Sequence-based diagnostics and precision medicine in bacterial and viral infections: from bench to bedside

Jonathan Pham[a], *LingHui David Su*[b], *Kimberly E Hanson*[a,c] *and Catherine A Hogan*[b,d]

Purpose of review
Nucleic acid sequence-based organism identification plays an important role in the diagnosis and management of transplant and cancer-associated infectious diseases. Here, we provide a high-level overview of advanced sequencing technologies, discuss test performance, and highlight unmet research needs with a focus on immunocompromised hosts.

Recent findings
Next-generation sequencing (NGS) technologies are powerful tools with a growing role in managing immunocompromised patients with suspected infection. Targeted NGS (tNGS) can identify pathogens directly from patient specimens, especially for mixed samples, and has been used to detect resistance mutations in transplant-related viruses (e.g. CMV). Whole-genome sequencing (WGS) is increasingly used for outbreak investigations and infection control. Metagenomic NGS (mNGS) is useful for hypothesis-free testing and can simultaneously assess pathogens and host response to infection.

Summary
NGS testing increases diagnostic yield relative to standard culture and Sanger sequencing but may be limited by high cost, turnaround times, and detection of unexpected organisms or commensals of uncertain significance. Close collaboration with the clinical microbiology laboratory and infectious diseases is recommended when NGS testing is considered. Additional research is required to understand which immunocompromised patients are most likely to benefit from NGS testing, and when testing should ideally be performed.

Keywords
immunocompromised, next generation, sequencing, transcriptomics

INTRODUCTION

Molecular diagnostic testing has revolutionized the detection and identification of pathogens directly in clinical specimens. Rapid multiplex polymerase chain reaction (PCR) panels are widely available for common infectious syndromes and are now considered integral to the routine care of immunocompromised hosts. These assays are designed to detect the most common community-acquired pathogens and may miss important opportunistic microbes [1]. In addition, current multiplex PCR platforms provide limited antimicrobial resistance (AMR) information. Sequencing can narrow this diagnostic gap by allowing clinicians to evaluate for a wider range of pathogens and AMR markers than is possible with multiplex testing [2].

'First generation' sequencing implies standard Sanger sequencing, whereas next-generation sequencing (NGS) encompasses methods that enable massively parallel or deep sequencing [3▪▪]. NGS assays may be designed to be targeted (tNGS) towards an organism or group of organisms or can be designed as 'shotgun' metagenomic (mNGS) approaches where all of the microbial DNA and/or

[a]Department of Pathology, University of Utah and ARUP Laboratories, Salt Lake City, Utah, USA, [b]British Columbia Centre for Disease Control, Vancouver, British Columbia, Canada, [c]Department of Medicine, Division of Infectious Diseases, University of Utah, Salt Lake City, Utah, USA and [d]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada

Correspondence to Catherine A Hogan, 655 West 12th Avenue, Room 4029, Vancouver, BC, Canada V5Z 4R4.
E-mail: catherine.hogan@bccdc.ca

Curr Opin Infect Dis 2023, 36:228–234
DOI:10.1097/QCO.0000000000000936

KEY POINTS

- Sequencing has various applications, including pathogen detection directly from specimens, antimicrobial resistance gene detection, isolate identification, and strain typing.
- tNGS and mNGS increase diagnostic yield relative to Sanger sequencing and culture, but at the current time, they cannot replace CMT.
- Host gene expression profiling is an emerging approach that may help differentiate infectious inflammation from noninfection as well as differentiate viral from bacterial infections.

RNA is analyzed in a clinical sample without knowing in advance what organism(s) are likely to be present [4]. This is also referred to as 'unbiased' or 'agnostic' sequencing. Whole-genome sequencing (WGS) implies the analysis of the entire genomic content of an organism and is currently most frequently performed using a cultured isolate [5]. A summary of the advantages and disadvantages of sequence-based diagnostics are described in Table 1. This review highlights recent studies of advanced sequencing methods for viruses and bacteria affecting immunocompromised populations.

SANGER SEQUENCING AND TARGETED NEXT GENERATION SEQUENCING

Sanger sequencing is the method most commonly used in clinical microbiology and is considered the operational 'gold standard' [8,9]. The primary genetic target for bacterial Sanger sequencing is the highly conserved *16S* rRNA gene [10,11], which is present in the majority of bacteria [12,13]. The gene also includes nine hypervariable regions (V1–V9) [13–15] (Fig. 1) that confer different levels of discriminatory power among bacteria [16]. PCR of the 16S rRNA gene followed by Sanger sequencing can be performed on cultured isolates or directly from specimens, including normally sterile body fluids as well as fresh or fixed tissue. This approach has demonstrated utility over culture, including the identification of potential pathogens in 10% of culture-negative cases and positive impact on clinical management decisions in 5% of patients [17]. This approach is also useful when only fixed pathology tissue specimens are available as well as for rare and/or fastidious organisms [18,19].

