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MAJOR ARTICLE

Impact of Metagenomic Next-Generation Sequencing of Plasma Cell-free DNA Testing in the Management of Patients With Suspected Infectious Diseases

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Metagenomic next-generation sequencing (mNGS) of cell-free DNA is an emerging modality for the diagnosis of infectious diseases, but studies on its clinical utility are limited. We conducted a retrospective single-center study including all patients who had plasma mNGS sent at the University of Michigan between 1 January 2021 and 25 July 2022. Test results were assessed for clinical impact. A total of 71 tests were sent on 69 patients; the mean ± SD age was 52 ± 19 years; and 35% of patients were immunocompromised. Forty-five (63%) mNGS test results were positive and 14 (31%) had clinical impact—from starting new antimicrobials (n = 7), discontinuing antimicrobials (n = 4), or changing antimicrobial duration (n = 2) or by affecting surgical decision making (n = 1). Twenty-six (37%) mNGS test results were negative and only 4 (15%) were impactful, leading to discontinuation of antimicrobials. Overall, just 25% of mNGS tests were clinically relevant. There was no significant difference in the proportion of tests that were clinically relevant between negative and positive results ($P = .16$) or if patients were immunocompromised ($P = .57$). Plasma mNGS was most frequently impactful (in 50% of patients) when included in the diagnostic workup of cardiovascular infection but less impactful in other clinical syndromes, including fever of unknown origin and pulmonary infection. Our findings underscore the need to further study this testing modality, particularly with prospective research including negative controls, before it is considered for widespread use.

Keywords. cell-free DNA; infections; metagenomic next-generation sequencing.

In recent years, advances in diagnostic technologies for infectious diseases (IDs)—including the development of automated systems for microbial identification and the use of matrix-assisted laser desorption ionization–time of flight—have increased the capability for rapid identification of relevant human pathogens in clinical samples. While these assays have excellent test performance and generally allow for accurate organism identification, they require growth of an organism in culture before identification can be attempted [1]. This is particularly challenging for cases in which a sample of infected material is unable to be obtained or when the suspected etiologic agent cannot be grown in the microbiology laboratory. Despite the current landscape of diagnostic testing, there remains a subset of patients in whom an infection is suspected but no etiologic agent can be identified.

Metagenomic next-generation sequencing (mNGS) utilizes high-throughput sequencing technologies to rapidly and accurately identify DNA/RNA from presumptive pathogens [2]. Although mNGS could presumably be utilized on any patient sample, the use of microbial cell–free DNA (mcf-DNA) allows for the use of an easily obtained sample, such as plasma. mcf-DNA is postulated to be released from dead and dying cells; fragments of genomic DNA from pathogenic organisms have been isolated in the mcf-DNA of patients with active infection [3, 4]. When mcf-DNA is sequenced and compared with existing libraries, a presumptive identification of the infecting organism can be made. mNGS testing of mcf-DNA is currently available through only 1 company, Karius; this testing is done on plasma and utilizes a comparator library of >1000 human pathogens [5]. Testing at several institutions has demonstrated that mNGS of mcf-DNA can identify bacterial, viral, and fungal pathogens in patients who are immunocompromised (IC) and have infections [6–10]. However, the test has not been well validated in patients without infections (negative controls), and interpretation of results can be challenging, particularly if a potentially colonizing organism is identified. Testing requires shipping the sample to the centralized

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Karius laboratory and is expensive; not all insurance companies authorize payment for this assay.

Our institution began offering mNGS of mcf-DNA in January 2021. We sought to determine the real-world clinical impact of mNGS of plasma mcf-DNA testing in the management of patients with suspected IDs.

METHODS

Study Design

This retrospective observational study included adult patients with mNGS testing obtained as part of clinical care. The study was performed at Michigan Medicine, a 1000-bed tertiary care hospital affiliated with the University of Michigan, located in southeastern Michigan.

We utilized EMERSE (Electronic Medical Record Search Engine) to identify all patients treated at Michigan Medicine for whom mNGS plasma testing was obtained from 1 January 2021 to 25 July 2022 [11]. Patients who were identified by EMERSE as having an mNGS test ordered by an outside facility were not included in the study.

The medical record was reviewed to obtain data regarding demographic information; mNGS testing date, indication, and results; additional pertinent ID workup temporally related to the episode of interest (culture- and nonculture-based microbiologic testing and radiologic findings); and final diagnosis and treatment.

