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The clinical utility of metagenomic next-generation sequencing in the management of fever in patients with hematological disorders

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ABSTRACT

Patients with hematological malignancies frequently present with severe and intricate infections that pose life-threatening risks. Conventional pathogen detection methods offer limited clinical insights and therapeutic guidance. This retrospective study evaluated the clinical application of metagenomic next-generation sequencing (mNGS) in hematologic patients who remained febrile despite prolonged antibiotic therapy, which means unresponsive to antibiotic therapy. This retrospective analysis included 204 patients with hematologic malignancies, undergoing conventional pathogen detection and peripheral blood mNGS. The cohort was stratified into neutropenia and non-neutropenia groups to compare the diagnostic and therapeutic implications of mNGS versus conventional microbiological tests (CMT). Among the 204 patients with mNGS, the overall positive detection rate was significantly higher than that of CMT (68.1% vs 30.9%, P<0.001). In both the neutropenia and non-neutropenia group, mNGS demonstrated a higher positivity rate for bacteria than for CMT (bacteria, 36.4% vs 15.6%, P<0.01). mNGS proved notably advantageous for bloodstream infections with clinically relevant drug-resistant strains, particularly in the neutropenia cohort (26.4% vs 12.5%, P<0.001). Using a composite reference standard, mNGS manifested sensitivity and specificity rates of 78.4% and 61.9%, respectively. Patients in the neutropenia group derived superior clinical benefit from mNGS, including higher diagnostic accuracy and treatment efficacy (diagnosis, 56.4% vs 40.6%, P=0.036; treatment, 49.3% vs 31.3%, P = 0.016). Additionally, the 30-days mortality rate was notably higher among mNGS-positive patients who tested compared to those who tested negative (17.3% vs 1.5%, P<0.001). mNGS demonstrated clinical relevance in patients with hematologic malignancy who received prolonged antibiotic treatment and holds promise in predicting patient survival prognosis.

Keywords: diagnosis, infection, metagenomic next-generation sequencing (mNGS), clinical utility

Abbreviations:
mNGS: metagenomic next-generation sequencing
CMT: conventional microbiological tests
HSCT: hematopoietic stem cell transplant
CRB: carbapenem-resistant bacteria

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INTRODUCTION

Infectious diseases significantly contribute to patient mortality and morbidity, imposing a substantial burden on global public health and economic development.[1] Individuals with hematologic malignancies commonly suffer from infections that may result from chemotherapy, radiation therapy, or hematopoietic stem cell transplantation (HSCT). Domestic guidelines emphasize the necessity for prompt blood culture and other traditional microbiological assessments at the onset of fever in patients with hematological diseases. Furthermore, empirical antibiotics should be promptly considered and administered; however, traditional microbiological testing methods are hindered by prolonged turnaround times, low culture yields, and limited capacity to detect viruses.[2]

Metagenomic next-generation sequencing (mNGS), characterized by its high throughput, deep sequencing, and efficient performance, has emerged as a promising molecular diagnostic technique for precise pathogen identification.[3] It introduces a novel technical approach for rapidly confirming microbiological diagnoses and holds significant potential for clinical applications. Despite its advantages, the application of mNGS in patients with fever due to hematological diseases who are continuously treated with antibiotics remains unclear.

Previous studies have suggested that discontinuing antimicrobial therapy for more than 3 days prior to sample collection may be necessary to further enhance the positivity rate of mNGS for infections.[4] Currently, the clinical utility of mNGS in patients with hematological malignancies who remain febrile despite prolonged antibiotic therapy remains inconclusive and requires further validation. This study aimed to evaluate the clinical diagnostic and therapeutic value of mNGS for hematological malignancies under prolonged antibiotic exposure.

MATERIAL AND METHODS

Study design and data collection

We conducted a retrospective analysis of patients with hematologic conditions admitted to the Hematology Department of Nanfang Hospital, Southern Medical University, Guangzhou, China, between January 1, 2022 and January 1, 2023 who were suspected of infection and underwent mNGS testing. Patients were included in the study if they met the following criteria: (i) diagnosed with hematological diseases; (ii) experience fever >38.3 °C; (iii) demonstrated persistent fever after 72 hours of antibiotic treatment, with minimal or no reduction in peak temperature; (iv) had peripheral blood of mNGS and conventional microbiological tests (CMT) such as culture, cryptococcus antigen detection, (1-3)-β-D-glucan test (G-test), glucan mannan test (GM test), direct microscopic examination (DME), tuberculosis test (TB test), Xpert MTB/RIF test and polymerase chain reaction (PCR) were performed within one week. The exclusion criteria were as follows: (i) patients with hematologic diseases; (ii) patients with incomplete clinical data (iii) mNGS test results did not meet quality control standards or were suspected of contamination; (iv) repeat tests during 7 days after the first mNGS tests; (v) lack of conventional paired microbiological tests. This study was conducted in accordance with the declaration of Helsinki. and approved by Institutional Review Board and Ethics Committee of Nanfang Hospital, Southern Medical University (approval number, NFEC-2024-341).

