Open Forum Infectious Diseases

MAJOR ARTICLE

Effect of Interpretation of Positive Metagenomic Next-Generation Sequencing Reports on the Infection Diagnosis in Patients With Hematological Disorders

Chunhui Xu,[1,2,3,a] Yuyan Shen,[1,2,a] Shulian Chen,[1,2,a] Teng Liu,[3] Xin Chen,[1,2] Yuetian Yu,[4] Li Liu,[1,2] Runzhi Ma,[1,2] Lining Zhang,[1,2] Xin Liu,[1,2,©] Lukun Zhou,[5] Guoqing Zhu,[1,2] and Sizhou Feng[1,2]

[1]State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, China, [2]Tianjin Institutes of Health Science, Tianjin, China, [3]Microbiology laboratory, Tianjin Union Precision Medical Diagnostic Co., Ltd, Tianjin, China, [4]Department of Critical Care Medicine, Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China, and [5]Department of Hematology, Zhongda Hospital, Southeast University, Nanjing, People's Republic of China

Background. Metagenomic next-generation sequencing (mNGS) has become a crucial diagnostic tool for infectious diseases in patients with hematological disorders. However, despite the abundant microbial information provided by positive mNGS reports, interpreting these results remains challenging due to the lack of standardized criteria.

Methods. We surveyed 92 clinicians to identify common challenges in understanding mNGS reports. Microbiologists then provided additional "report interpretation cards" (RICs) for positive mNGS results alongside original reports. The aim of using RICs was to determine whether each detected microorganism was likely cause of infection. After a 3-month period, a panel of clinical experts retrospectively reviewed 281 cases, involving 728 detected microorganisms, to assess RIC accuracy.

Results. In total, 82.6% of clinicians (76 of 92) experienced difficulties in interpreting mNGS reports. After receiving RICs, 97.8% of clinicians (90 of 92) reported satisfaction. The overall concordance rates between interpretation and adjudication in the 281 cases was 79.0% (222 of 281). In 203 cases in which multiple microorganisms were detected, 37.9% (77 of 203) and 37.4% (76 of 203) were interpreted and adjudicated as mixed infections. Among the 728 microorganisms, interpretation and adjudication revealed concordance rates of 93.9% (154 of 164), 95.7% (88 of 92), and 72.3% (339 of 469) for bacterial, fungal, and viral infections, respectively. In 68.7% of the cases (193 of 281), mNGS positively influenced pathogen diagnosis.

Conclusions. Not all microorganisms detected by mNGS are responsible for infection, and appropriate interpretation is essential. The provision of interpretations by microbiologists aids clinicians in accurately using mNGS for infection diagnosis.

Keywords. diagnosis; hematological disorders; infection; interpretation; next generation sequencing.

Patients with hematological disorders have compromised immune function due to underlying conditions and treatments, making infections a common complication and a major contributor to disease and death [1–3]. Early and accurate microbiological diagnosis is crucial in guiding precise and successful antimicrobial therapy for patients [4–6]. The diagnosis of infections in patients with hematological diseases faces significant challenges, primarily for the following reasons: (1) patients with hematological disorders often present with inconspicuous infection symptoms, making it difficult to obtain specimens from the infection site [6, 7]; (2) prior use of antimicrobials may lead to decreased sensitivity of cultures, direct microscopic examination, and serological tests [8, 9]; and (3) in developing countries, molecular methods for diagnosing infections, such as 16S polymerase chain reaction (PCR) for bacteria, are often inaccessible; PCR testing for *Aspergillus* is not readily available in routine clinical practice [10].

Metagenomic next-generation sequencing (mNGS) is increasingly being applied in clinical settings due to its rapid, culture-independent, and unbiased nature [11–13]. For diagnosing infections in patients with hematological disorders, mNGS offers advantages such as high sensitivity, broad pathogen detection, and reduced influence of antimicrobial drugs [14, 15]. Nonetheless, due to the lack of standardized interpretation criteria for mNGS results, clinicians face challenges in interpreting positive reports [16–19]. mNGS results showed a

Received 07 August 2024; editorial decision 05 February 2025; accepted 06 February 2025; published online 7 February 2025

[a]C. X., Y. S., and S. C. contributed equally to this work.

