Journal of Microbiology, Immunology and Infection 57 (2024) 11—19

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Original Article

Diagnostic performance and clinical impacts of metagenomic sequencing after allogeneic hematopoietic stem cell transplantation

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Received 2 July 2023; received in revised form 10 October 2023; accepted 17 November 2023
Available online 28 November 2023

KEYWORDS
Infection;
mNGS;
Metagenomic sequencing;
Diagnosis

Abstract *Background:* Metagenomic Next-Generation Sequencing (mNGS) is a rapid, non-culture-based, high-throughput technique for pathogen diagnosis. Despite its numerous advantages, only a few studies have investigated its use in patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT).
Methods: We conducted a retrospective analysis of 404 mNGS tests performed on 264 patients after allo-HSCT. The tests were divided into three groups (Phase A, B, C) based on the time spent hospitalized post-transplantation, and we evaluated the analytical performance of mNGS in comparison with conventional microbiological tests (CMT), while also analyzing its clinical utility for clinical impacts.
Results: Metagenomic sequencing demonstrated a significantly higher rate of positive microbiological findings as compared to CMT (334/404 (82.7 %) vs. 159/404 (39.4 %), respectively, $P < 0.001$). The detection rates by both mNGS and CMT varied across the three-phase (mNGS: A-60/89 (67.4 %), B-147/158 (93.0 %), C-125/157 (79.6 %), respectively, $P < 0.001$; CMT: A-21/89 (23.6 %), B-79/158 (50.0 %), C-59/157 (37.6 %), respectively, $P < 0.001$). The infection sites and types of pathogens were also different across the three phases. Compared to non-GVHD cases, mNGS detected more *Aspergillus* spp. and Mucorales in GVHD patients (*Aspergillus*:

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https://doi.org/10.1016/j.jmii.2023.11.002

12/102 (11.8 %) vs. 8/158 (5.1 %), respectively, $P = 0.048$; Mucorales: 6/102 (5.9 %) vs. 2/158 (1.3 %), respectively, $P = 0.035$). Forty-five (181/404) percent of mNGS tests yielded a positive impact on the clinical diagnosis, while 24.3 % (98/404) of tests benefited the patients in antimicrobial treatment.

Conclusion: mNGS is an indispensable diagnostic tool in identifying pathogens and optimizing antibiotic therapy for hematological patients receiving allo-HSCT.

Introduction

Infections are the most significant and common complications that can occur following allogeneic hematopoietic stem cell transplantation (allo-HSCT). Unfortunately, these infections are associated with a high mortality rate that is related to the treatment.[1] Prior to immune reconstitution, the infection risk and its associated types of pathogens vary according to different time periods after allo-HSCT.[2,3] Graft versus host disease (GVHD) or immunosuppressive drugs render the patients more susceptible to opportunistic infections of mixed pathogens.[1,4,5] Accurate and timely diagnosis of pathogens are critical for the clinical management of infections, especially for patients undergoing allo-HSCT. However, in immunocompromised patients, the wide range of potential pathogens capable of causing infections, along with inadequate sensitivity of conventional microbiological tests (CMT) has made diagnosis extremely challenging in a real-world setting.[6,7] A previous study that investigated the cause of death after HSCT have found that microbial infection of unknown etiology is the main culprit.[8] While CMT tests such as culture, direct microscopic examination (DME) and histopathology lack sensitivity in patients receiving empiric antibiotics, the nucleic acid amplification tests (NAAT) are highly sensitive but limited to a handful of common pathogens.[9,10] Therefore, a rapid, accurate and comprehensive method of pathogen detection has become critical.

Metagenomic next-generation sequencing (mNGS) has recently been gaining traction as a culture-independent, high-throughput nucleic acid sequencing-based agnostic testing for pathogen identification from clinical specimens.[11,12] Compared to conventional methods, mNGS has several advantages, including the ability to detect a wide spectrum of pathogens within a clinically actionable time frame.[13,14] With the decrease of sequencing time and cost, mNGS has shown promise in a variety of infectious diseases, including bloodstream infections (BSIs), respiratory infections, central nervous system infections (CNSIs), sepsis, invasive fungal infections (IFIs), urinary tract infections (UTIs) and endocarditis.[15–23] Nevertheless, few studies have explored the utility of mNGS in allo-HSCT patients with hematological disorders.[24,25] Therefore, the aim of our study was to evaluate the diagnostic performance and clinical impact of mNGS in a cohort of hematological patients who underwent allo-HSCT.