Neutropenia was defined[5] as an absolute neutrophil count (ANC) of <500/mm^3. The clinical committee retrospectively diagnosed different types of infections based on a comprehensive consideration of clinical symptoms, laboratory tests, radiological imaging, clinical medical technology evaluation, and treatment response, with reference to the Centers for Disease Control

and Prevention/National Healthcare Safety Network (CDC/NHSN) surveillance definitions.[6] The diagnostic capabilities and agreement between NGS and CMT were assessed through comparative evaluation and subgroup analyses of neutropenic and non-neutropenic cohorts.

Detection methods and criteria for mNGS reports

At least 3mL of blood sample was collected from the antecubital vein, stored in a nucleic acid preservation tube at room temperature, and transported the same day to Precision Medicine Center laboratory of Nangfang Hospital. mNGS testing was conducted using a magnetic bead-based microbial kit. A QIAamp Viral RNA Mini Kit (QIAGEN) was used to extract DNA. The library quality was assessed using quantitative PCR performed on the Agilent 2100 system (Agilent Technologies) and a Qubit 4.0 fluorometer (Thermo Fisher). The MGISEQ-2000 platform (BGI) was employed for high-throughput mNGS sequencing. After filtering out low-quality and short reads, as well as human host sequences, the remaining data were aligned with entries in the microbial genome database, including National Center for Biotechnology Information (NCBI) database, NCBI RefSeq database, U.S. Food and Drug Administration dAtabase for Reference Grade micrObial Sequences (FDA-ARGOS), Genome Taxonomy Database (including 10634 bacterial, 1179 fungal, 5050 viral, 282 species of parasites, and 355 other intracellular genomes, such as Mycobacteria, Chlamydia and Mycoliasma).

Given the absence of a universally recognized standard experimental protocol and varying interpretations of NGS results, we adopted the following criteria in our study, informed by expert consensus and previous research: (i) For bacteria and fungi, a genus-level relative abundance >30% was considered indicative. (ii) For bacteria (excluding *Mycobacterium tuberculosis*), viruses, and fungi (excluding *Pneumocystis jiroveci*), a microorganism was deemed positive if the number of reads mapped stringently at the species level (SMRN) was ≥3. (iii) *Mycobacterium tuberculosis* and *Pneumocystis jirovecii* were considered positive if the reads were specifically aligned. (iv) Parasites were considered positive if SMRN was ≥100. (v) Positive microorganisms exhibited higher SMRN than the negative controls.

Interpretation and clinical impact of NGS

Given the lack of consensus regarding the interpretation of NGS reports in clinical practice, NGS results are interpreted jointly by hematologists, clinical microbiologists, and radiologists. Based on the medical history, clinical presentation, relevant laboratory tests, and imaging results, interpretations were categorized into five groups: definite, probable, possible, unlikely, and false negative. (i) Definite: the NGS results corresponded with the CMT results conducted within a week. (ii) Probable: the microorganisms detected by NGS were highly probable to have initiated and propagated the infection. (iii) Possible: NGS indicates that these microorganisms may be associated with infection, but they are not commonly considered primary causes of infection in clinical practice. (iv) Unlikely: the microorganisms identified through NGS may not necessarily be the primary cause of the infection, or their detection results may differ from those obtained by traditional methods. (v) False negative: although the NGS result was negative, the case was still evaluated as an infection.

To better evaluate the performance of mNGS in clinical infection management, we established the following criteria. In terms of diagnosis, (i) positive impact: NGS results were faster than CMT or aid in pathogen identification. (ii) No impact: both of the NGS and CMT were positive, but the pathogen identified by NGS were clinically irrelevant or slower. (iii) Negative impact: clinical suspicion of specific pathogen infection, but NGS results were negative. In terms of treatment, (i) positive impact: NGS results led to modification in antibiotic administration, including selection and dosage, or supported maintaining current antibiotics when the NGS

results are positive and the pathogen was already covered, thus providing precise therapeutic guidance. (ii) No impact: antibiotics were not adjusted in response to positive or negative NGS results, or the patient may have been discharged or died prior to the availability of the report. (iii) Negative impact: the NGS led to antibiotic treatment that was not warranted. The diagnostic impact evaluation centers on discerning whether NGS results identify the pathogen responsible for the infection, whereas the treatment impact assessment evaluates the adjustment of antibiotic therapy within a week of receiving the NGS report based on these findings.

Statistical analysis

Continuous variables are represented as medians and ranges, while categorical variables are expressed as counts and percentages. The Mann-Whitney U test was used to compare differences in continuous variables, and the chi-square test was used for categorical variables. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using clinical composite diagnosis as the reference standard. The concordance of test results was assessed using kappa statistics. $P < 0.05$ was considered statistically significant. Statistical analysis and graphical representation were conducted using SPSS software, version 29.0 software (IBM) and GraphPad Prism 10.0 software (GraphPad Software).