Correspondence: Sizhou Feng, MD, PhD, National Clinical Research Center for Blood Diseases, Chinese Academy of Medical Sciences, Institute of Hematology and Blood Diseases Hospital, No. 288 Nanjing Road, Tianjin 300020, China; Guoqing Zhu, MSc, National Clinical Research Center for Blood Diseases, Chinese Academy of Medical Sciences, Institute of Hematology and Blood Diseases Hospital, No. 288 Nanjing Road, Tianjin 300020, China (szfeng@ihcams.ac.cn).

Open Forum Infectious Diseases®

https://doi.org/10.1093/ofid/ofaf076

high positive rate, but not all identified microorganisms are pathogenic, emphasizing the crucial need for interpreting the results [20, 21].

To date, research on how to interpret microorganisms detected by means of mNGS is lacking, and a standard for determining whether they are pathogens remains elusive. The purpose of the current study was to understand the challenges clinicians face and assess the accuracy and clinical value of interpretations provided by microbiologists who are provided with clinical information on the patients at the time of mNGS.

METHODS

Ethical Considerations

This study was approved by the institutional review board (IRB) and ethics committee of the Institute of Hematology and Blood Diseases Hospital (IRB no. QTJC2023059-EC-1). The ethics committee approved the waiver of informed consent owing to the retrospective nature of the review. All human data from patient records were confirmed for collection in accordance with the relevant guidelines and regulations.

Microbiologists Interpretation

Since our hospital started performing mNGS in January 2020, our previous studies have shown that pathogen mNGS has played a significant role in the etiological diagnosis of infections in patients with hematological diseases [10, 14, 21]. Although mNGS had higher positivity rates than conventional microbiological tests [10], clinicians commonly expressed concerns in interpreting the reports. To understand the questions clinicians raised about report interpretation, we conducted a survey in August 2022 among 92 clinicians who had previously submitted samples for mNGS (Table 1).

Table 1. Clinicians' Concerns From the Survey and Interpretation Information Needed by Clinicians

Concerns and Information Needed	Clinicians, No. (%)
Clinicians' concerns	
1. When mNGS shows low microorganism read counts, clinicians cannot determine whether they have clinical significance.	59 (64.1)
2. When mNGS detects multiple microorganisms, clinicians face challenges in distinguishing between infection, colonization, and contamination.	43 (46.7)
3. Clinicians do not understand some terms in mNGS testing (eg, *abundance, reads,* and *coverage*)	32 (34.8)
4. Clinicians may be unfamiliar with the characteristics of rare pathogenic microorganisms.	30 (32.6)
Interpretation information needed by clinicians	
1. Pathogen interpretation	91 (98.9)
2. Treatment recommendations	79 (85.9)
3. Microbiological characteristics	77 (83.7)
4. Local antimicrobial susceptibility test data	70 (76.1)
5. Clinical and radiological characteristics	65 (70.7)
6. Resistance epidemiology for rare microorganisms	5 (5.4)
7. Additional testing and diagnostics	2 (2.2)

Abbreviation: mNGS, metagenomic next-generation sequencing.

After recognizing the concerns expressed by clinicians, starting in October 2022, microbiologists provided with patients' clinical information began offering interpretation services for positive reports—termed "report interpretation cards" (RICs) (Supplementary materials). These microbiologists were also tasked with reporting mNGS results. The RICs are provided to clinicians along with mNGS reports, with a turnaround time of 24–48 hours. To assess clinicians' satisfaction and additional needs related to RICs, a follow-up survey was conducted among those who participated in the first survey (Table 1).

The RICs include a list of microorganisms in the mNGS report, pathogen interpretation (determining which microorganisms are etiological pathogens), and detailed information about the microorganisms. The microorganisms were categorized as (1) suspected pathogen, (2) indeterminate, or (3) nonpathogen (Supplementary Figure 1 and Supplementary materials).

Clinical Adjudication

The clinical adjudication occurred 3 months after the infection diagnosis and management. During the diagnosis and treatment phase, microbiologists independently interpreted the mNGS results and provided both the results and the RICs to the clinicians, who then used them to guide infection management. To assess the accuracy of pathogen interpretation, a clinical expert panel was formed, consisting of 3 hematologists, including one with extensive experience in infections, who served as the chair. The clinical adjudication process included 2 components: microorganism assessment and clinical impact assessment. For microorganism assessment, the panel independently assessed the microorganisms, blinded to the RIC. They reviewed the medical records and mNGS results to classify the microorganisms into 4 categories: (1) definite pathogen, (2) probable pathogen, (3) possible pathogen, or (4) unlikely pathogen (Supplementary Figure 2). Categories 1–3 were considered suspected pathogens. After microorganism assessment, the panel adjudicated the clinical impact of mNGS and the RIC based on the clinicians' management of the infection. The clinical impact of mNGS was classified as positive, negative, or none (Supplementary Table 1).