Materials

Study design

We reviewed patients at the Institute of Hematology and Blood Diseases Hospital who had mNGS for suspected infections after allo-HSCT from January 2021 to December 2022. For this retrospective study, the inclusion criterion was patients who had mNGS testing ordered post-transplantation, with the decision made by clinicians. Typically, transplant patients underwent mNGS testing due to the following reasons: 1) The patient suffered from a life-threatening infection and was in critical conditions, such as sepsis; 2) The patient's initial anti-infection treatment was ineffective; 3) Conventional tests were negative and no definitive evidence was available for the etiological diagnosis. We excluded: 1) patients with non-infectious etiologies (as confirmed by other tests that ruled out infections); 2) patients whose samples failed quality control; and 3) patients with incomplete medical records. Based on the criteria (Fig. 1), 404 mNGS tests (involving 264 patients and 343 infectious episodes) were included in the analysis. This study had been approved by the Ethics committee and Institutional Review Board (IRB number: QTJC2022043-EC-1). Informed consent was waived due to the retrospective design of our study.

The mNGS tests were categorized into three groups based on the duration of hospitalization post transplantation.[1,3] A's mNGS was conducted during the pre-engraftment phase (before reaching an absolute neutrophil count $> 0.5 \times 10^9$ cells/L for 3 days). B's mNGS was performed during the early post-engraftment phase (within first 100 days post-transplant, excluding pre-engraftment period). C's mNGS was conducted after 100 days post-transplant.

Diagnosis of infection was according to the CDC/NHSN surveillance definitions.[26] The definition of viremia (for CMV and EBV) is positive result in real-time qPCR testing of plasma (with the positive thresholds for CMV and EBV being 1000 copies/ml and 500 copies/ml, respectively). These EBV and CMV real-time qPCR tests were routinely conducted to monitor the viral levels in patients post transplantation. Neutropenia refers to a ANC $< 0.5 \times 10^9$ cells/L.[27] All patients had CMT ordered by their clinicians, including bacterial and fungal culture, viral PCR, DME, *Aspergillus* GM test (Bio-Rad Laboratories, Hercules, CA, USA), (1,3)-β-D-glucan (BDG) test (Dynamiker Biotechnology Co., Ltd, Tianjin, China), GeneXpert MTB/RIF

