB)	Negative	*Mycobacterium tuberculosis*	Surgical	Isoniazid+rifampicin+pyrazinamide+ethambutol
PT-4	41/M	Positive (TB)	Negative	*Mycobacterium tuberculosis*	Surgical	Isoniazid+rifampicin +pyrazinamide
PT-5	46/M	Positive (TB)	Negative	Negative	Surgical	Rifampicin+pyrazinamide +ethambutol+moxifloxacin
PT-6	47/M	Positive (non-TB)	Negative	*Streptococcus intermadius*	Surgical	Moxifloxacin
PT-7	63/F	Positive (non-TB)	*Escherichia coli*	Negative	Surgical	Moxifloxacin
PT-8	73/F	Positive (TB)	Negative	Negative	Surgical	Rifampicin+pyrazinamide +ethambutol+moxifloxacin
PT-9	48/F	Positive (non-TB)	Negative	*Mycobacterium tuberculosis*	Surgical	Isoniazid+rifampicin+pyrazinamide+ethambutol
PT-10	66/M	Positive (non-TB)	Negative	*Staphylococcus aureus*	Surgical	Vancomycin
PT-11	77/F	Positive (TB)	Negative	*Mycobacterium tuberculosis*	Surgical	Isoniazid+rifampicin+pyrazinamide+ethambutol
PT-12	68/F	Positive (non-TB)	*Staphylococcus aureus*	*Staphylococcus aureus*	Surgical	Vancomycin
PT-13	59/F	Positive (non-TB)	Negative	Negative	Surgical	Cefoperazone sulbacta
PT-14	81/F	Positive (TB)	Negative	*Mycobacterium tuberculosis*	Surgical	Isoniazid+rifampicin+pyrazinamide+ethambutol
PT-15	82/M	Positive (non-TB)	Negative	Negative	Surgical	Levofloxacin
PT-16	65/F	Positive (non-TB)	*Proteus mirabilis*	*Proteus mirabilis;* human herpesvirus	Surgical	Levofloxacin
PT-17	54/M	Positive (non-TB)	Negative	*Candida albicans*	CT-guided	Itraconazole+moxifloxacin
PT-18	76/M	Negative	——	Negative	CT-guided	None
PT-19	75/F	Positive (TB)	*Mycobacterium tuberculosis*	*Mycobacterium tuberculosis*	CT-guided	Isoniazid+rifampicin+pyrazinamide+ethambutol
PT-20	61/M	Positive (TB)	——	*Mycobacterium tuberculosis*	CT-guided	Isoniazid+rifampicin+pyrazinamide+ethambutol
PT-21	64/M	Negative	Negative	Negative	CT-guided	None
PT-22	46/F	Positive (non-TB)	Negative	*Staphylococcus aureus*	Surgical	Linezolid+meropenem
PT-23	61/M	Negative	Negative	Negative	Surgical	None
PT-24	70/M	Positive (non-TB)	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	Surgical	Vancomycin
PT-25	72/M	Positive (TB)	Negative	*Mycobacterium tuberculosis*	Surgical	Isoniazid+rifampicin+pyrazinamide+ethambutol

M, male; F; female; Positive (non-TB) refers to nontuberculous inflammatory pathology; Positive (TB) refers to inflammatory pathology with tuberculous granuloma.

shown in Table 4. Both CRP level and NEUT% decreased dramatically after treatment. Additionally, while both the WBC and ESR were also reduced, the change was not statistically significant. Moreover, the ODI, NRS score, and JOA score showed significant changes after treatment (Table 5).

Discussion

In the current study, the sensitivity of mNGS in identifying the pathogens in patients with a nontuberculous inflammatory pathology was 72.7%, whereas the corresponding sensitivity of bacterial culture was 36.4%. Among patients with tuberculous granuloma, the sensitivity of mNGS was increased to 80%, the sensitivity of bacterial culture was 11.1%. Furthermore, there was no significant difference in specificity between culture and mNGS. The results suggest that mNGS has higher sensitivity but a similar specificity as culture in identifying the pathogens of SI.

The diagnosis of SI is often delayed because the clinical presentations are not sufficiently specific. Previous studies have reported a delay of two to six months between the first symptoms and diagnosis of SI [23–25]. Failure to recognize the condition and properly treat it can lead to catastrophic consequences. In this context, pathogen identification through culture technique is crucial to the diagnosis and treatment of SI patients; however, a definite etiological diagnosis is difficult to obtain through culture due to this low sensitivity [26,27]. Many factors are associated with lower sensitivity, including prior exposure to

Table 2
Diagnostic efficacy in patients with nontuberculous pathology

	PPV	NPV	Sensitivity	Specificity
Culture	100% (45.4%–100%)	30.0% (10.3%–60.8%)	36.4% (15.0%–64.8%)	100% (40.0%–100%)
mNGS	100% (62.8%–100%)	57.1% (25.0%–84.3%)	72.7% (42.9%–90.8%)	100% (38.3%–100%)
p value	>.99	.350	.219	>.99

PPV, positive predictive value; NPV, negative predictive value.