Patient Consent Statement

This study was approved by the University of Michigan Institutional Review Board, and informed consent was deferred due to its retrospective nature.

mNGS Procedure and Definitions

mNGS plasma testing was performed by Karius; qualitative and quantitative results were requested. When testing was recommended, a 10-mL sample of whole blood was collected from the patient, centrifuged and the resulting 5 mL of plasma were shipped to the centralized laboratory according to specimen collection and preparation instructions provided by the manufacturer website [12]. mNGS results were classified as positive or negative based on the interpretation provided by the manufacturer.

Date of testing was defined as the date that mNGS testing was ordered in the electronic medical record. Date of testing was compared with the date that standard ID workup had been completed. Standard ID workup was considered complete (1) if relevant cultures were collected and incubated for >48 hours, (2) if serologic testing was sent and the result received, and (3) if relevant cross-sectional imaging (computed tomography, magnetic resonance imaging) was completed without a clear diagnosis established.

Clinical Impact of mNGS

Clinical impact was evaluated per the medical decisions made by the treating team after interpreting the mNGS test results. mNGS results were considered to have a clinical impact if they altered the initial choice or duration of antimicrobial therapy recommended by the treating physician. Results were considered not to have a clinical impact (1) if the initial antibiotic plan was not changed as a result of the mNGS test, (2) if the final diagnosis was already established by other means before the mNGS result returned, (3) if the treating ID physician determined that the result was not clinically relevant, or (4) if the patient expired or was enrolled in hospice before the mNGS result was available for interpretation. Adjudication of the final diagnosis and determination of the clinical impact of the mNGS result were performed by an ID physician not involved in the care of any patient in the investigational cohort, based on the decisions and actions of the treating physicians reported in the medical record. Diagnoses were ultimately based on all test results (including 16S rRNA sequencing, when a positive and clinically consistent result was available) and a longitudinal review of the patient's clinical characteristics.

We conducted descriptive data analyses of all variables using *t* test and Fisher exact test to determine differences between groups. All statistical analyses were completed with SPSS (version 29.0.1.0; IBM).

RESULTS

Demographics and Sample

In total, 71 mNGS tests were sent for 69 patients. The mean ± SD age of patients tested with mNGS was 52 ± 19 years, and 24 (35%) were IC (Table 1). Two patients had 2 mNGS tests sent (1 had 2 tests sent 9 days apart and 1 had 2 tests sent 3.5 months apart); both patients were IC. Prior ID workup was complete in 53 (75%) of 69 patients in whom mNGS was sent. A final diagnosis consistent with infection was established in 44 patients (64%): 15 diagnoses were made by conventional means, 8 by

Table 1. Demographic Information of Patients Who Had mNGS Testing

Demographic Indicator	No.	%
Age, y, mean ± SD	52 ± 19	…
Gender: male	42	63
Immune status		
Immunosuppression	24	35
Solid organ transplant recipient[a]	1	…
Hematologic cancer/stem cell transplant recipient	17	…
Other[b]	6	…
Nonimmunosuppressed	45	65

Abbreviation: mNGS, metagenomic next-generation sequencing.

[a]Liver n = 1.

[b]Immunosuppressing medications (n = 4), chronic granulomatous disease (n = 1), common variable immunodeficiency (n = 1).