RESULTS

Patient baseline characteristics and sample collection

After excluding non-conforming cases, 204 clinical samples were enrolled in our study, comprising 140 patients with neutropenia and 64 patients without neutropenia (Figure 1). Among them, there were 114 males and 90 females, with a median age of 43 (ranging from 15 to 71), prevalent underlying diseases included acute myeloid leukemia (49.5%), followed by acute lymphoblastic leukemia (23%), myelodysplastic syndromes (11.8%), lymphoma (8.8%), and other

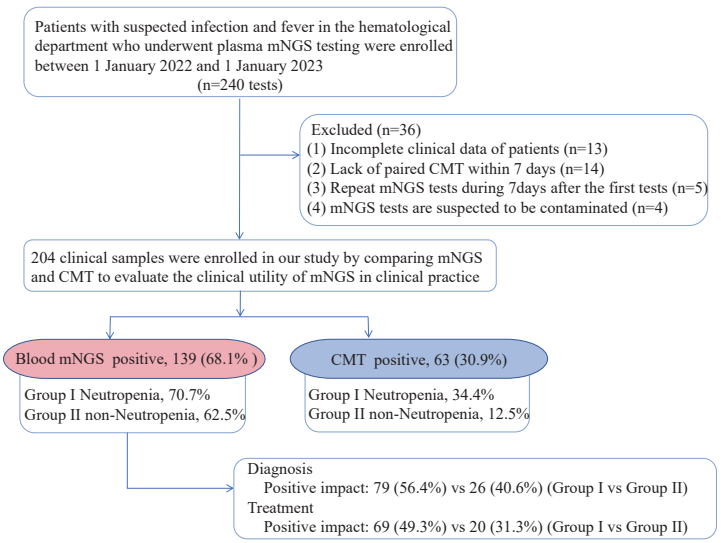

Fig. 1 Overview of study workflow
mNGS: metagenomic next-generation sequencing
CMT: conventional microbiological tests

Table 1 Samples characteristics and baseline of the two study groups

Samples characteristics	Total	Group I Neutropenia	Group II Non-neutropenia	P value
Number of tests	204	140	64	–
Median age (range)	43.0 (15–71)	40.0 (16–70)	48.5 (15–71)	0.118
Male, n (%)	145 (71.1%)	98 (70.0%)	47 (73.4%)	
Underlying diseases, n (%)				<0.001
Acute myeloid leukemia	101 (49.5%)	77 (55.0%)	24 (37.5%)	
Acute lymphoblastic leukemia	47 (23%)	33 (23.6%)	14 (21.9%)	
Lymphoma	18 (8.8%)	8 (5.7%)	10 (15.6%)	
Myelodysplastic syndromes	24 (11.8%)	15 (10.7%)	9 (14.1%)	
Other diseases	14 (6.9%)	7 (5.0%)	7 (10.9%)	
Laboratory examination, median (range)				
WBC, 10^9/L	1.15 (0.04–72)	0.67 (0.04–72)	4.72 (1.07–19.79)	<0.001
ANC, 10^9/L	0.09 (0–17.77)	0.01 (0–0.49)	3 (0.84–17.77)	<0.001
CRP, mg/L	98.66 (0.66–359.17)	119.65 (2.57–359.17)	68.77 (0.66–312.35)	<0.001
PCT, ng/mL	0.56 (0.03–63.07)	0.56 (0.04–63.07)	0.46 (0.03–57.27)	0.622
Therapy of underlying diseases, n (%)				
HSCT	65 (31.9%)	38 (27.1%)	27 (42.2%)	0.032
Chemotherapy	112 (54.9%)	87 (62.1%)	25 (39.1%)	0.002
Immunosuppressive therapy	11 (5.4%)	8 (5.7%)	3 (4.7%)	0.763
Antibiotic exposure time, median (range)	22 (3–99)	22 (3–66)	21 (1–99)	0.072
30-day mortality, n (%)	25 (12.3%)	16 (11.4%)	9 (14.1%)	0.595

WBC: white blood cell
ANC: absolute neutrophil count
CRP: C-reactive protein
PCT: procalcitonin
HSCT: hematopoietic stem cell transplant

hematologic disorders (6.9%), with HSCT in 31.9%, chemotherapy in 54.9%, chimeric antigen receptor T-cell therapy (CART-T) in 5.4%, accompanied by a median antibiotic exposure of 22 days (range, 3–99; Table 1). Notably, no significant differences were observed between the two groups in terms of sex, age, previous antibiotic exposure, procalcitonin levels, or 30-day mortality. However, concerning the management of underlying conditions, a higher fraction of patients in the non-neutropenia cohort received chemotherapy.

Most cases manifested as single-site infections, followed closely by instances of infections involving two or more sites, with cases presenting only with fever without a discernible source of infection being the least prevalent (Figure 2A). No significant differences were observed in the distribution of the infection sites between the two groups. In these two groups of patients, the lower respiratory tract was the most common site of infection, with subsequent bloodstream, gastrointestinal, skin tissue, mucosal, and central nervous system infections (Figure 2B). Additionally, skin and soft tissue infections were more prevalent in the neutropenic group than in the non-neutropenic group.