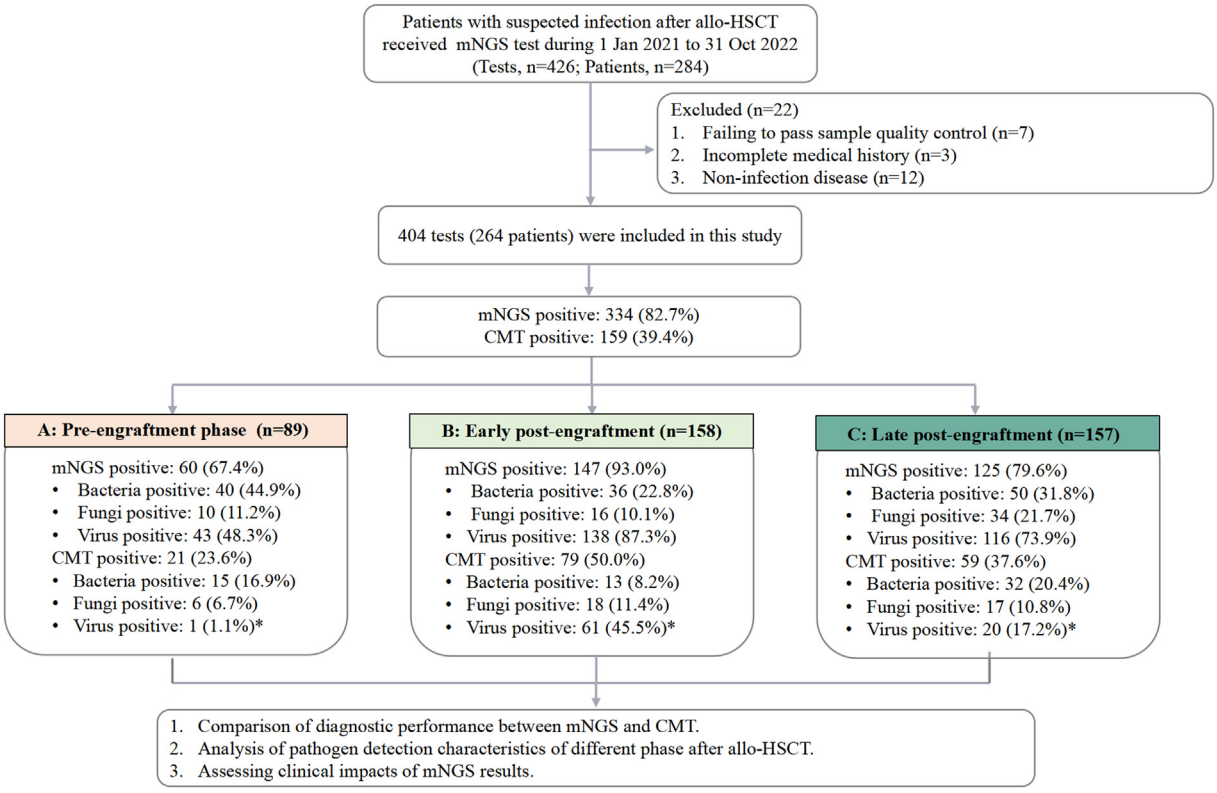

Figure 1. A schematic of the study profile. Abbreviations: allo-HSCT, allogeneic hematopoietic stem cell transplantation; mNGS, metagenomic next-generation sequencing; CMT, conventional microbiological tests; *, Only cases with viral PCR test were included.

(Cepheid, Sunnyvale, CA) and Cryptococcal antigen tests (Dynamiker Biotechnology Co., Ltd. Tianjin, China). Clinical impacts of mNGS were determined by a panel of two hematologists, one clinical microbiologist and one radiologist based on a detailed grading criteria established in an earlier study (Table S1).[28]

Metagenomic Next-Generation Sequencing

For peripheral blood, 600 μl plasma was obtained and nucleic acid (cell-free DNA) was extracted using TIANamp Micro DNA Kit (Tiangen Biotech). For other specimens, genomic DNA was extracted by TIANamp Micro DNA Kit (Tiangen Biotech) after enzymatic treatment and beads-beating. Sequencing libraries were prepared by DNA fragmentation, end-repair, adaptor ligation and PCR amplification. Qubit was used to evaluate the quality of libraries. Sequencing was performed on MGISEQ-200/2000. Short (<35 base pairs), low-quality reads and human sequences were removed and the remaining reads were aligned to an in-house database for identifying microbial species.

Criteria for a positive mNGS test

To determine the positive mNGS result microbes, we employed a methodology anchored in the evaluation of Stringently Mapped Read Numbers (SMRN) coupled with a Negative Control (NTC). NTC, composed of human stem cells without microorganisms, is tested alongside clinical samples to monitor potential contamination from reagents and the environment. A positive mNGS result is ascertained in this study when the following criteria are met.

(1) The microbe was considered positive when its relative abundance was the highest within its genus, and its SMRN must surpass that in the NTC.
(2) For Bacteria (excluding *Mycobacterium tuberculosis*), Fungi (excluding *Pneumocystis jiroveci*), and Viruses: A microbe is considered positive if it has an SMRN $\geq$ 3 at the species level and its relative abundance at the genus level is >30 %.
(3) *M. tuberculosis* and *P. jiroveci* (PJ) are considered positive with the alignment of at least one SMRN.
(4) For Parasites: SMRN$\geq$100, and the species must not be present in the NTC.

Since the read depth was not same among different samples, normalized mapped read number to 20M was used to compare with plasma virus detection between three groups.

Statistical analysis

SPSS Version 25.0 software (SPSS Inc., Chicago, IL, USA) were performed for statistical analysis. Mann—Whitney U-test, Chi-square, Fisher exact test, correlation analysis were used for discrete variables where appropriate. $P < 0.05$ was considered statistically significant.

Results

Patient characteristics and clinical samples

The average age of 264 patients (154 males and 110 females) was 39.3 years old. We divided the 343 infectious episodes into three groups based on different stages, and the basic characteristics and laboratory test results were shown in Table 1. Significant differences were found in the transplantation methods, occurrence of GVHD, prior exposure to antibiotics, and laboratory data such as white blood cell (WBC), absolute neutrophil count (ANC), C-reactive protein (CRP), procalcitonin (PCT) level. The details of the collected specimens were shown in Fig. S1. Ninety patients (involving 169 infection episodes) underwent more than one mNGS test.

Infection sites and pathogens detected after allo-HSCT

For the 343 infectious episodes, the sites of infection among the three groups were shown in Fig. 2. In the Pre-engraftment phase (Group A), gastroenteritis was most common at 44.6 %, followed by both perianal and oral mucositis at 31.3 %. In the early post-engraftment (Group B), the leading infections were viremia (37.7 %), pneumonia (36.7 %), and gastroenteritis (21.0 %). In the late phase (Group C), pneumonia was predominant at 59.8 %, with gastroenteritis (12.9 %) and viremia (12.7 %) trailing. We observed differences in microbial detection rates of both CMT and mNGS among the three groups. Both mNGS testing (A-67.4 %, B-93.0 %, C-79.6 %, $P < 0.001$) and CMT (A-23.6 %, B-50.0 %, C-37.6 %, $P < 0.001$) showed the highest detection

Table 1 Case characteristics and baseline of the three study groups.