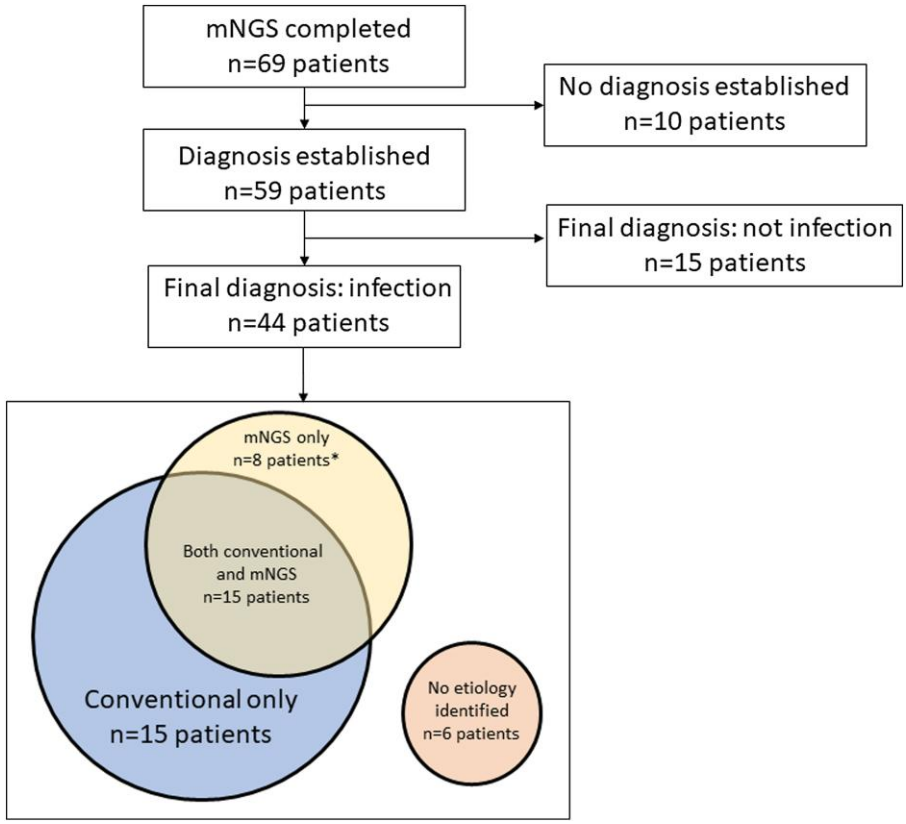

Figure 1. Comparison of conventional vs molecular next-generation sequencing (mNGS) modalities for the diagnosis of infection syndromes in 69 patients. *Culture-negative endocarditis/suspected endovascular infection (n = 5; *Neisseria* spp, *Prevotella* spp, *Staphylococcus aureus, Streptococcus thermophilus/Sarcina ventriculi, Veillonella* spp), systemic/fever of unknown origin (n = 1; *Enterococcus faecium*), pulmonary (n = 1; *Rhizopus* spp), and intra-abdominal (n = 1; polymicrobial).

mNGS, and 15 by conventional means and mNGS. In 6 patients with a syndrome consistent with infection, conventional testing and mNGS results were negative, and etiology remained unknown (Figure 1).

mNGS Assay

mNGS testing results were positive in 45 patients (63%). The most common condition for which this testing was requested was the evaluation of culture-negative endocarditis/suspected endovascular infection (n = 24, 33%), followed by suspected systemic infection/fever of unknown origin (n = 19, 26%) and suspected pulmonary infection (n = 17, 24%).

Of the positive test results, 14 (31%) directly affected patient management: antibiotics were prescribed in 7 patients (50%); they were discontinued or narrowed in 4 (29%); and antibiotic duration was extended in 2 (14%). In 1 patient whose surgical procedure was previously deferred, the procedure was reconsidered and ultimately performed per the mNGS result (Figure 2).

Overall, 31 (69%) positive test results did not affect patient care. In these cases, the diagnosis had already been established by standard culture-based mechanisms (n = 8, 26%); the patient was already undergoing appropriate empiric therapy and the clinical course was not altered further (n = 9, 29%); the mNGS result was deemed irrelevant (n = 9, 29%); and the patient either died or went home with hospice before the mNGS result was received (n = 5, 16%).

mNGS testing was negative in 26 cases (37%) and affected patient care in 4 (15%), all through the discontinuation or narrowing of antibiotics (Figure 2). In 6 cases, mNGS test results were considered "discordant" because the test was negative or the assay detected a different pathogen from what was ultimately identified through standard culture-based diagnostic methods or 16S universal polymerase chain reaction testing (Table 2). In all instances, the diagnosis made through standard diagnostic modality or universal polymerase chain reaction prevailed as the true diagnosis over the mNGS test result.

Of 71 mNGS test results, 18 (25%) had a clinical impact on patient management. There were no significant changes in impact whether the test result was positive or negative (45% vs 15%, $P = .16$). In both patients who had mNGS repeated, neither the first nor the second test was clinically impactful.

