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Original Article

Applying metagenomic next-generation sequencing to analyze infections in immunosuppressed patients with chronic kidney disease: A single-center retrospective study

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Abstract

Introduction: This retrospective study evaluated the diagnostic value and clinical application of metagenomic next-generation sequencing (mNGS) for detecting infections in immunosuppressed CKD patients.

Methodology: Data from immunosuppressed CKD patients who were suspected of having an infection and admitted to Jinling Hospital from 2018–2022 were retrospectively analyzed. The patients were divided into the conventional microbiological testing (CMT)-confirmed infection group (Group I), clinically diagnosed infection group (Group II), and exclusion of infection group (Group III), and the efficiencies of microbiological detection with mNGS and CMT were compared.

Results: In the 303 patients included in this study, 2 (1, 3) types of immunosuppressants were used for a median duration of 7 (2, 50) months. In Group I, 38.79% of the mNGS results were completely consistent with the CMT results, 27.88% were partially consistent, and 33.33% were inconsistent. In Group II, 57.69% of the infecting pathogens were detected by mNGS. Furthermore, 2 patients in Group III had positive NGS results. mNGS outperformed CMT in terms of the time to a positive test and the detection of mixed or rare microbial pathogens ($p < 0.05$). The sensitivity and accuracy of the detection of infectious pathogens were greater for mNGS than for CMT ($p = 0.014$).

Conclusions: mNGS can improve the sensitivity and accuracy of infectious pathogen detection in immunosuppressed CKD patients. mNGS is a promising emerging technique for detecting pathogens in CKD patients, with potential benefits in speed and sensitivity, and may provide more diagnostic evidence for the detection of mixed, opportunistic, and rare infectious pathogens.

Key words: Chronic kidney disease; immunodeficiency; infection; metagenomic next-generation sequencing.

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Introduction

In recent years, with the widespread use of immunosuppressants among chronic kidney disease (CKD) patients, their prognosis has improved significantly [1]. However, long-term immunosuppression leads to damage to the body's physical barriers and immune system and increases susceptibility to infection by various pathogens, which severely affects prognosis [2]. Among the possible complications, infection has become the primary cause of death in patients with lupus nephritis (LN) [3]. After infection in immunosuppressed CKD patients, both inaccurate antibiotic coverage of pathogens and inadequate drug doses impair treatment efficacy and lead to increased mortality. Therefore, accurate and timely pathogen identification is essential. At present, body fluid culture is the most commonly used method for pathogen identification, but this method has limitations, such as low sensitivity, long culture time, and the fact that antibiotic use affects the results. Although histopathology is the gold standard for the diagnosis of invasive fungal infection, it is difficult to obtain infected tissue samples in clinical practice. The diagnostic efficiency of microbiological antigen and antibody detection in immunocompromised patients is also low. Although polymerase chain reaction (PCR) is fast and sensitive, it can be used to detect only specific pathogens rather than unknown pathogens, so its clinical application has certain limitations [4].

Metagenomic next-generation sequencing (mNGS) does not rely on pathogen culture and is instead based on direct extraction of the nucleic acids of all microorganisms from a sample for high-throughput sequencing. Unlike conventional microbiological testing (CMT), mNGS is unbiased, has wide coverage, and requires no prior knowledge of possible pathogens [5]. Since it was first used to confirm evidence of *Leptospira santarosai* infection in a boy with severe combined immunodeficiency in 2014 [6], it has shown good detection efficiency in blood, cerebrospinal fluid (CSF), deep sputum, and bronchoalveolar lavage fluid (BALF) samples from patients with unexplained fever