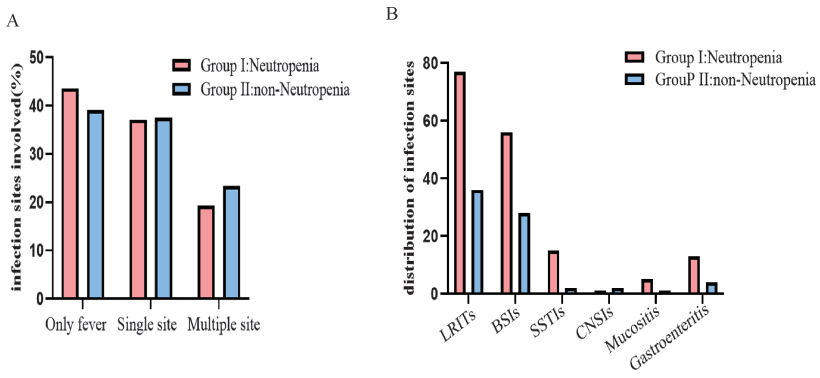

Fig. 2 The number and distribution of infection sites between the two groups in this study
Fig. 2A: The number of infection sites involved between two groups
Fig. 2B: The infection sites distribution involved between two groups
LRTIs: lower respiratory tract infections
BSIs: bloodstream infections
SSTIs: skin and soft-tissue infections
CNSIs: central nervous system infections

Diagnostic performance of bacteria detection between mNGS and blood culture

Our study aims to compare the detection rates between the blood culture group and the mNGS group that those patients accept the detection method of blood culture and mNGS. Compared to blood culture, which demonstrated a positivity rate of only 7.8%, mNGS identified a broader range of microbial species, consisting of 69 bacterial strains, with 27 distinct species. The distribution of strains between the two groups was differed significantly, with 47.8% *Gram-negative bacilli*, and *Gram-positive cocci* 31.9%. Statistical significant differences were observed in the distribution between the two groups ($p< 0.001$; Figure 3A).

In addition to its broader detection capabilities, mNGS revealed a broader spectrum of bacterial species and identified a higher proportion of clinically relevant drug-resistant strains in both patient cohorts, with a median antibiotic exposure duration of approximately 20 days, mNGS detected a greater number of drug-resistant bacteria (Figure 3B). Among these, *Carbapenem-resistant bacteria (CRB)* was the most prevalent resistant strain (9/16), followed by *Methicillin-resistant Staphylococcus aureus (MRSA)* (3/16) and *Multiple resistant bacteria (MDR)* (2/16). The remaining cases included one instance each of *Vancomycin-resistant Staphylococcus aureus (VRSA)*, *Vancomycin-resistant Enterococcus (VRE)*, and *Extended-Spectrum β-Lactamases (ESBLs)*. Notably, significant statistical variance was observed in the positivity rate for drug-resistant bacteria between the two groups (Group I, 26.4% vs Group II, 12.5%, $P < 0.001$).

Capability of mNGS for pathogens detection

In this study, all patients who remained febrile after 72 hours of antibiotic exposure underwent CMT and mNGS within one week. Among the 204 patients analyzed using the mNGS method for pathogen detection, 146 cases tested positive, yielding an overall positivity rate of 68.1%, compared to 30.9% for CMT (Figure 3C).

The positivity rate of mNGS was markedly higher than that of CMT in both groups. However, the positivity rate of CMT in the neutropenic group was significantly higher than that in the non-neutropenic group (Group I, 34.4% vs Group II, 12.5%, $P < 0.001$). In contrast, no statistically significant difference was observed in the mNGS positivity rates between the two groups (Group I, 73.6% vs Group II, 67.2%).

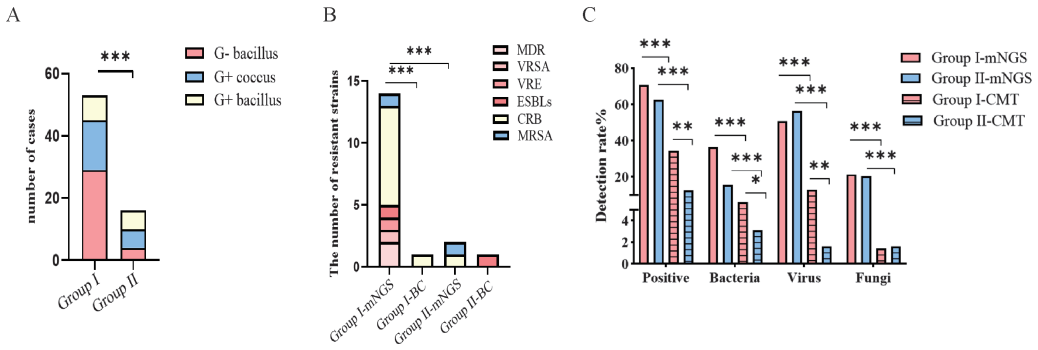

Fig. 3 Difference in pathogenic microorganisms between two groups

Fig. 3A: The distribution of strains between two groups

Fig. 3B: The relevant drug-resistant strains of metagenomic next-generation sequencing (mNGS) and blood culture between two groups

Fig. 3C: The detection rate comparison of mNGS and conventional microbiological tests (CMT)

G– bacillus: Gram-negative bacillus

G+ coccus: Gram-positive coccus

G+ bacillus: Gram-positive bacillus

MDR: multiple drug-resistant bacteria

VRSA: Vancomycin-Resistant Staphylococcus aureus

VRE: Vancomycin-Resistant Enterococcus

ESBLs: Extended-Spectrum β-Lactamases

CRB: Carbapenem-Resistant bacteria

MRSA: Methicillin-Resistant Staphylococcus aureus

mNGS exhibited higher rates of positivity for bacteria, fungi, and viruses than CMT (Figure 4A). Notably, within the neutropenic group, the positive rate for bacteria in the mNGS was significantly higher than those in the non-neutropenic group (Group I, 36.4% vs Group II, 15.6%, $P < 0.001$).