Samples characteristics	Total (n = 343)	A: Pre-engraftment phase (n = 83)	B: Early post-engraftment (n = 128)	C: Late post-engraftment (n = 132)	*P*
Mean age ± SD	38.77 ± 12.33	40.00 ± 11.92	37.81 ± 12.68	38.31 ± 12.49	0.416
Male, n (%)	197 (57.4)	54 (65.1)	70 (54.7)	73 (55.3)	0.271
Underlying diseases, n (%)					0.201
Acute myeloid leukemia	127 (37.0)	28 (33.7)	40 (31.3)	59 (44.7)	
Myelodysplastic syndromes	76 (22.2)	24 (28.9)	27 (21.1)	25 (18.9)	
Acute lymphoblastic leukemia	87 (25.4)	18 (21.7)	38 (29.7)	31 (23.5)	
Aplastic anemia	15 (4.4)	4 (4.8)	9 (7.0)	2 (1.5)	
Chronic Myeloid Leukemia	17 (5.0)	3 (3.6)	6 (4.7)	8 (6.1)	
Lymphoma	11 (3.2)	4 (4.8)	3 (2.3)	4 (3.0)	
Other diseases	10 (2.9)	2 (2.4)	5 (3.9)	3 (2.3)	
Transplantation method, n (%)					**0.015**
Haplo	253 (73.8)	68 (81.9)	98 (76.6)	87 (65.9)	
MSD	83 (24.2)	15 (18.1)	25 (19.5)	43 (32.6)	
URD	7 (2.0)	0 (0.0)	5 (3.9)	2 (1.5)	
Laboratory examination					
WBC, 109/L, median (IQR)	1.93 (0.24—3.64)	0.03 (0.01—0.11)	2.87 (1.96—4.73)	2.16 (0.69—4.03)	**< 0.001**
ANC, 109/L, median (IQR)	1.08 (0.04—2.35)	0.00 (0.00—0.03)	1.84 (1.19—3.38)	1.19 (0.11—2.49)	**< 0.001**
CRP, mg/L, median (IQR)	42.94 (14.48—90.17)	58.19 (31.44—110.62)	29.36 (7.80—70.00)	50.02 (16.91—91.21)	**0.001**
PCT, ng/mL, median (IQR)	0.15 (0.09—0.37)	0.20 (0.09—0.59)	0.13 (0.07—0.31)	0.17 (0.07—0.35)	**0.019**
aGVHD	78 (22.7)	0 (0.0)	41 (32.0)	37 (28.0)	**< 0.001**
cGVHD	24 (7.0)	0 (0.0)	0 (0.0)	24 (18.2)	—
Previous antibiotic exposure	327 (95.3)	83 (100.00)	124 (96.9)	120 (90.9)	**0.005**
Immunosuppressive drugs	314 (91.5)	83 (100.00)	128 (100.00)	103 (78.0)	—

Abbreviations: Haplo, Haploidentical; MSD, Matched-sibling Donor; URD, Unrelated Donor; WBC, White cell count; ANC, absolute neutrophil count; CRP, C-reactive protein; PCT, procalcitonin; aGVHD, acute Graft-versus-host disease; cGVHD, chronic Graft-versus-host disease.