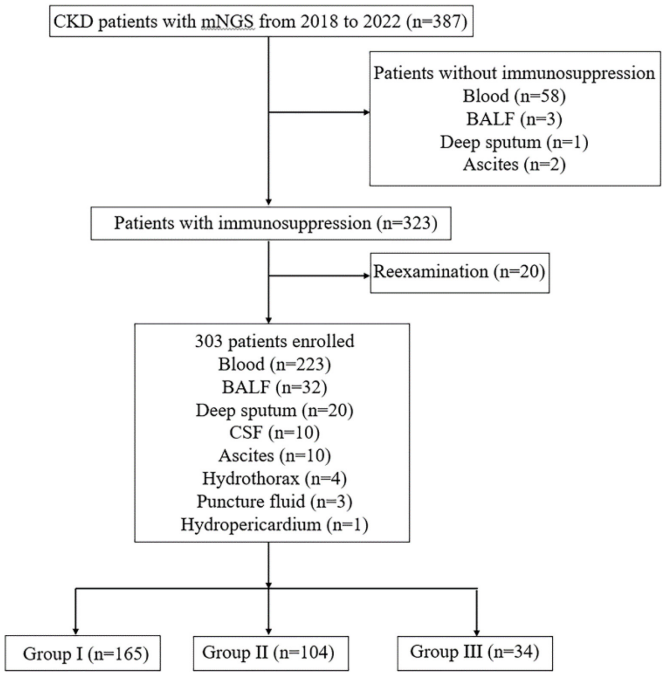
[7], sepsis [8], and transplantation [9]. To date, there has been no research on the clinical application of mNGS in CKD patients with infection. In this study, data from patients with suspected infection admitted to the center were retrospectively analyzed, the detection abilities of mNGS and CMT were evaluated, and the application value of mNGS for the differential diagnosis of infection in immunocompromised CKD patients was explored.

Methodology

Patients

Immunosuppressed CKD patients with suspected infection at the National Clinical Research Center of Kidney Diseases, Jinling Hospital, from August 2018 to April 2022 were selected as the study subjects. The criteria for suspected infection included the following: (1) persistent axillary temperature ≥ 37.3°C; (2) clinical symptoms of infection, such as cough, respiratory distress, fatigue, skin redness and swelling, abdominal pain, diarrhea, and headache; (3) increased levels of inflammatory markers, such as C-reactive protein (CRP) and procalcitonin (PCT); and (4) chest X-ray, computed tomography (CT), cardiac ultrasound and other imaging examinations suggesting the presence of infectious lesions. The criteria for immunosuppression included (1) a CD4+ T-cell count < 200 cells/μL; (2) receiving an organ or hematopoietic stem cell transplant; (3) receiving prednisone at 20 mg/day for more than 14 days before infection or a cumulative dose of prednisone > 600 mg; and (4) receiving other immunosuppressants [10]. All patients underwent CMT (including testing of blood, deep sputum, BALF, nasopharyngeal swab, and puncture fluid cultures as well as testing for microbiological antigens and antibodies and bacterial, fungal, and viral biomarkers) according to their clinical needs before receiving antibiotics. Culture and mNGS samples were obtained and sent for testing simultaneously. CMT was completed by Jinling Hospital, and mNGS was completed by Willingmed Corp., Beijing, China. The exclusion criteria included the following: (1) death or withdrawal of treatment within 3 days after admission and incomplete CMT results; (2) identification of pathogens before admission; (3) the presence of mNGS results upon reexamination after antibiotic therapy; and (4) fever of unknown origin, with the cause of fever not identified during hospitalization.

Figure 1. Screening process for enrolled patients.



Patient sample processing and DNA+RNA extraction for mNGS

Whole blood samples (≥ 4 mL) were collected in anticoagulation tubes and transported at 4°C after collection. Other body fluid samples (BALF, deep sputum, hydrothorax, ascites, and CSF) were collected from patients into sterile tubes with anticoagulant according to standard procedures and transported at 4–8 °C after collection.

For broad coverage of the potential pathogens in the samples, both DNA and RNA were extracted. DNA was extracted using a PathoXtract Basic Pathogen Nucleic Acid Kit (WYXM03211S, Willingmed Corp., Beijing, China), and RNA was extracted using a PathoXtract Virus DNA/RNA Isolation Kit (WYXM03009S, Willingmed Corp., Beijing, China) according to the manufacturer's protocol. DNA and RNA were eluted with 50 μL of nuclease-free water. DNA and RNA were mixed, and then reverse transcription of the RNA to complementary DNA (cDNA) was performed by using a SuperScript Double-Stranded cDNA Synthesis Kit (11917020, Invitrogen).

Library construction, sequencing, and data analysis

For cell-free DNA (cfDNA) library construction, the KAPA DNA HyperPrep Kit (KK8504, KAPA, Kapa Biosystems, Wilmington, MA, United States) was used according to the manufacturer's protocol. Genomic DNA libraries were constructed using Illumina DNA Prep (M) Tagmentation (20018705, Illumina) according to the manufacturer's protocol. Pooled libraries were sequenced on a NextSeq 550Dx system using a 75 bp, single-end sequencing kit

(Illumina), and at least 20 million sequencing reads were acquired from each sample.