After the expert panelists completed their evaluation of the clinical value of the mNGS results, they were provided with the RIC results and analyzed the accuracy of the RIC. A major error in interpretation occurred when the expert panel classified an mNGS result as a suspected pathogen, while microbiologists had interpreted it as either a nonpathogen or an indeterminate pathogen. A minor error occurred when the expert panel classified an mNGS result as an unlikely pathogen but the microbiologists had interpreted it as a suspected pathogen.

This study conducted a clinical adjudication of 300 RICs provided to clinicians from October 2022 to March 2023. Among these, 281 RICs were included in the final analysis. Exclusions were due to nonhematological diagnoses (6 cases), incomplete medical records (8 cases), or lack of follow-up information (5 cases).

Statistical Analysis

SPSS 25.0 software (SPSS) was used for statistical analysis. Continuous variables were expressed as median and range, and categorical variables as percentages. We used χ^2 or Fisher exact tests to compare differences in categorical variables. A significance level of $P < .05$ was applied to determine statistical significance.

RESULTS

Concerns and Needs in Interpretation of mNGS Reports

Among 92 clinicians, 82.6% (76 of 92) expressed concerns regarding positive reports. Furthermore, 48.9% (45 of 92) raised multiple questions (Table 1). The most common challenge was determining the clinical significance of microorganisms with low read count (in 64.1% [59 of 92 clinicians]). After the provision of RICs, clinician satisfaction reached 97.8% (90 of 92). The information most needed included pathogen interpretation (98.9% [91 of 92]), treatment recommendations (85.9% [79 of 92]), and microbiological characteristics (83.7% [77 of 92]) (Table 1).

Clinical Characteristics of Patients Included in This Study

The study involved 281 RICs (representing 281 reports from 225 patients). Patient characteristics are detailed in Supplementary Table 2. Underlying diseases mainly consisted of hematological cancers, with the most common being acute myeloid leukemia (39.1% [88 of 225 patients]). The most infection was respiratory tract infection (59.6% [134 of 225 patients]), followed by mucosal infection (26.7% [60 of 225]) and bloodstream infection (8.4% [19 of 225]). The most common sample type was blood samples (78.8%), followed by bronchoalveolar lavage fluid (10.6%) and cerebrospinal fluid (4.8%) samples (Supplementary Figure 3).

Case-Based Analysis of Interpretation and Adjudication

In the 281 infection cases, the detection rates for bacteria, fungi, viruses, and parasites were 42.7% (120 of 281), 26.7% (75 of 281), 86.1% (242 of 281), and 1.1% (3 of 281), respectively. For interpretation, 97.5% of bacteria (117 of 120), 100% of fungi (75 of 75), and 24.4% of viruses (59 of 242) were interpreted as suspected pathogens. In the adjudication, the percentages of bacteria, fungi, and viruses interpreted as suspected pathogens were 92.5% (111 of 120), 97.3% (73 of 75), and 26.4% (64 of 242), respectively (Figure 1*A*). All detected parasites were both interpreted and adjudicated as suspected pathogens. In addition, the positive agreement rate in cases interpreted and adjudicated as suspected pathogens was 70.1% (197 of 281) (Figure 1*B*). A total of 25 cases were interpreted as nonpathogens and adjudicated as unlikely pathogens, accounting for 8.9% (25 of 281). Another 12.1% of cases (34 of 281) were interpreted as indeterminate but adjudicated as unlikely pathogens. The overall concordance rate between interpretation and adjudication in all cases was 79.0% (222 of 281).