Of 71 serum mNGS tests, 26 (36%) were from 24 patients who were IC (Table 1): 20 were positive and 6 were negative

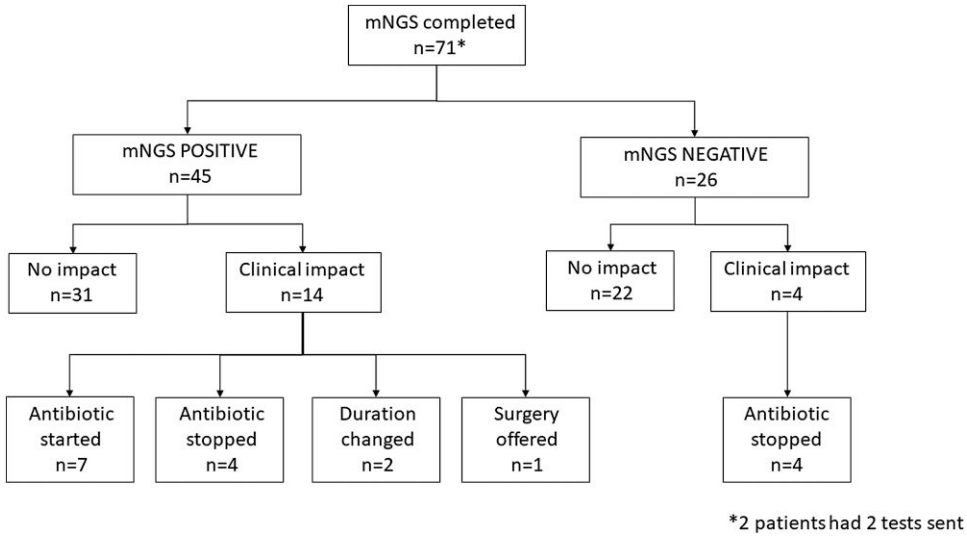

Figure 2. Clinical utility of molecular next-generation sequencing (mNGS) of 71 tests when stratified by positive or negative result.

(2 patients had 2 tests). Of 26 tests, 8 (30%) had either a positive or a negative result that was deemed to have a clinical impact (4 each).

The clinical impact of mNGS test results between patients who were immunosuppressed and nonimmunosuppressed was comparable ($P = .57$). In patients who were immunosuppressed, 66% of mNGS negative test results had a clinical impact on patient management, as opposed to 20% of positive test results ($P = .05$). Among 45 mNGS tests performed in patients who were non-IC, 10 of 25 (40%) positive results had a clinical impact in patient care, while none of the 20 negative results were considered clinically relevant.

When analyzed by indication for testing, the evaluation of culture-negative endocarditis or endovascular infection was most impactful. Of the 24 mNGS tests sent for evaluation of culture-negative endocarditis/suspected endovascular infection, 16 were positive and 8 were negative (Table 3). Twelve (50%) mNGS test results sent for this indication were clinically impactful: 6 (50%) for discontinuing antibiotics or antimicrobial spectrum, 5 (42%) for starting a new antibiotic or prolonging duration of antibiotics, and 1 (8%) related to surgical planning. Ten of these impactful tests were positive and 2 were negative; neither test result was more likely to be impactful than the other ($P = .08$).

Tests sent for systemic infection/fever of unknown origin and pulmonary infection were impactful in only 3 (16%) and 2 (12%) cases, respectively. Further clinical impact of mNGS results stratified by clinical syndrome is summarized in Table 4.

DISCUSSION

Our study showed that the results of commercially available plasma mNGS tests had a low impact on the management of patients within our medical center. In our cohort, having a positive or negative mNGS test result was clinically impactful in only 25% of patients tested.

Our findings are similar to those reported by Hogan et al [13]. In a retrospective multicenter study, the authors evaluated the clinical impact of plasma mNGS tests performed for any indication in 82 patients, including 39 adults and 53 patients who were IC. In their study, mNGS test results had a clinical impact in 11% of patients. Similarly, in a prospective study of 58 patients with central nervous system infection, Wilson et al showed the clinical usefulness of mNGS in cerebral spinal fluid in 12% of patients [14]. In a recent series of 29 patients, Freeman Weiss et al showed a positive clinical impact of mNGS in 45% of cases; a negative impact was noted in 10% of cases [15].