Bioinformatic analysis

The raw FASTQ data were subjected to Trimmomatic v0.40 for quality control and evaluation, during which low-quality or undetected sequences, sequences contaminated by splices, high-coverage repeats, and short-read-length sequences were filtered out. High-quality sequencing data were compared with the human reference genome GRCh37 (hg19) using Bowtie2 v2.4.3 to remove human host sequences. The remaining sequences were aligned with a previously established reference database using Kraken2 (version 2.1.0) with the PlusPF database to annotate pathogen genomes and identify pathogens present in the sample. The classification was performed using 8 threads and a confidence threshold of 0.9. Unclassified sequences were written to a separate file for further analysis. The resulting classification was analyzed to determine the taxonomic composition of the sample. The reference database was established by using genomic data for bacteria, fungi, viruses, parasites, and other pathogenic microorganisms obtained from NCBI GenBank. These data were subjected to genomic filtering, screening, and validation to establish a reference database of pathogenic microorganisms that was suitable for clinical application.

Positive pathogens were identified based on the pathogen-specific reads per ten million (RPTM) values. For viral pathogen detection, a read count ≥ 3 was used as an empirical threshold for virus detection. Positivity for bacterial and fungal pathogens required meeting an RPTM threshold of ≥ 8 in blood samples and sterile body fluids. For BALF, deep sputum, and other nonsterile samples, positivity for bacteria and fungi required reaching an RPTM threshold of ≥ 20. Positivity for certain pathogens (including Cryptococcus and Mycobacterium) was defined as RPTM ≥ 1.

Table 1. Clinical characteristics of enrolled patients.

Characteristics	Patients (n = 303)
Age (years)	49 (34, 60)
Male, n (%)	186 (61.39)
Immunosuppressants (types)	2 (1, 3)
Course of immunosuppressants (months)	7 (2, 50)
CKD, n (%)	
Post-renal transplantation	72 (23.76)
LN	56 (18.48)
MN	34 (11.22)
Chronic renal failure	33 (10.89)
AAGN	20 (6.60)
Nephrotic syndrome	20 (6.60)
FSGS	16 (5,28)
IgA nephropathy	13 (4.29)
Cryoglobulinemia associated glomerulonephritis	8 (2.64)
Renal amyloidosis	5 (1.65)
DN	5 (1.65)
anti-GBM nephritis	5 (1.65)
MGRS	3 (0.99)
ADPKD	3 (0.99)
POEMS-RI	2 (0.66)
IgM nephropathy	2 (0.66)
HSPN	2 (0.66)
SS-RI	2 (0.66)
MM-RI	1 (0.33)
MPGN	1 (0.33)
Infection sites, n (%)	
Lung	221 (72.94)
Abdomen	33 (10.89)
Skin	25 (8.25)
Bloodstream	16 (5.28)
Urine	13 (4.29)
Intracalvarium	5 (1.65)
Endocarditis	1 (0.33)
Pericarditis	1 (0.33)

CKD: chronic kidney disease; LN: lupus nephritis; MN: membranous nephropathy; AAGN: antineutrophil cytoplasmic antibody-associated glomerulonephritis; FSGS: focal segmental glomerulosclerosis; DN: diabetic nephropathy; anti-GBM: anti-glomerular basement membrane; MGRS: monoclonal gammopathy of renal significance; ADPKD: autosomal dominant polycystic kidney disease; POEMS: polyneuropathy, organomegaly, endocrinopathy, M-protein, skin changes; RI: renal injury; HSPN: Henoch-Schonlein purpura nephritis; SS: Sjogren's syndrome; MPGN: mesangial proliferative glomerular nephritis.

Statistical analysis

SPSS 26.0 statistical software was used for data processing. All measurement data in this study exhibited a skewed distribution and are expressed as the median (quartile 1, quartile 3). The Kruskal–Wallis H test was used to compare the data among the three groups. Count data are expressed as percentages, and comparisons between groups were performed by Fisher's exact test or the χ^2 test. $p < 0.05$ was considered to indicate statistical significance.

Results

Clinical characteristics and patient grouping

A total of 303 patients who met the above criteria were included in this study (Figure 1), including 186 (61.39%) males and 117 (38.61%) females. The median age at admission was 49 (34, 60) years. From the onset of CKD to the time of admission, 2 (1, 3) types of immunosuppressants were used, and the median medication time was 7 (2, 50) months. Within 2 months before admission, 48 (15.84%) patients had received high-dose immunosuppressive therapy (such as methylprednisolone, cyclophosphamide, and rituximab), and 253 (83.50%) patients had received prednisone at a median dose of 20 (5, 30) mg/d. The median CD4+ T-cell count was 149.50 (81.25, 262.25)/μL. Fifty-one (16.50%) patients met the sepsis 3.0 criteria [11], and 62 (20.46%) patients died during this hospitalization. The distributions of different types

Table 2. Comparison of clinical characteristics among the three groups.