Cases With Multiple Microorganisms Detected

Distinguishing between infection, colonization, and contamination when multiple microorganisms are detected can be challenging. In our survey, 46.7% of clinicians expressed concern about the difficulty of accurately identifying pathogens in these cases. We analyzed the interpretation and adjudication of suspected pathogens in 281 cases, of which 72.2% (203 of 281) had multiple microorganisms reported (Supplementary Table 3). However, only 37.9% (77 of 203) and 37.4% (76 of 203) of these cases were interpreted or adjudicated, respectively, as mixed infections. Furthermore, in 27.8% (78 of 281) only one microorganism was reported, yet 57.7% (45 of 78) and 50.0% (39 of 78) of these cases were interpreted or adjudicated, respectively, as suspected pathogens. In 26.0% of cases (73 of 281), no suspected pathogen was detected after clinical adjudication.

Microbial-Based Analysis of Interpretation and Adjudication

A total of 728 microorganisms were detected, including bacteria (n = 164), fungi (n = 92), DNA viruses (n = 451), RNA viruses (n = 18), and parasite (n = 3). Among these, 45.5% (331 of 728) were interpreted as suspected pathogens, 14.4% (105 of 728) as indeterminate, and 40.1% (292 of 728) as nonpathogens. For the microorganisms interpreted as suspected pathogens, the concordance rate of clinical adjudication was 91.5% (30.5% definite, 42.0% probable, and 19.0% possible). The microorganisms not interpreted as suspected pathogens (92.9% [369 of 397]) were adjudicated as nonpathogenic (Figure 2*A*).

The spectrum of microorganism detection is illustrated in Figure 2*B*. Bacteria, fungi, and viruses were interpreted as suspected pathogens in 97.0% (159 of 164), 100.0% (92 of 92), and 16.4% (77 of 469) of cases, respectively. Meanwhile, 92.1% (151 of 164), 95.7% (88 of 92), and 19.0% (89 of 469) were adjudicated as suspected pathogens. RNA viruses, such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and human respirovirus, were all interpreted/adjudicated as suspected pathogens. In contrast, <20% of DNA viruses, including cytomegalovirus (CMV), Epstein-Barr virus (EBV), and herpes simplex virus, were interpreted or adjudicated as suspected pathogens.

Analysis of interpretation and adjudication results revealed concordance rates of 93.9% (154 of 164), 95.7% (88 of 92), and 72.3% (339 of 469), respectively, for bacterial, fungal, and viral pathogens (Supplementary Table 4). Minor errors were observed in the interpretation of bacteria, fungi, and viruses.