It has been suggested that plasma mNGS testing may be particularly useful in the diagnosis of infections in IC hosts, particularly those with impaired T-cell response [16, 17]. These patients are at high risk for infections, and differential diagnosis is broad. Clinical presentation of infections in patients who are IC is atypical, and culture-based testing has low yield in some settings [6, 8, 18]. The reported impact of plasma mNGS testing in the diagnosis and antibiotic management has been cited as 47% to 61% in patients with febrile neutropenia, 53% in adults with hematologic malignancies and in recipients of stem cell transplantation, and 45% in recipients of solid organ transplantation [8, 18–20]. Our study found no difference in clinical impact of mNGS test results between patients who were IC and non-IC, possibly due to the underrepresentation of the former in our cohort. Among patients who were IC, a negative test result more frequently led to a change in management, allowing for discontinuation or narrowing of antibiotic spectrum.

Table 2. Discordant Results Between Plasma mNGS Testing and Alternative Identification Strategies

Clinical Infection Syndrome	mNGS Result	Alternative Identification	Final Diagnosis
Breast abscess	Negative	*Actinomyces* spp on abscess culture	*Actinomyces* breast abscess
Brain lesion	*Klebsiella pneumoniae* (216 mpm)	Gram-positive cocci on brain biopsy (Gram stain) and *S intermedius* detected on 16S universal PCR from brain biopsy	*S intermedius* brain abscess
Cardiac device infection (ICD/PPM)	*S epidermidis* (708 mpm), *S thermophilus* (242 mpm)	*K aerogenes*[a] on extracted device lead culture	*S epidermidis* PPM pocket infection with concurrent *K aerogenes* device lead infection
Mitral valve endocarditis, sternal hardware infection/ osteomyelitis	*S thermophilus* (77 mpm), *Burkholderia cepacia* (74 mpm)	*S enterica* detected on 16S universal PCR from debrided tissue	*S enterica* endocarditis and sternal osteomyelitis
Prosthetic valve endocarditis	Negative	*S infantarius/lutetiensis* detected on 16S universal PCR from extracted prosthetic aortic valve	*S infanterius/lutetiensis* prosthetic valve endocarditis
Cavitary lung lesion	Negative	*M avium* complex on bronchoalveolar lavage culture	*M avium* pulmonary infection

Abbreviations: ICD, implantable cardiac defibrillator; *M avium, Mycobacterium avium*; mNGS, metagenomic next-generation sequencing; mpm, DNA molecules per micrometer; PCR, polymerase chain reaction; PPM, permanent pacemaker; *S enterica, Salmonella enterica*; *S epidermidis, Staphylococcus epidermidis*; *S infantarius/lutetiensis, Streptococcus infantarius/ lutetiensis*; *S intermedius, Streptococcus intermedius*; *S thermophilus, Streptococcus thermophilus.*

[a] *Klebsiella aerogenes* (formerly *Enterobacter aerogenes*).

Table 3. mNGS Results and Clinical Impact in 24 Cases of Suspected Culture-Negative Endocarditis or Endovascular Infection

Clinical Impact (n = 12)				No Clinical Impact (n = 12)			
Age, y; Gender	Infection	mNGS Result	Comment	Age, y; Gender	Infection	mNGS Result	Comment
22 M	CNE	Negative	Discontinued ABx	78 M	CNE	Negative	…
78 M	Aorta	Negative	Discontinued ABx	60 M	CNE	Negative	…
61 F	CNE	*Prevotella* spp	Started ABx	60 F	CNE	Negative	…
65 F	CNE	*Prevotella* spp	Started ABx	66 F	CNE	Negative	…
18 M	CNE	*Staphylococcus aureus*	Surgery	61 M	CNE	Negative	…
56 M	CNE	*S thermophilus, Sarcina ventriculi*	Discontinued ABx	74 M	VAD drive line	*Escherichia coli*	Dx made by other means prior to mNGS result
77 M	CNE	*S mutans*	Duration of ABx lengthened	39 M	CNE	*Enterococcus faecium*	Patient on appropriate ABx prior to mNGS result
18 M	CNE	*Cardiobacterium hominis*	Discontinued ABx	57 M[a]	CNE	*S thermophilus*	Patient on appropriate ABx prior to mNGS result
89 M	Aortic graft	*Enterococcus faecalis, Klebsiella aerogenes,* Epstein-Barr virus	Started Abx	57 M[a]	CNE	*S thermophilus*	Dx made by other means prior to mNGS result
71 M	CNE	*Veillonella* spp, HHV-7	Discontinued ABx	68 M	ICD/PPM	*Staphylococcus epidermidis, S thermophilus*	Dx made by other means prior to mNGS result
36 M	CNE	*Neisseria sicca, Micrococcus* spp	Started ABx	68 M	CNE	Negative	…
19 F	CNE	*Streptococcus mitis/oralis*	Discontinued ABx	79 F	CNE	*S mutans*	mNGS result thought irrelevant