Clinical variables	Group I (n=165)	Group II (n=104)	Group III (n=34)	P	P1	P2	P3
Male, n (%)	99 (60.0)	71 (68.27)	16 (47.06)	0.076			
Age (years)	49.00 (35.50, 58.50)	51.00 (35.00, 59.75)	47.50 (29.50, 64.50)	0.792			
Immunosuppressants (types)	2.0 (2.0, 3.0)	2.50 (1.00, 3.00)	1.0 (1.0, 3.0)	0.029	1.000	0.144	0.024
Course of immunosuppressants (months)	11.0 (3.0, 60.0)	8.00 (2.00, 47.75)	1.00 (1.00, 17.25)	0.001	0.798	0.014	0.001
Prednisone in the last two months, n (%)	147 (89.09)	80 (76.92)	26 (76.47)	0.015	0.007	0.957	0.088
Dose of prednisone (mg/d)	25.00 (8.75, 40.00)	10.0 (5.0, 30.0)	30.00 (3.75, 48.75)	0.001	0.002	0.037	1.000
High-dose immunosuppressive therapy, n (%)	28 (16.97)	11 (10.58)	9 (26.47)	0.074			
Axillary temperature (°C)	38.60 (37.80, 39.00)	38.10 (37.13, 38.98)	37.60 (36.90, 38.65)	0.001	0.011	0.755	0.005
WBC (*10^9/L)	7.09 (4.66, 11.68)	7.12 (5.25, 10.63)	8.96 (6.24, 12.13)	0.149			
PLT (*10^9/L)	148.0 (98.0, 198.0)	149.50 (98.00, 241.50)	145.50 (79.50, 269.00)	0.338			
TLC (*10^9/L)	0.45 (0.24, 0.74)	0.54 (0.28, 0.95)	0.67 (0.44, 1.25)	0.008	0.180	0.373	0.013
TNC (*10^9/L)	6.10 (3.78, 10.18)	5.49 (4.01, 9.38)	7.57 (3.93, 10.19)	0.684			
CD3 + T (/uL)	280.00 (166.25, 491.50)	339.0 (171.0, 637.0)	392.0 (209.0, 649.0)	0.067			
CD4 + T (/uL)	127.50 (71.50, 205.00)	177.00 (100.00, 335.50)	167.00 (91.50, 257.00)	0.005	0.005	1.000	0.309
CD8 + T (/uL)	136.00 (72.75, 219.00)	152.00 (58.50, 257.75)	161.0 (81.0, 354.0)	0.388			
IgG (g/L)	6.37 (4.62, 9.98)	7.79 (5.15, 10.40)	9.77 (5.24, 14.43)	0.032	0.411	0.547	0.041
PCT (ug/L)	0.63 (0.24, 2.22)	1.15 (0.27, 5.01)	0.73 (0.42, 1.53)	0.080			
CRP (mg/L)	69.30 (28.80, 140.45)	84.75 (20.20, 163.25)	36.95 (8.13, 95.28)	0.042	1.000	0.037	0.098
Deaths, n (%)	46 (27.88)	14 (13.46)	2 (5.88)	0.001	0.006	0.357	0.006

WBC: white blood cell; PLT: platelet; TLC: total lymphocyte count; TNC: total neutrophil count; PCT: procalcitonin; CRP: C-reactive protein; P: comparisons among the three groups; P1: comparison between Group I and Group II; P2: comparison between Group II and Group III; P3: comparison between Group I and Group III.

of CKD and infection sites are presented in Table 1.

Among the 303 patients, 165 patients were diagnosed with definite infections based on both microbiological and clinical criteria (Group I), and 104 patients were diagnosed with infection based on only clinical criteria (Group II). Thirty-four patients were ultimately diagnosed with noninfectious diseases (Group III). There were significant differences among the three groups in the types of immunosuppressants used and the duration of their use, the proportion of patients who used prednisone and the dose used, tacrolimus, axillary temperature on admission, total lymphocyte count, $CD4^+$ T-cell count, IgG levels, CRP levels, and in-hospital mortality ($p < 0.05$) (Table 2).