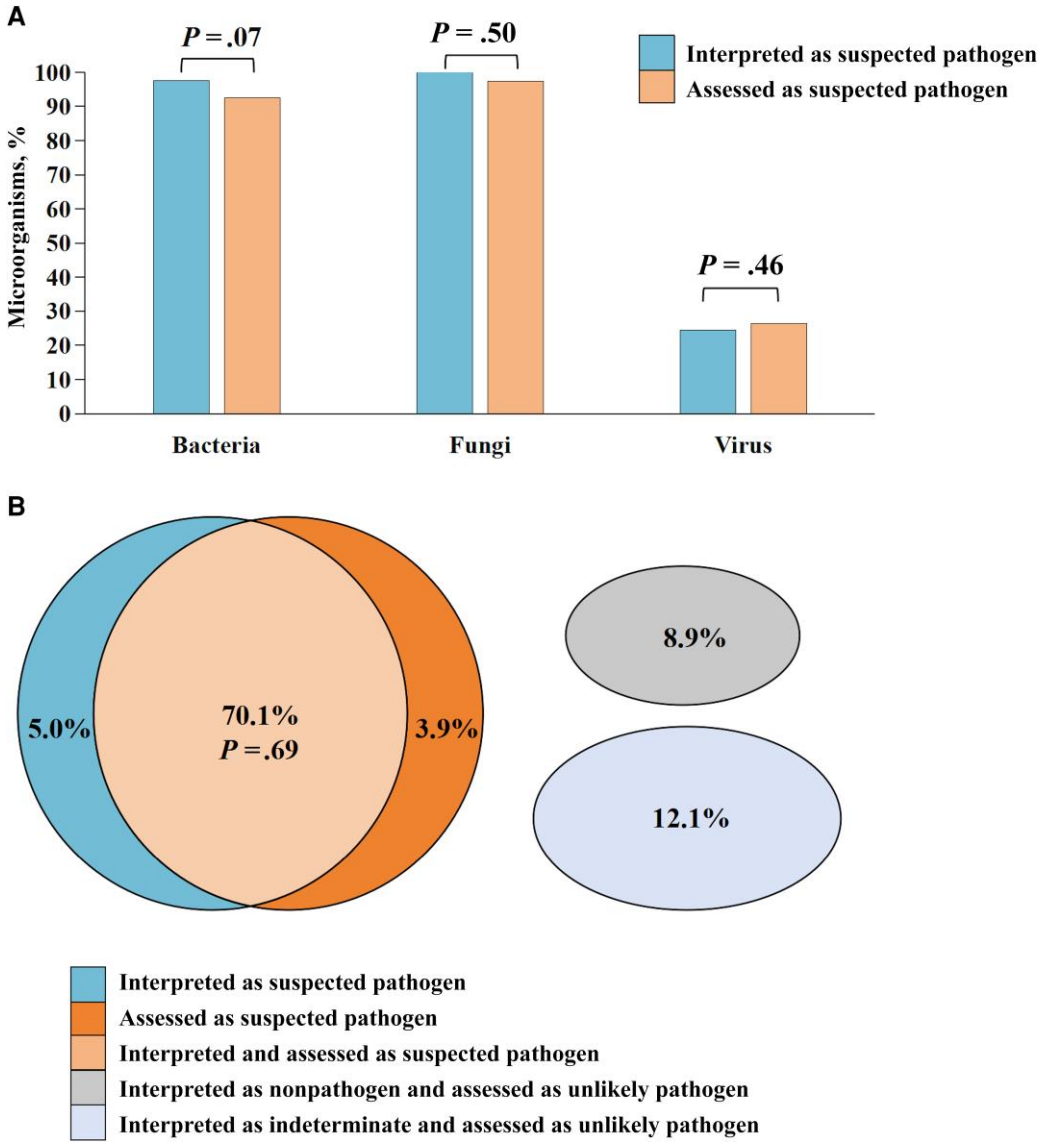

Figure 1. Detection rates and concordance in interpretation and adjudication. *A*, Interpretation and adjudication (assessment) of microorganisms. *B*, Concordance comparison of interpretation and adjudication as suspected pathogens.

In terms of major errors, 0.6% (1 of 164) of bacterial interpretations involved a major error, which was for *Staphylococcus aureus*. In viral interpretations, 5.8% (27 of 469) were identified as major errors, including CMV (n = 7), EBV (n = 7), herpes simplex virus (n = 5), BK polyomavirus (n = 3), human herpesvirus (HHV) 7 (n = 2), HHV-6B (n = 1), human polyomavirus 3 (n = 1), and human polyomavirus 4 (n = 1).

Clinical Impact of mNGS and Report Interpretation

Among 281 cases, mNGS had a positive clinical impact on infection management in 68.7% (193 of 281) (Supplementary Table 5). The positive impact was more significant in cases in which the microorganisms were interpreted as suspected pathogens, compared with those classified as indeterminate (87.7% vs 15.9%, $P < .001$) or nonsuspected (87.7% vs 3.8%, $P < .001$). For detection of bacteria, fungi, and viruses, the positive impact percentages were 87.5% (105 of 120), 96.0% (72 of 75), and 18.6% (45 of 242), respectively. In addition, the positive impact of mNGS was greater in cases in which the virus was interpreted as a suspected pathogen (55.9% vs 6.6%; $P < .001$).

DISCUSSION

While mNGS has become an important tool in diagnosing pathogenic microorganism infectious, difficulties in interpreting mNGS reports are common among clinicians [20, 22–24].