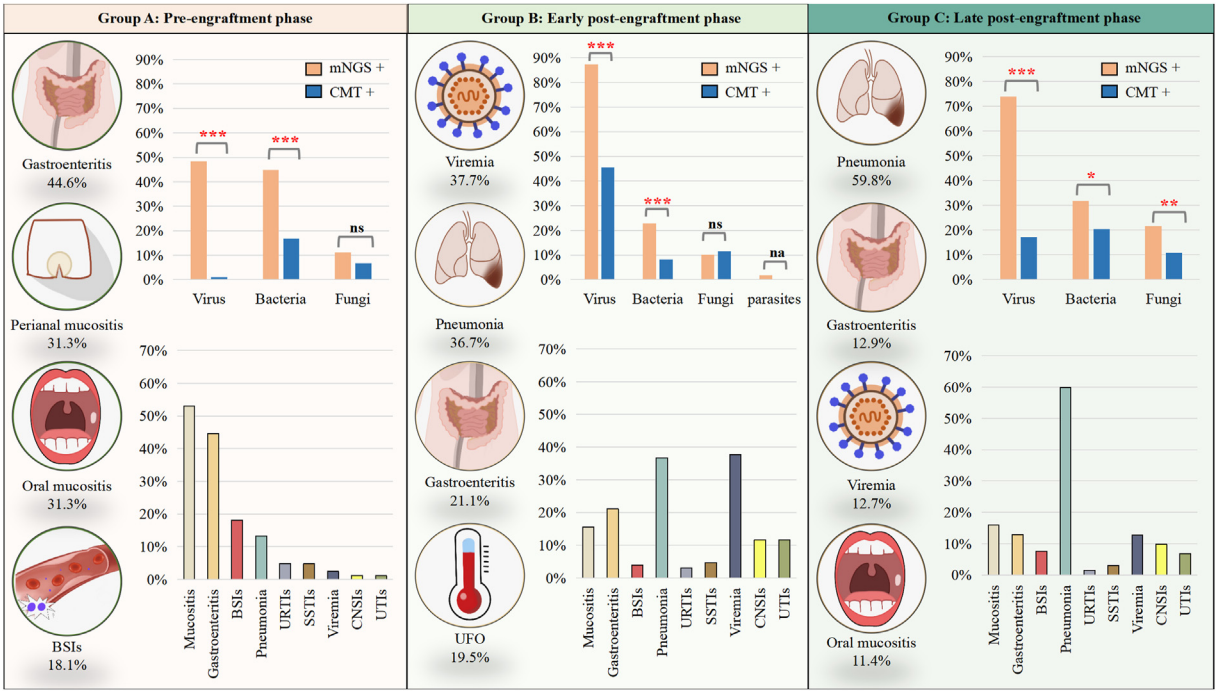

Figure 2. Distribution of the infection sites involved and the comparison of the detection rates among three phases after allo-HSCT. Abbreviations: mNGS, metagenomic next-generation sequencing; CMT, conventional microbiological tests; BSIs, blood stream infections; SSTIs, skin and soft-tissue infections; UTIs, urinary tract infections; CNSIs, central nervous system infections; URTI, upper respiratory tract infections; *$P < 0.05$; **$P < 0.01$; ***, $P < 0.001$; ns, no statistically significant; na, not appropriate.

rate in Group B, followed by Groups C and A (Fig. S2A-D). This trend was also observed in the mNGS results for plasma samples (Fig. S3). Our results indicated that *Klebsiella*, *Escherichia*, and *Pseudomonas* spp. were the most-frequently detected bacterial species after allo-HSCT and were also the most commonly detected in plasma samples. The detection rate of these bacteria in Phase A was higher than in the other two phases (Fig. S2E). Among fungal pathogens, only PJ's detection rate varied notably, peaking in Phase C. For respiratory samples, *Acinetobacter* and *Pseudomonas aeruginosa* were the most commonly detected bacteria, while *Aspergillus* spp. and PJ were the most frequently identified fungi.

Since detecting viruses in plasma is common but not always a sign of infection, we conducted an analysis of the detection rates of different viruses. Among viruses commonly found in plasma, the top five were CMV (56.2 %), Torque teno virus (TTV) (43.8 %), EBV (21.0 %), BK virus (BKV) (15.2 %), and Herpes simplex virus type 1 (HSV-1) (9.5 %). The frequency and abundance of viral reads also varied at different stages after allo-HSCT (Fig. S4). Since the pathogenicity of TTV was still under debate, it was not included in the analysis of pathogens.

Pathogens identified in patients with and without GVHD

We compared the detection rates of bacteria and fungi in infectious episodes with and without GVHD (Fig. S5A-D). Bacterial detection was higher in GVHD cases regardless of the diagnostic method (mNGS: 37.3 % vs. 22.2 %, $P = 0.008$; CMT: 20.6 % vs. 9.5 %, $P = 0.011$), especially in severe Grade III-IV acute GVHD. This trend was similar with mNGS findings both in plasma and respiratory samples (Fig. S6A-B). Although fungal differences weren't statistically significant overall, GVHD cases had higher detections of *Aspergillus* (11.8 % vs. 5.1 %, $P = 0.048$) and Mucorales (5.9 % vs. 1.3 %, $P = 0.035$) (Fig. S5E-F). In plasma, *Aspergillus* was more detected in GVHD patients (8.1 % vs. 2.1 %, $P = 0.032$). Notably, only patients with GVHD mNGS tested positive for *Aspergillus* and Mucorales in respiratory samples (Fig. S6 C-D).