Comparison of mNGS and CMT

Among the 165 patients in Group I, 98 had positive cultures, 65 had positive serum (1,3)-beta-D-glucan test (G test) results, 23 had positive serum galactomannan (GM) antigen test results, and 3 had positive interferon-gamma release assay results (IGRAs). The results of mNGS and CMT were completely consistent in 64 patients, partially consistent in 46 patients, and inconsistent in 55 patients. In Group II, 60 of the 104 patients were found to harbor infectious pathogens by mNGS. A total of 86 patients (31.97%) in Groups I and II were treated with antibiotics that were adjusted according to the results of mNGS. In Group III, there were 3 cases each of diffuse alveolar hemorrhage, autoimmune hemolytic anemia, hemophagocytic syndrome, and reversible posterior white matter lesions; 2 cases of thrombotic thrombocytopenic purpura; and 1 case each of myelodysplastic syndrome, drug hypersensitivity syndrome, antiphospholipid antibody syndrome, Guillain–Barr syndrome, and interstitial lung disease. Among these, 3 cases were CMT positive and mNGS negative, and 2 cases were CMT negative and mNGS positive (Figure 2). Following the exclusion of infection, the patients in Group III discontinued antibiotic use and proceeded

Figure 2. Comparison of the distribution of mNGS and CMT results.

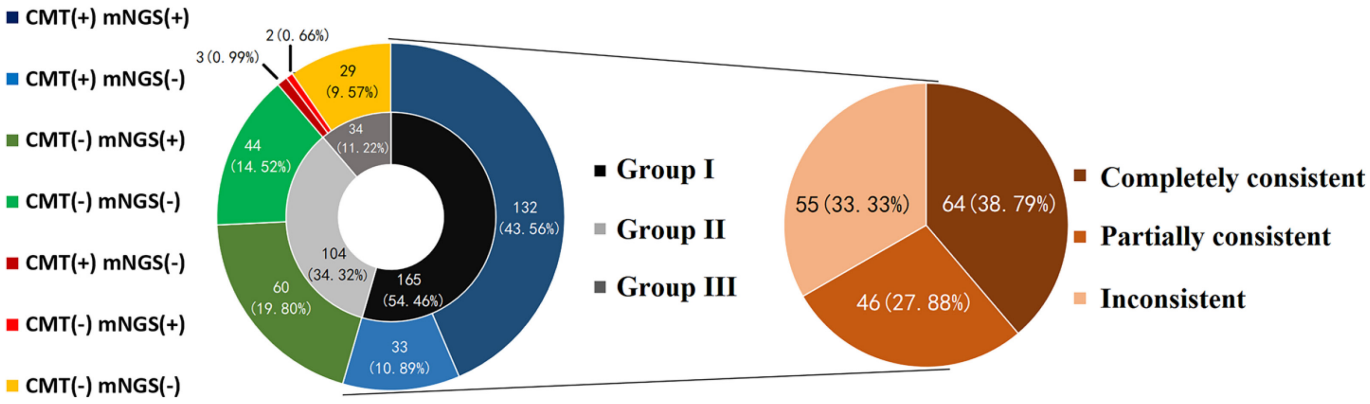
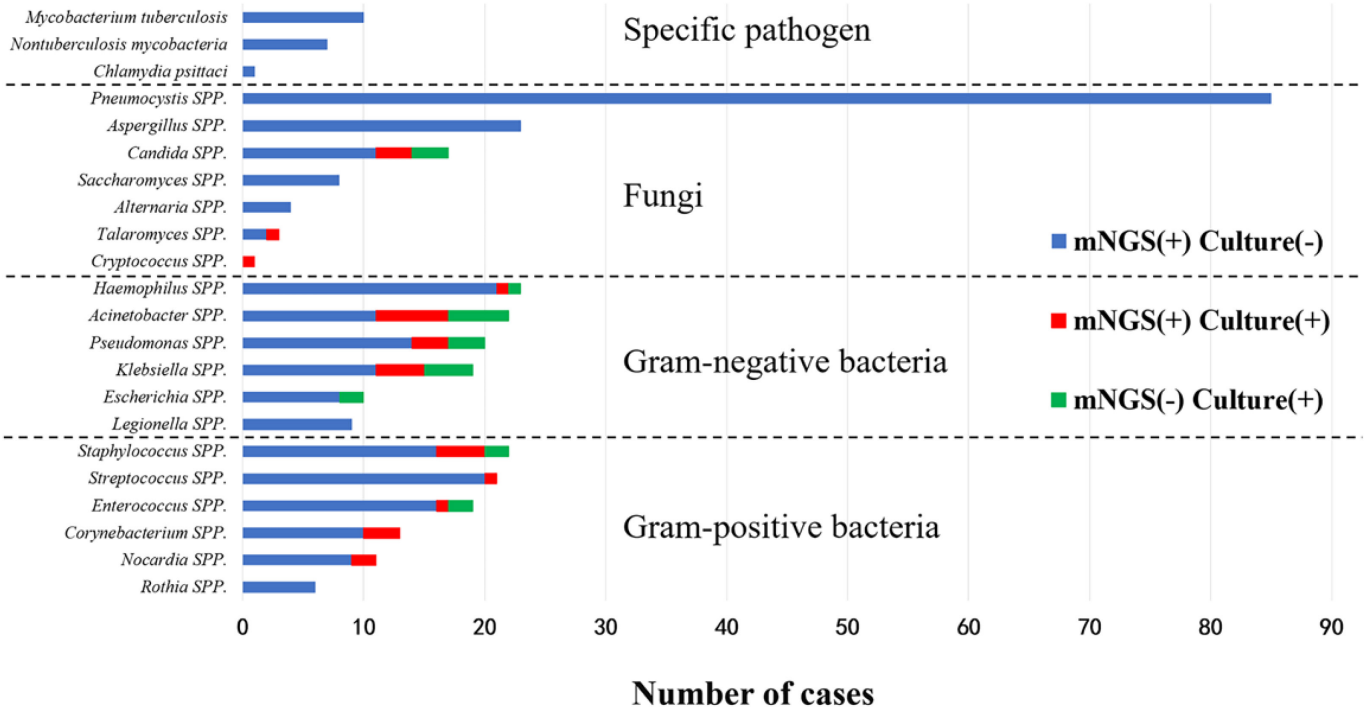