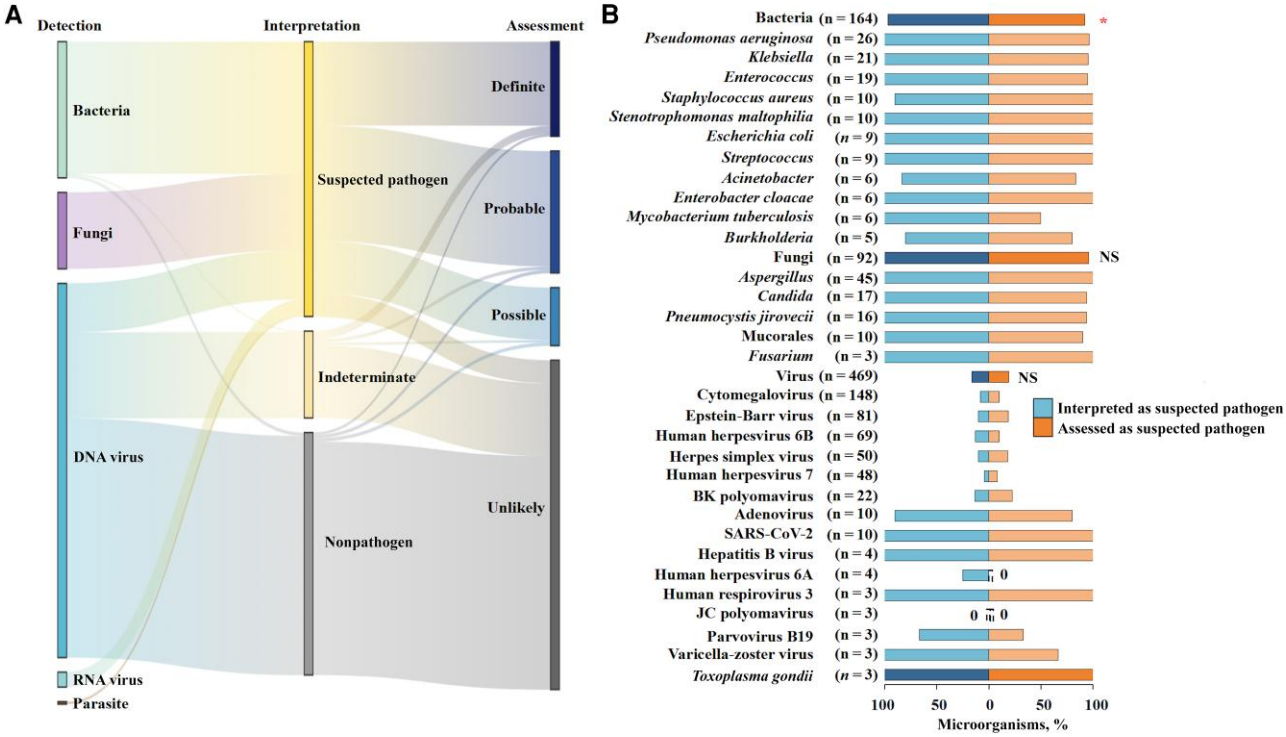

Figure 2. Interpretation and adjudication (assessment) of microorganisms and their distribution. *A*, Sankey diagram illustrating the interpretation and adjudication microorganisms. *B*, Detection of frequent microorganisms with metagenomic next-generation sequencing, along with interpretation and adjudication. $^*P \leq .05$. Abbreviation: NS, not statistically significant; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Our study evaluated the accuracy and clinical impact of interpretations provided by microbiologists. We showed a high concordance rate between interpretation and clinical adjudication, contributing to the better utility of mNGS.

More than 80% of clinicians had questions when presented with a positive mNGS report. We found that the inability to judge the clinical significance of microorganisms was the most common challenge in clinical practice (64.1%). Information needed by clinicians included pathogen interpretation, clinical information on microorganisms, recommendations for antimicrobial treatment, and epidemiological data.

Although mNGS has a high positivity rate in patients with hematological diseases, not all detected microorganisms are clinically significant [21, 25]. Among 281 cases with positive mNGS results, 24.6% of cases were interpreted and 26.0% were assessed as having no suspected etiological pathogens. The concordance rate between interpretation and adjudication was 79.0%. Furthermore, reporting of multiple microorganisms is common for patients with hematological diseases, but not all are indicative of mixed infections. In our study, >70% of cases had multiple microorganisms but <40% were adjudicated as mixed infections, and >700 microorganisms were identified. While viruses had the highest detection rate, they were least frequently classified as suspected pathogens. Specifically, up to 80% of viruses detected with mNGS, particularly DNA viruses such as CMV, EBV, HHV, and JC polyomavirus, were not identified as the cause of infections. Previous studies have reached similar conclusions [26, 27]. In contrast, RNA viruses were more likely to be suspected pathogens. Of the 18 RNA viruses detected across 15 cases, most were interpreted and adjudicated as suspected pathogens.