Abbreviations: ABx, antibiotics; CNE, culture-negative endocarditis; Dx, diagnosis; F, female; HHV, human herpesvirus; ICD/PPM, intracardiac device/permanent pace maker; M, male; mNGS, metagenomic next-generation sequencing; *S mutans, Streptococcus mutans*; *S thermophilus, Streptococcus thermophilus*; VAD, ventricular assist device.

[a] This patient had mNGS sent twice.

In our study, plasma mNGS test results were more impactful in clinical decision making when performed as part of the evaluation of patients with culture-negative endocarditis or endovascular infection. These findings are similar to those of Li et al, who found that the mNGS of blood specimens was impactful in 10 of 18 patients with culture-negative endocarditis [21]. However, data are scarce to support the widespread use of plasma mNGS over traditional methods of diagnosing culture-negative endocarditis [21–24]. Case reports have demonstrated that pathogens implicated with culture-negative endocarditis can be detected by mNGS, but direct comparisons between mNGS and standard serologic testing are not available

Table 4. Impact of 71 mNGS Test Results by Clinical Syndrome

	No. of Tests		
Clinical Infection Syndrome	Impact	Nonimpact	Total (% Impact)
Cardiovascular	12	12	24 (50)
Systemic/FUO	3	16	19 (16)
Pulmonary	2	15	17 (12)
Gastrointestinal	0	3	3 (0)
Brain abscess	0	3	3 (0)
Skin/soft tissue	0	2	2 (0)
Bone/joint	1	1	2 (50)
ENT	0	1	1 (0)
Total	18	53	71 (25)

Abbreviations: ENT, ear, nose, throat; FUO, fever of unknown origin; mNGS, molecular next-generation sequencing.

[22, 23]. Performance of mNGS on valve tissue could have a role in the diagnosis of infectious endocarditis (IE). In a prospective study of patients undergoing valve surgery, Zeng et al evaluated 99 patients with proven IE (defined by blood culture and valve culture) and 10 negative controls without IE. In this study, the mNGS sensitivity of valve tissue was 89% and the specificity was 100% when compared with standard of care (blood culture plus valve culture) [25]. While these results are promising, more studies are needed.

Currently, Karius is the only mNGS test commercially available in the United States, and it does not have Food and Drug Administration clearance; this has implications for hospital reimbursement when this test is sent. Although the test is increasingly used in the United States, most literature available to support its use includes case reports, case series, and retrospective and prospective studies and a variety of clinical impact definitions as an endpoint for outcome [6–10, 18–25]. Furthermore, the clinical performance of this testing modality has not been fully studied. Prospective studies are needed to determine the clinical performance of mNGS vs standard-of-care diagnosis (eg, culture-based or well-established nonculture-based diagnostic tools). In particular, negative controls (ie, patients who have neither infection nor suspicion of infection) are needed to better assess for test performance characteristics. Understanding the diagnostic performance of plasma mNGS in different clinical contexts and patient populations will help define the role and use of this assay in clinical practice.

Limitations to this study include its retrospective design, single-center review of mNGS, and small sample size. It is possible that a larger sample or a sample that included a larger proportion of patients with specific types of infections (eg, culture-negative endocarditis) would be more useful in assessing the impact of mNGS in specific situations. Patient selection, timing of mNGS testing, and interprovider ordering practices and test interpretation may have also affected the impact in patient management in our cohort. Last, our study was not designed to determine the performance of mNGS but rather to evaluate local order practices and its clinical impact on patient management.

At this point, mNGS has emerged as a promising tool for the diagnosis of infections, particularly among patients with febrile neutropenia and in the clinical setting of culture-negative endocarditis/endovascular infection. However, further studies are warranted before widespread adoption of this testing is considered standard of care. Given the data suggesting a low clinical impact of plasma mNGS results, the paucity of data regarding clinical performance, and the significant costs, this assay should be a test of last resort until more data and rigorous evaluation of test clinical performance are available.

Notes

Author contributions. K. A. L. and M. H. M. collaborated equally in study design, data collection and analysis, and manuscript preparation.

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