Figure 3. Comparison of mNGS and culture in the detection of different pathogens.



with aggressive treatment for their primary disease. Among them, 32 patients recovered, while 2 patients died from irreversible alveolar hemorrhage and catastrophic antiphospholipid antibody syndrome.

According to the mNGS results, there were 50 patients without pathogen infections, 121 patients with fungal infections, 54 patients infected with gram-positive bacteria, and 86 patients infected with gram-negative bacteria. Human herpesvirus type 1 and JC polyomavirus were detected in the CSF of 2 patients, and these patients were diagnosed with herpes simplex virus encephalitis and progressive multiple leukoencephalopathy (PML), respectively. The distributions of the mNGS and culture results during the detection of different pathogens are shown in Figure 3. Among them, 85 cases of *Pneumocystis* spp. infection, 23 cases of *Aspergillus* spp. infection, 10 cases of *Mycobacterium tuberculosis* infection, 9 cases of *Legionella* spp. infection, 8 cases of *Saccharomyces* spp. infection, 7 cases of nontuberculous *Mycobacterium* infection, 6 cases of *Rothia* spp. infection, 4 cases of *Alternaria* spp. infection and 1 case of *Chlamydia psittaci* infection could be identified only by mNGS.

Consistency and efficiency of detection

The assessment of the consistency between mNGS and culture results for the detection of bacteria and fungi in blood, BALF, deep sputum, serous effusion, and CSF yielded kappa values of 0.043, 0.158, 0.055, 0.051, and 0.615, respectively, whereas the consistency was 47.53%, 62.50%, 40.0%, 33.33%, and 90.0%, respectively. mNGS had an earlier positive detection time and could be used to detect more species of bacteria and fungi than culture ($p < 0.05$) (Table 3). The rate of detection of mixed infections with more than 2 species of bacteria or fungi was higher with mNGS than with culture (24.42% vs. 3.30%; $p < 0.001$). The

Table 3. Comparison of mNGS and culture in the positive detection time and types of infectious pathogen species.

	Positive detection time of mNGS (days)	Positive detection time of culture (days)	*p*
Blood	2.0 (1.0, 2.0)	3.00 (2.25, 4.00)	< 0.001
BALF	2.0 (1.0, 2.0)	4.00 (2.75, 6.25)	< 0.001
Deep sputum	2.0 (1.0, 2.0)	3.0 (2.0, 4.0)	0.002
Serous effusion	2.0 (1.0, 2.0)	5.00 (2.75, 5.75)	0.006
	Bacteria and fungi detected by mNGS (types)	**Bacteria and fungi detected by culture (types)**	***p***
Blood	1.0 (1.0, 2.0)	1.0 (1.0, 1.0)	0.024
BALF	3.5 (2.0, 5.0)	1.0 (1.0, 2.0)	< 0.001
Deep sputum	3.0 (2.0, 4.0)	1.0 (1.0, 2.0)	0.001
Serous effusion	1.0 (1.0, 2.0)	1.0 (1.0, 2.5)	0.888

mNGS: metagenomic next-generation sequencing; BALF: bronchoalveolar lavage fluid;

sensitivity (71.38% vs. 61.33%, p = 0.014) and accuracy (73.93% vs. 64.69%, p = 0.014) of mNGS for the detection of infectious pathogens were greater than those of CMT, whereas there was no significant difference in specificity, positive predictive value (PPV), or negative predictive value (NPV) (Table 4).