Compared with the 7.3% positive clinical impact reported by Hogan et al [28] and the 14.0% reported by Zhu et al [29], our study demonstrates a more significant positive impact of mNGS on infection management. This difference may be attributed to several factors. The first is differences in the criteria for evaluating positive clinical impact. Consistent with our study, Hogan et al [28] categorized cases in which mNGS results confirmed clinical diagnoses but did not lead to therapeutic modifications as having a positive impact, as these results supported clinical decision making. In contrast, Zhu et al [29] classified such cases as having no impact. As a second factor, our study included only positive mNGS results and interpreted them, which could have increased the proportion of positive impact. Third, in our study pathogen-specific PCR tests such as *Aspergillus* PCR and bronchial pan-Mucorales PCR were not used due to their limited availability. In contrast, Hogan et al [28] routinely used these tests, which may have influenced the proportion of positive clinical impacts in their study. The fourth and final factor is differences in the turnaround time for mNGS reports. In

our study, mNGS reports were completed within 24–48 hours, whereas with Hogan et al the average turnaround time was 74.4 hours. This difference may have further influenced the evaluation of positive clinical impact.

This study has several limitations. First, it was a single-center retrospective study focusing primarily on the concerns and interpretation of positive mNGS reports, without the analysis of negative mNGS reports. Second, conventional microbiological tests were relatively limited, which may lead to variations in the proportion of interpretations or adjudications as suspected pathogens. Third, while our adjudication criteria considered patients' clinical status and conventional microbiological test results, it still mainly relied on judgments from an expert panel, introducing potential bias.

Overall, the interpretation of positive mNGS reports had a positive impact, addressing clinicians' doubts concerning positive mNGS reports. This service assisted clinicians in promptly and accurately identifying pathogens, particularly in patients with hematological disorders. However, in adjudicating the practical utility of the RIC service in clinical practice, larger-scale studies are still needed to obtain more comprehensive data support.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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

Author contributions. Methodology, formal analysis, and writing—original draft: C. X., Y. S., and S. C. Data curation: T. L., X. C., Y. Y., L. L., R. M., L. Zhang, X. L., and L. Zhou.

Supervision: T. L., X. C., Y. Y., L. L., R. M., L. Zhang, X. L., L. Zhou, G. Z., and S. F. Conceptualization, funding acquisition, resources, and writing—editing: G. Z. and S. F. All authors reviewed the manuscript.

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

Financial support. This work was supported by the Chinese Academy of Medical Sciences Innovation Fund for Medical Sciences (grants 2021-I2M-1-017, 2023-I2M-2-007, 2021-I2M-1-039), National Natural Sciences Foundation of China (82470208), National Key R&D Program of China (2024YFC2510500), and Special Fund Project for Science and Technology Innovation Strategy of Nanjing [YKK21273].

Potential conflicts of interest. The authors: No reported conflicts.