Concordance of mNGS and CMT for potential microbes

The comparative diagnostic efficacies of the mNGS and CMT are presented in Table 2. Among the 204 specimens analyzed, 11 cases (5.4%, 11/204) tested positive in both mNGS and culture, while 109 (53.4%, 109/204) tested negative in both method. There were 79 cases (38.7%) with positive mNGS but negative culture results and 5 cases (2.5%) with positive culture but negative mNGS results, with a sensitivity of 68.8% and a specificity of 58.0% (kappa, 0.086, 95%) when compared to blood culture.

Regarding CMT, 49 specimens (24.1%, 49/204) tested positive for both mNGS and CMT, 51 specimens (25.0%, 51/204) tested negative. Furthermore, 90 specimens (44.1%, 90/204) tested positive for mNGS but negative for CMT, and 14 specimens (6.8%, 14/204) tested positive for CMT but negative for mNGS. In comparison to CMT, mNGS exhibited a sensitivity of 77.8% and a specificity of 36.2% (Kappa, 0.105). Among the specimens that tested positive in both mNGS and CMT, the rate of double positivity was notably higher between two groups (Group I, 30.0% vs Group II, 10.9%, $P < 0.001$), with 21 specimens showing entirely consistent results between mNGS and CMT, 14 cases showing partially consistent results, and 14 cases showing entirely discordant results between mNGS and CMT (Figure 4B).

Table 2 Agreement of plasma metagenomic next-generation sequencing (mNGS) results versus blood culture, blood culture plus plasma virus DNA test, all conventional microbiological testing, and the composite clinical standard

	mNGS positive	mNGS negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Kappa	Agreement	P-value
Blood culture[a] positive	11	5	68.8%	58.0%	12.2%	95.6%	0.086	58.8%	0.039
Blood culture negative	79	109							
Blood culture positive or virus DNA[b] positive	32	3	91.4%	34.3%	22.4%	95.1%	0.116	44.1%	0.02
Blood culture positive or virus DNA negative	111	58							
CMT[c] positive	49	14	77.8%	36.2%	35.3%	21.5%	0.105	49.0%	0.048
CMT negative	90	51							
Composite clinical standard[d] positive	127	35	78.4%	61.9%	88.8%	42.6%	0.345	75.0%	<0.001
Composite clinical standard negative	16	26							

[a] The blood culture was only included within a week of the NGS specimen collection.
[b] Plasma virus DNA tests included CMV, EBV, qPCR.
[c] Patients who received conventional microbiological tests (CMT) included culture, sample DME, BDG test, GM test, antigen detection, and virus NAAT within 7 days of the NGS specimen collection.
[d] mNGS results were classified as definite, probable, possible, unlikely, or false-negative causes of the cases. In the definite, probable, and possible situations, the NGS results were evaluated to be positive in accordance with the composite clinical standard, while in the unlikely and false-negative situations, they tended to be negative.
mNGS: metagenomic next-generation sequencing
PPV: positive predictive value
NPV: negative predictive value
CMT: conventional microbiological tests
DME: direct microscopic examination
CMV: cytomegalovirus
EBV: Epstein-Barr Virus
qPCR: quantitative polymerase chain reaction
BDG: (1-3)-β-D-glucan
GM: galactomannan
NAAT: nucleic acid amplification test

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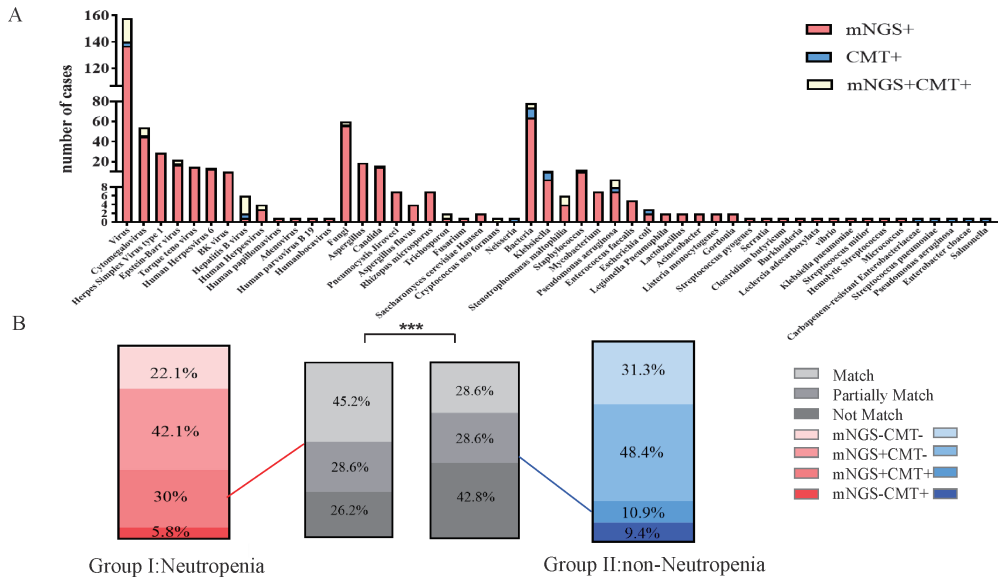

Fig. 4 Comparison of microbial distribution and results between metagenomic next-generation sequencing (mNGS) and conventional microbiological tests (CMT)
Fig. 4A: Distribution of microbes detected by NGS and CMT
Fig. 4B: Comparison of outcomes and consistency in double-positive results between NGS and CMT
***P < 0.001.