Discussion

Traditional methods for the diagnosis of infectious diseases require the identification of pathogens and the analysis of their characteristics. At present, there are thousands of known pathogens, and CMT can identify only a small proportion of common pathogens [12]. The detection results depend on the judgment of the physician and the skills of the laboratory staff. In addition, empirical antibiotic treatment can lead to false-negative results, inadequacies in body fluid sampling, a small pathogen load, a long culture period, and other effects, resulting in many pathogens in the clinic not being identified [13]. CKD patients taking immunosuppressants have some clinical manifestations that are different from those of the general population when common pathogens are the etiological agents of infection. They are also susceptible to infections caused by rare pathogens and newly emerging pathogens and need to receive effective anti-infection treatment early [2].

In recent years, with the development of genomics and the reduction in detection cost, mNGS has facilitated great advancement in the diagnosis of infections that are difficult to detect because of its high sensitivity, speed, and efficiency, but there are also some challenges associated with this method [14]. A study conducted at Huashan Hospital in Shanghai, China, used mNGS to test a variety of samples, including blood, BALF, and puncture fluid, from immunocompromised patients taking corticosteroids. The sensitivity and specificity of mNGS in the diagnosis of infection were 80.6% and 87.1%, respectively, and the results were not affected by the degree of immunosuppression. The success rate of antibiotic therapy guided by mNGS was significantly higher than that of empirical therapy (81.8% vs. 52.6%) [15]. Another study, conducted at St. Jude Children's Research Hospital, USA, showed that mNGS analysis of blood could be performed to detect infectious pathogens during the first three days of bloodstream infection, with a predictive sensitivity of 75% and a specificity of 91% [16]. However, researchers from Texas Children's Hospital, USA, reported that CMT and mNGS results were similar and that mNGS had little additional diagnostic value. When other pathogens were identified by mNGS, antibiotics were adjusted in only 26% of patients [17]. A study from Peking Union Medical College Hospital, China, revealed that all mNGS-derived criteria had low sensitivity for diagnosing invasive pulmonary aspergillosis. A combination of mNGS and CMT may be the best diagnostic strategy [18].

This study revealed that the use of mNGS can improve the sensitivity and accuracy of pathogen detection and the rate of detection of mixed infections in immunocompromised CKD patients, shorten the time to positive detection, and eliminate the need for clinical prediction. It can be used to quickly detect pathogens that are difficult to culture, such as *Pneumocystis jirovecii, Aspergillus, Mycobacterium tuberculosis, Legionella*, and rare pathogens, such as *Talaromyces marneffei*, *Nocardia*, nontuberculous *Mycobacterium*, and *Chlamydia psittaci*, exhibiting important clinical application value. There was no significant difference in specificity, PPV, or NPV between mNGS and CMT, and the NPV was low for both methods. In 42.31% of the patients in the clinically diagnosed infection group (Group II), the pathogen could not be detected via mNGS, and infection could not be ruled out based on only mNGS negativity. The low NPV values of mNGS and CMT may be related to the fact that CKD patients without infection were not included in this study, resulting in a small proportion of

Table 4. Comparison of the diagnostic efficiency of mNGS and CMT.

	Group I+II (n = 269)	Group III (n = 34)	
CMT (+) (n = 168)	165	3	
CMT (-) (n = 135)	104	31	
mNGS (+) (n = 194)	192	2	
mNGS (-) (n = 109)	77	32	
	CMT (95% CI)	**mNGS (95% CI)**	***p***
Sensitivity	61.33% (55.21%, 67.14%)	71.38% (65.51%, 76.62%)	0.014
Sensitivity	91.18% (75.19%, 97.69%)	94.12% (78.94%, 98.97%)	1.000
PPV	98.21% (94.46%, 99.54%)	98.97% (95.93%, 99.82%)	0.666
NPV	22.96% (16.35%, 31.15%)	29.36% (21.22%, 38.97%)	0.256
Accuracy	64.69% (59.35%, 70.15%)	73.93% (69.05%, 78.95%)	0.014