The diagnostic performance of mNGS and CMT

Among the 404 tests, mNGS had a significantly higher positive rate (82.7 %) than CMT (39.4 %, $P < 0.001$) (Fig. S7). Furthermore, mNGS detected more viruses, bacteria, and fungi than CMT (Virus: 73.5 % vs. 24.3 %, $P < 0.001$; Bacteria: 31.2 % vs. 14.9 %, $P < 0.001$; Fungi: 14.9 % vs. 10.2 %, $P = 0.043$). *Toxoplasma gondii* was only identified via mNGS, exclusively in plasma samples, involving two patients. mNGS detected bacteria in 21.5 % (74/344) of tests and fungi in 10.5 % (38/363) where CMT was negative. Conversely, CMT was positive in 5.5 % (19/344) of tests where mNGS found no fungi. The main fungi identified by mNGS were *Aspergillus* spp., PJ, and Mucorales. Of the 404 mNGS tests, 377 had cultures within 48 h, with a 10.1 % positive rate. Using culture as a reference, mNGS had a 97.4 % sensitivity and 64.4 % specificity, with a 67.4 % agreement rate. Compared to CMT, mNGS's sensitivity was 86.7 %, specificity 26.8 %, and a 50.2 % agreement rate (Table 2). Patients detected with bacteria/fungi/parasites by mNGS had worse prognosis (14-day improvement and 30-

Table 2 The diagnostic performance of mNGS results versus of culture, culture and PCR, and conventional microbiology tests.

	mNGS Positive	mNGS Negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Kappa, agreement
Culture* positive	37	1	97.4	64.4	23.3	99.5	0.254, 67.4 %
Culture negative	122	217					
Culture or PCR** positive	100	2	98.0	27.7	31.8	97.6	0.153, 45.7 %
Culture and PCR negative	214	82					
CMT positive	137	21	86.7	26.8	43.2	75.9	0.115, 50.2 %
CMT negative	180	66					

Notes: *Culture refers to bacterial and fungal culture; ** PCR testing includes CMV, EBV PCR tests, and GeneXpert testing. Abbreviations: mNGS, metagenomic next-generation sequencing; PPV, positive predictive value; NPV, negative predictive value; PCR, polymerase chain reaction; CMT, conventional microbiological tests.

day survival rates) than those with only viral detection or negative results (Fig. S8).

Clinical impacts of mNGS on etiological diagnosis and antibiotic adjustment

A total of 44.8 % (181/404) of mNGS tests had a positive impact on etiological diagnosis, and 24.3 % (98/404) led to proper antibiotic adjustment (Fig. 3A—C) (Table S1). In the cases with positive CMT results, 53.5 % (85/159) of mNGS tests had a positive impact due to detecting more pathogens. In the cases with negative CMT results, 39.2 % (96/245) of mNGS tests provided valuable etiological insights. However, 26.0 % (87/335) of mNGS positive results weren't deemed infectious after clinical review. In terms of antibiotic adjustments, In terms of antibiotic adjustments, 54.0 % (181/335) of the positive mNGS tests had a positive impact.

The positive impact of mNGS was more evident in non-plasma samples, such as BALF and cerebrospinal fluid (Fig. 3 D). For pathogen diagnosis, the positive impact was 62.7 % in non-plasma samples versus 41.2 % in plasma samples ($P = 0.001$). For antibiotic adjustment, the positive impact was 40.3 % in non-plasma samples compared to 21.1 % in plasma samples ($P = 0.002$). Furthermore, mNGS showed greater benefits in etiological diagnosis for patients with multi-site infections compared to those with single-site infections or fever of unknown origin (61.6 % vs. 42.5 % and 19.2 %, respectively, $P = 0.001$).

Discussion

We explored the differences in clinical utility of mNGS and CMT in terms of etiologic diagnosis and antibiotic management in hematological patients after allo-HSCT. Our results showed that mNGS outperformed CMT in detecting a wider range of pathogens and had a higher detection rate, providing clues for diagnosis and treatment of infectious diseases. Two previous studies have reported the application of mNGS in allo-HSCT patients, but the sample size was considerably larger in our study.[6,29] In addition, we analyzed the detection rate and types of pathogens during different stages after allo-HSCT, which can help clinicians choose appropriate diagnostic tests and know more about the characteristics of infections in this cohort.

Allo-HSCT patients have different risks of opportunistic infection and types of pathogen among three phase after allo-HSCT.[2] During the pre-engraftment phase, bacterial infections are common until neutrophil recovery, which is reminiscent of the cohort of febrile neutropenic patients after chemotherapy. Conditioning regimens can cause damage to the intestinal, perianal, and oral mucosa, leading to the entry of colonizing microorganisms into the bloodstream. In our study, the detection rate of bacteria (47.0 %) was the highest based on mNGS method. In the early post-engraftment phase, virus replication was significantly increased, and virus detection rates were the highest, as reflected in both CMT and mNGS results. It is notable that in many cases, viral detection is only a phenomenon and not the cause of infection. TTVs were commonly found in our study. Although rarely pathogenic, this virus was reported to be correlated with immune status in HSCT patients.[30,31] In the late post-engraftment phase, we noted the most common type of infection was pneumonia (close to 60 % of cases). In this phase, the detection rate of fungi rose to over 20 % by mNGS and the most frequently detected fungal pathogen was *Aspergillus* spp., PJ, and Mucorales. For detecting PJ and Mucorales, mNGS played a crucial role due to the low sensitivity of cultures, gomori-methenamine silver staining and DME.[31,32]