Table 3
Diagnostic efficacy in patients with tuberculous granuloma pathology

	PPV	NPV	Sensitivity	Specificity
Culture	100% (16.8%–100%)	63.6% (42.9%–80.4%)	11.1% (0.6%– 49.3%)	100% (73.2%–100%)
mNGS	88.9% (54.3%–100%)	87.5% (62.7%–97.8%)	80.0% (44.2%– 96.5%)	93.3% (66.0%–99.7%)
p value	>.99	.143	.031	>.99

PPV, positive predictive value; NPV, negative predictive value.

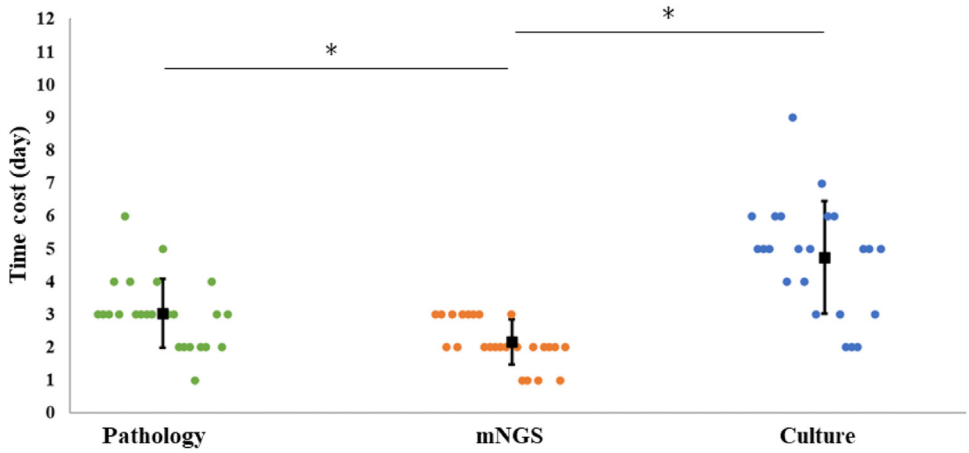

Fig. 3. Time cost of mNGS, culture and pathology.

antimicrobial therapy [28], inadequate specimen size (particularly when the bacterial inoculum is small) [29] and perhaps the specific characteristics of spondylodiscitis [30]. For many pathogenic microorganisms, such as *M. tuberculosis* [31], Brucella [32,33] and *C. acnes* [34], the appropriate culture technique is extremely difficult to identify and time-consuming. Quantitative real-time PCR has been reported to effectively improve the sensitivity of pathogen identification; however, it is only suitable for identifying specific pathogens rather than unknown pathogens [8,9]. The revolutionary mNGS conducts parallel sequencing of many millions of DNA molecules at a time and covers thousands of pathogens (including bacteria, fungi, and viruses). Recent studies have reported the great potential of mNGS in identifying the pathogens of infection diseases [35–38], such as identifying meningoencephalitis [39], fatal human encephalitis [40], chlamydia psittaci pneumonia [41], prosthetic joint infection [42], and post-transplantation infections [43–45], etc. mNGS can be used for the in-depth and rapid identification of pathogens and shows higher sensitivity than traditional culture technique [46–50]. Herein, the mNGS results were positive in more than 70% of the culture-negative patients (11/15), suggesting that mNGS was the only approach that identified the potential pathogens in 11 patients of our study. These results further demonstrated that mNGS is superior to culture technique in terms of sensitivity.

Table 4
Blood tests before and after treatment

	Before treatment (n=25)	After treatment (n=25)	p value
ESR, median (IQR)	22.0 (13.5–59.0)	17.0 (12.0–42.5)	.275
CRP, median (IQR)	14.0 (5.4–48.0)	2.8 (1.6–4.9)	.000
WBC, median (IQR)	6.2 (4.7–7.8)	6.2 (5.3–7.2)	.872
NEUT%, median (IQR)	67.6 (59.5–72.2)	59.3 (52.3–67.5)	.009

ESR, erythrocyte sedimentation rate; IQR, interquartile range; CRP, C-reactive protein; WBC, white blood count; NEUT, neutrophil.

Table 5
ODI, NRS and JOA before and after treatment

	Before treatment(n=25)	After treatment(n=25)	p value
ODI, median (IQR)	58.60% (43.58%–75.28%)	7.42% (2.95%–17.72%)	.000
NRS (neck /back/low back), median (IQR)	6 (3–7)	0 (0–1)	.000
NRS (hip/leg), median (IQR)	5 (1–6)	0 (0–1.5)	.000
JOA, median (IQR)	11 (9–15)	27 (24.5–29)	.000

ODI, The Oswestry Disability Index; IQR, interquartile range; NRS, Numerical Rating Scale; JOA, Japanese Orthopaedic Association Score.