Clinical impact of diagnosis and treatment using mNGS for infection

mNGS played a pivotal role in the diagnosis and therapeutic guidance of infections. Out of 204 cases, mNGS positively influenced the diagnosis in 105 cases (51.5%), had no discernible effect in 95 (46.6%), and adversely affected 5 (2.5%). In terms of treatment guidance, mNGS positively influenced 89 patients (43.6%) and had no impact on 115 patients (56.4%), with no instances of negative impact (Figure 5A).

The primary positive impact of mNGS on diagnosis, observed in 87 out of 204 cases (42.6%), was ability to identify pathogens, including at the species level. A significant therapeutic benefit

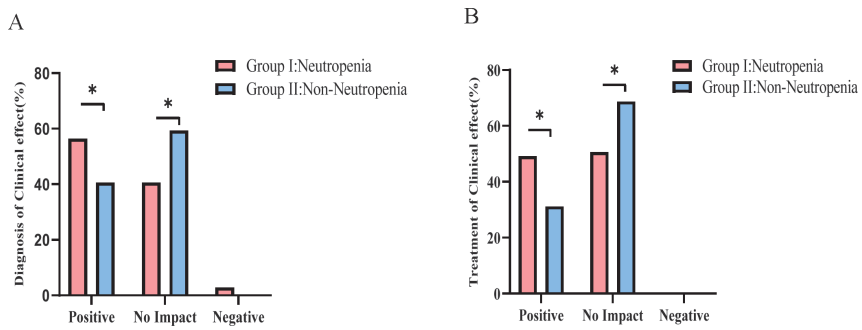

Fig. 5 Comparison of clinical impact on infection diagnosis and treatment of the two groups
Fig. 5A: Clinical impact on the diagnosis of infection in our study
Fig. 5B: Clinical impact on antibiotic treatment of infection in our study
*P < 0.05.

of mNGS was its role in adjusting antibiotic usage based on the results, which were observed in 53 cases (26.0%). The diagnostic and therapeutic impacts of mNGS results were more pronounced in the neutropenia group than in the non-neutropenia group (diagnosis, 56.4% vs 40.6%, P = 0.036; treatment, 49.3% vs 31.3%, P = 0.016; Figure 5B).

Moreover, our investigation highlighted a notable discrepancy. A 30-day mortality rate of 17.3% (24/139) among individuals testing positive for mNGS, in contrast with a lower rate of 4.2% (1/65) among individuals testing negative (Figure 6). Additionally, no statistically significant differences in mortality were observed between the neutropenia and non-neutropenia groups, regardless of mNGS findings.

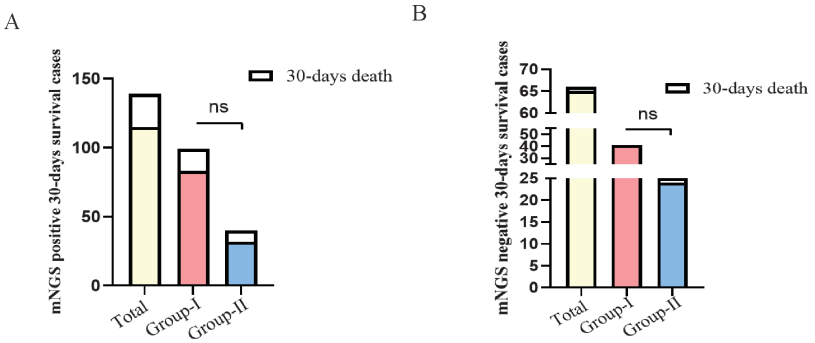

Fig. 6 The 30-day mortality cases of individuals testing for metagenomic next-generation sequencing (mNGS) between two groups

Fig. 6A: mNGS positive 30-days survival cases between two groups

Fig. 6B: mNGS negative 30-days survival cases between two groups

DISCUSSION

Patients with hematologic malignancies undergoing treatments such as chemotherapy, transplantation, and immunotherapy, often experience immunosuppression, rendering them highly susceptible to infections. Persistent fever, despite receiving antibiotic is a common complication in these patients, as infections remain prevalent complication in hematological disorder. Given the high incidence and mortality rates of infections, coupled with the low positivity of traditional pathogen detection, there is an urgent need to establish a diagnostic approach that can rapidly identify pathogenic microorganisms and their resistance profiles. Precise identification of pathogen varieties and implementation of targeted therapy are pivotal actions for effective infection management. This retrospective study evaluates the diagnostic and therapeutic performance of mNGS in patients with hematologic disorders who failed to respond to antibiotic therapy, compared with traditional microbiological detection. Additionally, the study assessed its ability to identify drug-resistant pathogens and evaluate the prognosis of infection in both neutropenic and non-neutropenic groups. Although mNGS cannot distinguish between viable and non-viable bacteria, positive results may reflect residual DNA from pathogens already neutralized by prior antibiotics, We emphasize that mNGS results were interpreted in conjunction with clinical symptoms, laboratory markers (eg, CRP, procalcitonin), and treatment response to mitigate overreliance on DNA-based detection alone.