CMT: conventional microbiological tests; mNGS: metagenomic next-generation sequencing; (+): positive; (-): negative; PPV: positive predictive value: NPV negative predictive value.

true negatives. An increase in the levels of inflammatory markers such as CRP is associated with the activity of anti-neutrophil cytoplasmic antibody-associated glomerulonephritis (AANG) [19] and LN [20]. Some patients with anti-basement membrane nephritis present with fever [21], and it is difficult to distinguish the chest CT manifestations of diffuse alveolar hemorrhage and pulmonary infection. The observation of the antidiastole associated with infection in CKD patients needs to be combined with assessments of medical history, symptoms, signs, and laboratory and imaging examinations, along with pathogen detection at multiple sites, to determine whether to intensify antibiotic treatment or increase the dose of immunosuppressive agents. Moreover, the consistency of mNGS and culture results during the analysis of blood, BALF, deep sputum, serous effusion, and other samples was low. Although the consistency of the results for the detection of bacteria and fungi in CSF was 90.0%, 2 patients could still be diagnosed with intracranial virus infection only by mNGS. mNGS can be used to detect a variety of pathogens in one sample, whereas culture can generally be used to detect only one type of pathogen. In some patients in this study, *Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterococcus faecium,* and other pathogens could not be identified by mNGS in blood but could be detected by culture in samples from other sites, suggesting that the samples used for mNGS should be obtained from the primary infected lesion when possible. The reliability of the mNGS detection method needs to be further improved; the two detection methods are complementary and need to be combined instead of used in isolation.

As it has the potential to detect any microorganism in the host, mNGS can be widely used to identify pathogen colonization before infection, analyze host and microbial gene expression, monitor pathogen load and antibiotic resistance after treatment, perform risk assessment of hospital-acquired bloodstream infection, and even perform epidemic investigations and identification of transmission routes in the future [22]. Like in studies of CKD, in studies of patients with tumors, rheumatism, diabetes, malnutrition, and other possible secondary immune deficiencies, mNGS needs to be used with larger sample sizes to assess different underlying diseases to determine their detection efficiency. Therefore, the changes in pathogenic opportunistic microorganisms in CKD patients with different levels of immunity and renal function warrant further investigation [23].

The limitations of this study are as follows. First, the clinical diagnosis of infection in a single-center retrospective study is somewhat subjective. Second, patients in whom the pathogens causing infection were clearly identified before admission were not analyzed by mNGS, which may have interfered with the evaluation of the detection efficiency. Third, in actual clinical practice, owing to the cost, mNGS could not be performed on samples from multiple patient sites, but CMT could be used to test samples from multiple sites, which interfered with the evaluation of the consistency between the two detection methods. Fourth, this study retrospectively analyzed the CMT results, which were submitted on the basis of the actual clinical requirements at the time rather than by following a uniform and comprehensive submission plan. This approach may have impacted the theoretical accuracy of the CMT results. Finally, the continuous improvement in mNGS detection technology and real-time updating of microbial databases over the past five years have affected the consistency of the results.

Conclusions

In summary, the use of mNGS can increase the sensitivity and accuracy of pathogen detection in immunocompromised CKD patients; shorten the time to positive detection; improve the detection rate for mixed, opportunistic, and rare infectious pathogens; and help inform the adjustment of anti-infective therapy in an accurate and timely manner, providing important clinical application value. As the cost of mNGS declines, it will likely experience broader adoption in developing countries.

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Authors' contributions

Study concept and design: Shijun Li and Zhe Li. Acquisition of data: Shuhua Zhu, Yuchao Zhou, and Jing Jiang. Statistical analysis: Yang Wang and Lixuan Lou. Analysis and interpretation of data: Zhe Li and Shutian Xu. Drafting the manuscript: Zhe Li. Critical revision of the manuscript: Shijun Li and Shutian Xu. All authors contributed to the article and approved the submitted version.

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Ethical Approval and Consent to participate

All procedures performed in the present study were in accordance with the ethical standards of the institutional

research committee of Jinling Hospital, Nanjing University School of Medicine (No. 2022DZKY-033-01) and with the tenets of the Declaration of Helsinki of 1964 and its later versions. As the present study was an observational and historical but not a prospective interventional study, the ethics committee waived the need for direct informed consent.

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Conflict of interests

No conflict of interests is declared.

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