Spinal tuberculosis accounts for over 40% of all spine infections [51], which is consistent with the results of this study. However, only one patient in the TB group showed a positive culture with *M. tuberculosis*, indicating the low sensitivity of bacterial culture in the diagnosis of spinal tuberculosis. Encouragingly, the sensitivity of mNGS in the diagnosis of spinal tuberculosis was significantly greater than that of culture (80%). In this study, the PPV and NPV of mNGS in the TB group were 88.9% and 87.5%, respectively. Based on these results, mNGS is a promising diagnostic technique for spinal tuberculosis. *Staphylococcus aureus* is the most common pathogen of pyogenic spinal [52,53], which is consistent with our results. Moreover, some opportunistic pathogens have also been identified, such as *P. mirabilis* and *Streptococcus intermedius*. These low-virulence, opportunistic pathogens have been reported as pathogens of SI [54–56]. It is worth mentioning that *C. acnes* were detected by mNGS as background bacteria in samples from multiple patients. The percentage of osteomyelitis caused by *C. acnes* varies from 2% to 18% [12]. A previous study has reported the positive culture of *C. acnes* in disc material from herniated discs, suggesting that this organism is involved in the development of disc herniation [4,12]. However, *C. acnes* are difficult to culture; consequently, its association with SI is not fully understood. On the other hand, this species is often considered a contaminant due to its widespread presence in the skin [4,57]. In this study, the identification of *C. acnes* in spinal tissues by mNGS suggests the potential pathogenic roles of *C. acnes* in SI. In addition, *Moraxella osloensis* is also a difficult-cultured bacteria [58], and is considered to be related to osteomyelitis [59,60]. In this study, it was identified by mNGS as background bacteria. The above results demonstrate the superiority of the mNGS technique in identifying rare opportunistic pathogens and those that are difficult to culture. mNGS has great importance in the treatment and prognosis of infectious diseases. First, the application of mNGS allows the early identification of antibiotic regimens, thus simplifying the use of antibiotics and even avoiding their overuse [61]. For example, for PT-16 and PT-23, empirical antituberculosis therapy was given before operation. According to the results of mNGS, the postoperative regimens for these patients included changing the antibiotics and stopping the antituberculosis therapy were selected after the operation, which avoided the potential adverse effects brought by the abuse of the antituberculosis drugs and improved the prognosis of the patients. Second, the application of mNGS can also increase doctors' confidence in making medical decisions. For example, PT-9 was suspected of spinal tuberculosis according to the preoperative clinical manifestations and auxiliary examination results. However, the postoperative culture results were negative, and the pathological results suggested bacterial inflammation but did not support the diagnosis of tuberculosis. The mNGS results, however, showed that the pathogen was *M. tuberculosis*. As this bacterium does not usually appear by sample contamination, for this patient, we formulated a standard antituberculosis treatment plan based on the mNGS results. Lastly, mNGS can identify the presence of drug resistant genes in *M. tuberculosis* (Supplementary Table 1), which contributed to establishing a sensitive antituberculosis regimen for each patient. Because of the above reasons, the prognostic indicators (inflammatory indicators and scores) of the patients in this study were significantly improved after treatment.

Identifying pathogens as early as possible is very important for the standard treatment of SI. In this study, the average time to obtain the culture results was 4.74 days, and the average time to obtain the histopathology examination results was 3.04 days. Remarkably, the mean time to obtain the mNGS results was lower, at 2.16 days. The results were consistent with those of other studies [17,62–64], indicating that mNGS technology promotes the rapid can help obtain the etiological diagnosis of SI in a shorter time, thus allowing patients to receive the appropriate treatment sooner. The higher diagnostic efficiency of mNGS will buy time for the treatment of patients with SI. Unfortunately, the cost of mNGS is currently much higher than that of culture and histopathology, which limits its widespread use in clinical practice. However, as the costs gradually decrease, mNGS will become more widely used as an important alternative technology in the etiological diagnosis of SI.

This study has several limitations. First, this study was a retrospective case series. There are challenges in comparing outcomes in such retrospective studies, the most important of which include indication or selection bias. Additionally, the sample size of this study was relatively small because SI is a relatively rare disorder, and mNGS was only performed for patients with good economic conditions that could afford it. Second, the presence of SI is clinically decided based on multiple criteria, including clinical presentations, radiological finding, laboratory tests and

therapeutic responses. There is no recognized diagnostic standard for SI. In this study, we used the histopathological results as the reference standard to calculate the sensitivity and specificity of mNGS and culture. As widely known, except for the typic tuberculous granuloma, pathological results only indicate inflammation and cannot differentiate inflammatory from infective pathology. Therefore, the histopathology is not a suitable diagnostic method for pyogenic infections. Moreover, the histopathology is unable to directly provide evidence of the etiology of the disease. In summary, the reasons mentioned above may lead to some bias in the final data. In the future, randomized controlled studies with large sample size and longer follow-up will be required to support the application of mNGS in the diagnosis and treatment of SI.

Conclusion

The mNGS technique showed a high sensitivity and specificity in identifying the pathogens of SI. Additionally, the high efficiency of mNGS significantly reduced time required to determine the etiological diagnosis. With further developments in precision medicine, mNGS will play a more crucial role in the diagnosis and treatment of SI.

Declaration of competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.spinee.2023.02.001.

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