References

1. Sahin U, Toprak SK, Atilla PA, Atilla E, Demirer T. An overview of infectious complications after allogeneic hematopoietic stem cell transplantation. J Infect Chemother **2016**; 22:505–14.
2. Chen CY, Tien FM, Sheng WH, et al. Clinical and microbiological characteristics of bloodstream infections among patients with haematological malignancies with and without neutropenia at a medical centre in northern Taiwan, 2008–2013. Int J Antimicrob Agents **2017**; 49:272–81.
3. Chen Y, Wang J, Gan X, et al. Application of plasma metagenomic next-generation sequencing improves prognosis in hematology patients with neutropenia or hematopoietic stem cell transplantation for infection. Front Cell Infect Microbiol **2024**; 14:1338307.
4. Hong M, Peng D, Fu A, et al. The application of nanopore targeted sequencing in the diagnosis and antimicrobial treatment guidance of bloodstream infection of febrile neutropenia patients with hematologic disease. J Cellular Molecular Medi **2023**; 27:506–14.
5. Schmidt-Hieber M, Teschner D, Maschmeyer G, Schalk E. Management of febrile neutropenia in the perspective of antimicrobial de-escalation and discontinuation. Expert Rev Anti Infect Ther **2019**; 17:983–95.
6. Freifeld AG, Bow EJ, Sepkowitz KA, et al. Executive summary: clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Diseases Society of America. Clin Infect Dis **2011**; 52:427–31.
7. Qi Y, Lin WQ, Liao B, Chen JW, Chen ZS. Blood plasma metagenomic next-generation sequencing for identifying pathogens of febrile neutropenia in acute leukemia patients. Sci Rep **2023**; 13:20297.
8. Donnelly JP, Chen SC, Kauffman CA, et al. Revision and update of the consensus definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. Clin Infect Dis **2020**; 71:1367–76.
9. Zhang M, Wang Z, Wang J, et al. The value of metagenomic next-generation sequencing in hematological malignancy patients with febrile neutropenia after empiric antibiotic treatment failure. Infect Drug Resist **2022**; 15:3549–59.
10. Xu C, Chen X, Zhu G, et al. Utility of plasma cell-free DNA next-generation sequencing for diagnosis of infectious diseases in patients with hematological disorders. J Infect **2023**; 86:14–23.
11. Gu W, Deng X, Lee M, et al. Rapid pathogen detection by metagenomic next-generation sequencing of infected body fluids. Nat Med **2021**; 27:115–24.
12. Gu W, Miller S, Chiu CY. Clinical metagenomic next-generation sequencing for pathogen detection. Annu Rev Pathol Mech Dis **2019**; 14:319–38.
13. Wang S, Ai J, Cui P, Zhu Y, Wu H, Zhang W. Diagnostic value and clinical application of next-generation sequencing for infections in immunosuppressed patients with corticosteroid therapy. Ann Transl Med **2020**; 8:227.
14. Feng S, Rao G, Wei X, et al. Clinical metagenomic sequencing of plasma microbial cell-free DNA for febrile neutropenia in patients with acute leukaemia. Clin Microbiol Infect **2024**; 30:107–13.
15. Wang D, Wang W, Ding Y, et al. Metagenomic next-generation sequencing successfully detects pulmonary infectious pathogens in children with hematologic malignancy. Front Cell Infect Microbiol **2022**; 12:899028.
16. Han D, Li Z, Li R, Tan P, Zhang R, Li J. mNGS in clinical microbiology laboratories: on the road to maturity. Crit Rev Microbiol **2019**; 45:668–85.
17. Batool M, Galloway-Peña J. Clinical metagenomics—challenges and future prospects. Front Microbiol **2023**; 14:1186424.
18. Chien JY, Yu CJ, Hsueh PR. Utility of metagenomic next-generation sequencing for etiological diagnosis of patients with sepsis in intensive care units. Bekal S, editor. Microbiol Spectr **2022**; 10:e00746–22.
19. Simner PJ, Miller S, Carroll KC. Understanding the promises and hurdles of metagenomic next-generation sequencing as a diagnostic tool for infectious diseases. Clin Infect Dis **2018**; 66:778–88.
20. Han D, Yu F, Zhang D, et al. The real-world clinical impact of plasma mNGS testing: an observational study. Microbiol Spectr **2023**; 11:e0398322.
21. Xu CH, Chen X, Zhu GQ, et al. Diagnostic performance and clinical impacts of metagenomic sequencing after allogeneic hematopoietic stem cell transplantation. J Microbiol Immunol Infect **2024**; 57:11–9.
22. Liu J, Zhang Q, Dong YQ, Yin J, Qiu YQ. Diagnostic accuracy of metagenomic next-generation sequencing in diagnosing infectious diseases: a meta-analysis. Sci Rep **2022**; 12:21032.
23. Schulz E, Grumaz S, Hatzl S, et al. Pathogen detection by metagenomic next-generation sequencing during neutropenic fever in patients with hematological malignancies. Open Forum Infect Dis **2022**; 9:ofac393.
24. Qian YY, Wang HY, Zhou Y, et al. Improving pulmonary infection diagnosis with metagenomic next generation sequencing. Front Cell Infect Microbiol **2021**; 10: 567615.
25. Wu J, Song W, Yan H, et al. Metagenomic next-generation sequencing in detecting pathogens in pediatric oncology patients with suspected bloodstream infections. Pediatr Res **2024**; 95:843–51.
26. Langelier C, Zinter MS, Kalantar K, et al. Metagenomic sequencing detects respiratory pathogens in hematopoietic cellular transplant patients. Am J Respir Crit Care Med **2018**; 197:524–8.
27. Seo S, Renaud C, Kuypers JM, et al. Idiopathic pneumonia syndrome after hematopoietic cell transplantation: evidence of occult infectious etiologies. Blood **2015**; 125:3789–97.
28. Hogan CA, Yang S, Garner OB, et al. Clinical impact of metagenomic next-generation sequencing of plasma cell-free DNA for the diagnosis of infectious diseases: a multicenter retrospective cohort study. Clin Infect Dis **2021**; 72:239–45.