GVHD and its treatment can aggravate and increase the risk of infections.[33] GVHD adds to the difficulty of diagnosing infections since its symptoms can mimic that of an infection, such as fever, diarrhea, and mucositis. Our results indicated that mNGS had value in GVHD patients, since the detection rate of bacteria was significantly increased, particularly in those with grade III-IV acute GVHD. When we considered *Aspergillus* spp. and Mucorales (the most common types of mold infections after allo-HSCT), the detection rates in GVHD patients was also significantly higher. This suggested that we need to be more vigilant about the possibility of invasive fungal infections in GVHD patients who do not respond to anti-bacterial treatment.

When comparing mNGS and CTM for diagnosing infections in patients undergoing allo-HSCT, mNGS showed a higher diagnostic rate, which was consistent with previous

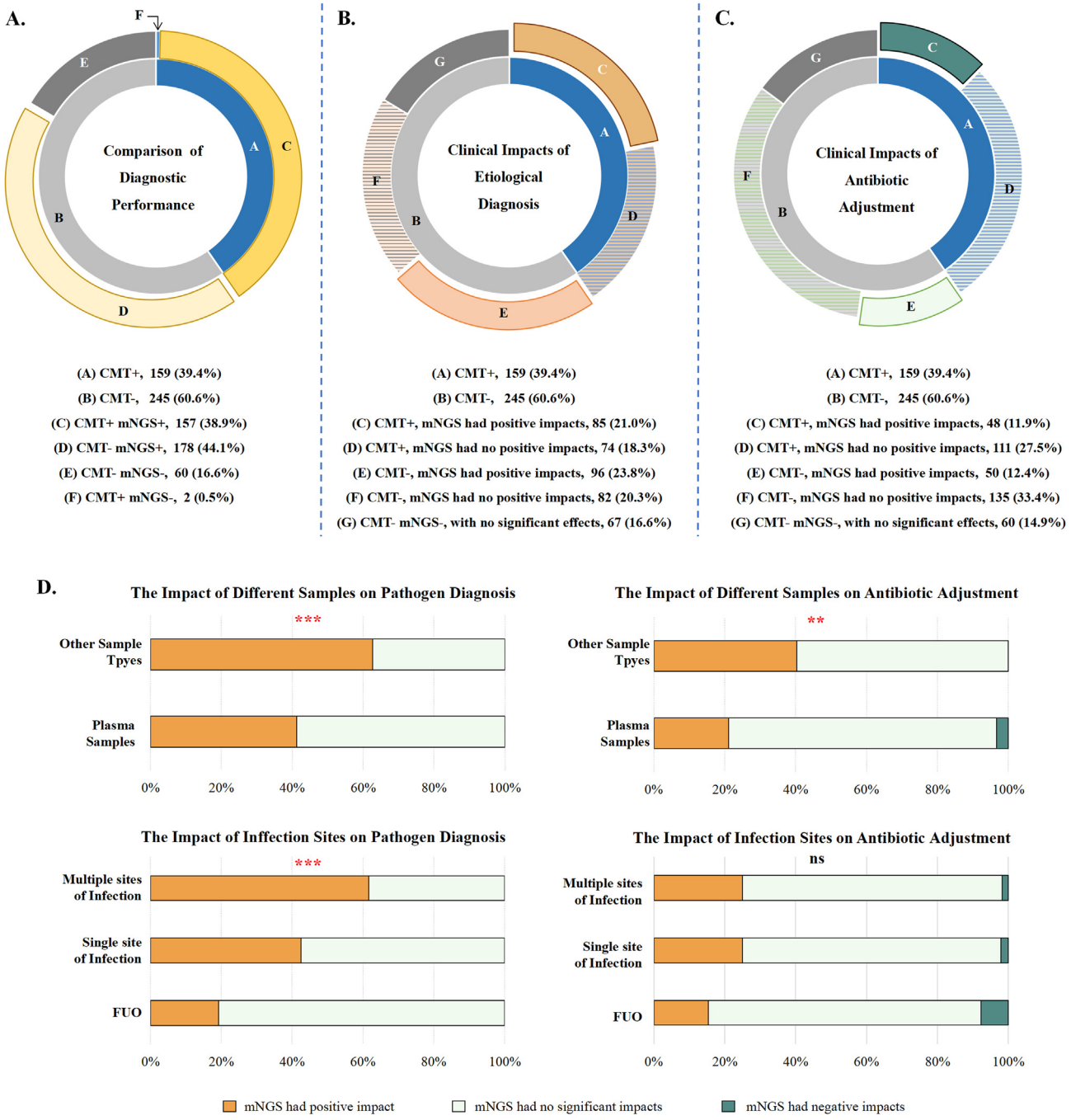

Figure 3. Clinical impacts on infection diagnosis and antibiotic adjustment. (A), Comparison of diagnosis performance between metagenomic next generation-sequencing (mNGS) and conventional microbiological tests (CMT). **(B)**, Clinical impacts of etiological diagnosis by mNGS between different CMT results. **(C)**, Clinical impacts of antibiotic adjustment by mNGS between different CMT results. **(D)**, Clinical impacts comparison of different sample types and timing of mNGS sample collection.

studies on HSCT patients.[6,29] However, the value of CMT should not be underestimated, and mNGS should be used as an adjunct to CMT.[12] Without GM test, we would miss 24.1 % of IFD diagnoses. Additionally, culture and antibiotic susceptibility tests can provide important information on bacterial resistance, which can guide treatment decisions. While mNGS can detect antibiotic resistance genes and provide drug-resistance information, the use of these databases in clinical diagnosis is still in development and has certain limitations.[34,35]

We conducted an evaluation of the clinical effectiveness of mNGS in terms of pathogen diagnosis and antibiotic adjustment, using a previously established Grading Criteria. Our findings indicated that mNGS has a positive impact on pathogen diagnosis in nearly 45.0 % of cases, and that antibiotic adjustments were made in 24.3 % of cases. It is

worth noting that mNGS was not only useful in cases with negative CMT, it can detect co-infecting pathogens or identify pathogens earlier than CMT.

Although mNGS has a higher positive rate than CMT, not all microorganisms detected by mNGS are necessarily pathogenic.[36] Therefore, mNGS findings need to be evaluated carefully. In 20.3 % of cases, the microorganisms detected by mNGS were not considered pathogenic, especially viruses. No standards existed for interpretation of mNGS results and factors such as the quality of sample and experimentation, the abundance of microorganisms, genomic coverage, and contamination monitoring must be taken into consideration when reporting and interpreting mNGS results.[37]