A significant difference in positivity rates was observed between the mNGS test and CMT, with rates of 68.1% and 30.9%, respectively, which were slightly lower than the 80.21% mNGS

rate reported by Tianjin Medical University General Hospital.[7] Exposure to antibiotics for less than 72 hours exerts a negligible influence on the positive rates of mNGS; however, the rates decrease moderately with prolonged antimicrobial therapy.[8] Extended antibiotic use in febrile patients, lasting over a week, contributed to the relatively low mNGS detection rate in our study, owing to the considerable cost of mNGS testing and the prevalent preference for conventional pathogen testing when infections are suspected.

A prospective study on bloodstream infections found that mNGS has a detection rate six times higher than that of blood culture (71% and 11 %), leading to over 53% of patients adjusting their antimicrobial therapy based on mNGS results and shorter hospital stay.[9] In this study, 27 bacterial species, 10 fungal species, and 12 viral species were identified, with bacteria and fungi exhibiting detection rates 4.4 and 14 times higher than blood culture, and viruses was 5.6 times higher than that of quantitative PCR, respectively. The difference in the frequency of detected bacteria between our study and clinical infectious diseases (CID) study may primarily stem from differences in detection methods, study populations, and pathogen spectrum coverage. Firstly, we used mNGS, which detects hard-to-culture bacteria like intracellular pathogens and anaerobes, while the CID study relied on traditional culturing methods, potentially missing some bacteria. Secondly, our cohort had many immunocompromised patients, prone to opportunistic infections, whereas the CID study likely focused on healthier populations, leading to differences in detected pathogens. Finally, mNGS covered a broader range of bacteria, including rare pathogens, while the CID study focused on common pathogens, potentially missing rare species, contributing to differences in detection frequency. In addition, it was observed that the neutropenic group exhibited higher positive rates for mNGS and CMT compared to the non-neutropenic group, consistent with other relevant retrospective studies.[10,11] This higher detection rate in the neutropenic group may be attributed to neutrophil deficiency and compromised immune function, which can lead to rapid pathogen proliferation, presenting challenges in pathogen eradication and resulting in severe infections. Furthermore, Gram-negative bacteria were predominant in the neutropenia group, while Gram-positive bacteria were more prevalent in the non-neutropenia group, primarily due to bloodstream infections being the most common type in our study. A previous study reported that 57.3% of bloodstream infection were caused by Gram-negative bacteria, with the remaining 42.7% caused by Gram-positive bacteria. *E.coli* and *Klebsiella pneumoniae* were the most common strains, accounting 22.7% and 13.3% of total cases, respectively.[12] Another study reported a higher proportion of *Gram-negative rods* compared to *Gram-positive cocci* (33% vs 7%) in non-HSCT patients, which they attributed to intense myelosuppression, immunosuppressive therapy, and immune reconstitution in patients who underwent HSCT.[13] In our study, the non-neutropenia group received more HSCT treatments than the chemotherapy and immunotherapy groups.

In recent years, misuse of antibiotic has fueled the rise of drug-resistant bacteria, posing a potential threat to global public health.[14] Global predictive models estimate approximately 4.95 million deaths from bacterial resistance in 2019, which is projected to reach 10 million by 2050.[15] mNGS can directly extract and clone DNA from samples to analyze bacterial genome, thus detecting antibiotic resistance genes (ARGs), which was discovered that genes encoding resistance to tetracyclines, β-lactams, and macrolide antibiotics are commonly occur in different populations.[16] In contrast to the mere detection of two resistant strains by blood culture, mNGS identified 19 strains, with CRB being the most prevalent, consistent with another Chinese study on CRE-related bloodstream infections.[17,18] Moreover, neutropenia group exhibited a notably higher count of drug-resistant strains compared to the non-neutropenic group, primarily ascribed to prolonged or broader application of antibiotics. The observed dominance of *CRB* (accounting for 56.3% of all resistant strains), followed by *MRSA* (accounting for 18.8%) and *multidrug-*