The timing and sample selection are crucial for mNGS testing. Primarily, obtaining specimens directly from the infection site is advocated over plasma samples. For instance, in pulmonary infections, the detection rate in BALF was markedly superior to that in plasma. Secondly, in instances where there is no identifiable infection site, it is ideal to collect plasma samples prior to the administration of antibiotics and during episodes of fever. While the detection rate of mNGS in patients with infections involving multiple sites was notably higher than those with fever alone, the diagnostic value of mNGS was significant for both groups. For hematological patients, fever of unknown origin (FUO) is common, and the etiology is often ambiguous with negative conventional microbiological tests (CMT). As a result, overuse of antibiotics is typical in these patients, leading to a rise of antimicrobial resistance. However, by potentially identifying the causative pathogen, mNGS offers a promising strategy to prevent the improper use of antibiotics. Lastly, employing a combined diagnostic approach is crucial. CMTs and mNGS testing results can validate each other and help clinicians identify pathogens more accurately and efficiently. Collecting multiple samples and using traditional methods in parallel with mNGS enhances pathogen detection.

Our study has limitations that should be carefully considered. Firstly, its retrospective and single-center design could lead to participant selection bias. Secondly, antibiotic use prior to mNGS testing may have interfered with the objective evaluation of diagnostic performance. Thirdly, the CMT we employed covered fewer bacterial and fungal molecular tests. Finally, we did not take the cost of mNGS testing into consideration for the assessment of clinical utility, which might potentially bias the findings.

In summary, our study suggested that mNGS had great potential for pathogen diagnosis in patients undergoing allo-HSCT. With improved methodologies and cost reduction, mNGS can serve as a valuable tool in addition to CMT.

Funding

This work was supported by the CAMS Innovation Fund for Medical Sciences (CIFMS) (grant numbers 2021-I2M-1-017 and 2021-I2M-C&T-B-080), Tianjin Municipal Science and Technology Commission Grant (21JCZDJC01170), and the Haihe Laboratory of Cell Ecosystem Innovation Fund (grant number HH22KYZX0036).

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available considering the privacy or ethical restrictions but are available from the corresponding author on a reasonable request.

Conflicts of interest

The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

Acknowledgments

The authors would like to thank all the reviewers who participated in the review, as well as Chao Liu for providing English editing services during the preparation of this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2023.11.002.