resistant strains (*MDR*, accounting for 12.5%) in our cohort may be attributed to the following reasons: Firstly, the prolonged median antibiotic exposure of 22 days (range 3–99 days), particularly the frequent use of broad-spectrum β-lactams and carbapenems in empiric therapy for neutropenic fever, creates strong selective pressure for CRB emergence. Secondly, chemotherapy/HSCT-induced mucosal damage facilitates translocation of gut-colonizing drug-resistant bacteria (eg, CRB, VRE), which are often undetectable by blood culture until invasive infection occurs. mNGS's ability to detect low-biomass pathogens may explain its higher CRB/MRSA yield in neutropenic patients compared to culture. However, ARG analysis was not conducted in this study, which remains further investigation. Currently, typical diagnostic methods for invasive fungal lung infections include fungal culture, 1-3-β-D-glucan testing, and galactomannan testing. However, these methods are associated with low positivity rates, time-consuming procedures, frequent detection of colonized fungi, limited sensitivity, and challenges in identifying precise pathogen species. Relevant researches[19,20] indicate that mNGS provides a more accurate and faster means of identifying pathogens in patients with invasive fungal infections (IFIs) compared to CMTs, confirming its value as a highly sensitive method for invasive pulmonary aspergillosis (IPA) and funtioning as a valuable adjunct to CMTs. Our findings revealed a positive rate of 21.1% for fungi in mNGS, identifying categories not detectable by conventional microbiology, such as *Aspergillus*, *Mucorales*, *Pneumocystis jirovecii*, *Fusarium*, *Trichosporon* and *Saccharomyces cerevisiae*.

In contrast to blood cultures, mNGS demonstrated superior specificity and sensitivity rates of 68.8% and 58%, respectively, for bloodstream infections. When compared to CMT, these figures were 77.8% and 36.2%, highlighting mNGS enhanced capability in identifying bloodstream infections. Additionally, in cases where both CMT and mNGS yielded positive results, approximately 71.4% of the pathogens showed complete or partial concordance, suggesting a degree of consistency between the two distinct detection methods.

To better evaluate the clinical impact of mNGS, we referenced relevant authoritative clinical studies,[21,22] which reported a positive diagnostic impact in 51.5% of cases and a positive therapeutic impact in 43.6% of cases. Among these, 53 cases involved adjustment to antibiotic, with the neutropenia group showing significantly higher diagnostic and curative performance compared to the non-neutropenic group; however, the role of mNGS in hematological malignancies demands attention, especially in neutropenia, despite broad-spectrum antibiotic therapy. Another study reported diagnostic and therapeutic effects of 47.8% and 38.6%, respectively, using plasma cell-free NGS, which was slightly below our study for the following reasons. Plasma cell-free NGS primarily detects circulating free DNA (cfDNA) in plasma samples, which originates from processes such as cell apoptosis, potentially limiting its ability to detect low-abundance pathogens. Additionally, pathogens may have very low concentrations after antibiotic therapy, restricting the role of guidance in the diagnosis and treatment of CMT.

A domestic study revealed a significant difference in the 28-day mortality rate between mNGS-positive and mNGS-negative patients with infectious diseases, with rates of 9.0% and 0%, respectively; however, no significant difference was found in the 90-day mortality rate. In contrast, our study showed 30-day mortality rates of 17.3% and 1.5% in the mNGS-positive and mNGS-negative groups, respectively. No differences were observed between the neutropenia and non-neutropenia subgroups. The higher mortality rate in our study may be attributed to the inclusion of patients with malignant hematological diseases, most of whom had severe immunodeficiency, resulting in more severe and complex infections compared to other disease conditions. However, mNGS positivity is associated with mortality, this association may be driven by higher baseline pathogen burden or degree of immunosuppression. We emphasize that mNGS may serve as a prognostic marker rather than the determinant of death itself.

However, our study has certain limitations. First, the sample size was relatively small, prompting the need for further studies with large sample size to validate our findings. Moreover, despite receiving antibiotic treatment for more than 72 hours, all enrolled patients continued to exhibit fever, potentially affecting the outcomes of mNGS to a certain degree. Second, the absence of standardized protocols for interpreting the mNGS results may introduce a degree of subjectivity in the analysis conducted in this study. Additionally, all mNGS samples were derived exclusively from blood specimens owing to the hematologic malignancies present in our patient cohort, characterized by a predominance of immunocompromised individuals with diminished platelet counts, precluding invasive procedures for conducting mNGS testing at suspected infection sites. Finally, mNGS cannot distinguish between viable and non-viable bacteria, we propose integrating mNGS with viability testing (eg, propidium monoazide-treated PCR or culture-based validation) in future studies to enhance specificity for active infections. This constraint imposes limitations on diagnostic and therapeutic efficacy.

CONCLUSION

In conclusion, our study suggests that even in cases where antibiotic therapy proves ineffective, mNGS maintains a notably high positivity rate and plays a crucial role in identifying pathogen species, which underscores its superior diagnostic and remedial utility in clinical contexts, particularly in neutropenia cohorts. The positive rate of mNGS may also correlate closely with the 30-day mortality rate, although further clinical data are required to substantiate this association.

DECLARATIONS

Authors' contributions

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

(1) Jiaying Cheng and Xuejie Jiang, conceiving and designing the study.

(2) Jiaying Cheng, Jinle Ni, Yu Zhao, Ling Jiang, Yun Huang, Yujiao Zhang, Ping Yan, Zhiquan Long and Haipeng Fu, collecting the data.

(3) Jiaying Cheng, Jinle Ni, Yu Zhao, Ling Jiang, Yun Huang, Yujiao Zhang, Ping Yan, Zhiquan Long and Haipeng Fu, analyzing and interpreting the data.

(4) Jiaying Cheng, writing the manuscript.

(5) Xuejie Jiang, providing critical revisions that are important for the intellectual content.

(6) All authors approved the final version of the manuscript.

Conflicts of interest

All authors declare that they have no conflicts of interest.

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