Next-generation sequencing (NGS) is a high-throughput sequencing method with higher resolution and accuracy than Sanger sequencing [20]. Due to the massive parallel reading capacity of NGS, sequencing the entire biome in the sample is

Table 1. Advantages and disadvantages of sequencing-based technologies 'ORIGINAL'

	Common uses in clinical microbiology	Advantages	Disadvantages
Sanger sequencing [2,6,8]	16S rRNA bacterial sequencing Sources: direct specimen (fresh or fixed tissue, normally sterile body fluids), isolates	Most accessible Cheaper than other sequencing technologies	Laborious Limited resolution with polymicrobial samples
Targeted next-generation sequencing (tNGS) [5,19]	16S rRNA bacterial sequencing Antiviral resistance testing Sources: same as 16S	Can differentiate polymicrobial infections High throughput capacity	Expensive Complex
Metagenomic next-generation sequencing (mNGS) [1,3▪▪,6]	Patients with undifferentiated fever or suspected infection without any cause identified by routine testing Sequence all genomic content (DNA and/or RNA) Sources: direct specimen – plasma mcfDNA, CSF, respiratory	Hypothesis-free Able to detect the DNA and RNA of all organisms simultaneously Ability to characterize the host response, if desired	Expensive Complex Requires significant bioinformatics expertise Environmental microorganism and host contamination that requires host depletion or target enrichment strategies May detect clinically insignificant microorganisms (e.g., transient, commensal) Send out testing to a reference laboratory may delay results
Whole genome sequencing (WGS) [3▪▪,7]	Outbreak investigation Longitudinal follow-up for differentiation of new vs. chronic infection Sources: isolates, direct specimen	Can differentiate polymicrobial infections High throughput capacity	Expensive Complex Requires significant bioinformatics and taxonomic expertise

CSF, cerebrospinal fluid; DNA, deoxyribonucleic acid; RNA, ribonucleic acid.

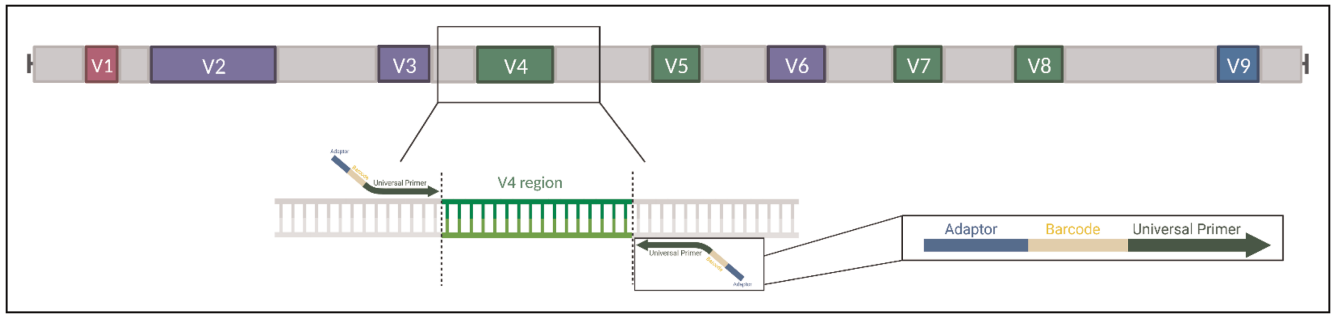

FIGURE 1. Map of the bacterial 16S rRNA genome regions 'ORIGINAL'.

possible; however, targeted NGS (tNGS) is currently more commonly used for clinical purposes [21]. For tNGS, a metataxonomic target, such as the 16 s rRNA gene, is enriched before NGS to maximize the accuracy and efficacy of the assay [22].

As with Sanger sequencing, tNGS can be performed on an extensive range of samples, including normally sterile body fluids and biopsy tissue [17,23–25]. A recent retrospective study demonstrated that the average NGS positivity rate was 87% higher compared to Sanger sequencing, which was most pronounced in lung tissue (+300%), and least pronounced in eye fluid (no change) [23]. This observation reflects the complexity of the lung microbiome and supports the superiority of NGS for resolving polymicrobial samples. In addition, the clinical sensitivity of NGS was 11% higher than conventional culture overall and 22% higher in patients on antibiotic therapy [23]. Certain clinical syndromes may be better suited for the NGS testing approach. For example, 16S rRNA gene sequencing on heart valves was shown to successfully identify the etiological agent in 75% of patients with culture-negative infective endocarditis [26].

Another important clinical application of Sanger and tNGS is the assessment of antiviral drug resistance. Using the cytomegalovirus (CMV) genome as an example, the UL97 kinase, UL54 DNA polymerase, UL27 early gene, and UL56 partial terminase complex can be amplified and sequenced to look for drug resistance mutations [27–29]. A potential benefit of tNGS for the clinical laboratory is the ability to analyze multiple resistance genes in a single reaction. Additionally, tNGS can detect low-frequency variants making up as little as 1-2% of the total population, while Sanger sequencing generally requires variant frequencies on the order of 10–40% for detection. A recent study demonstrated that tNGS could detect minority UL97 and UL54 variants, and case reports have shown earlier detection of emerging resistance using NGS [30,31]. Whether detecting CMV drug-resistant variants earlier or at frequencies <10% improves treatment outcomes, however, has not been established and requires additional study.

METAGENOMIC NEXT-GENERATION SEQUENCING

Unbiased sequencing approaches are particularly attractive for immunocompromised patients when the differential diagnosis is broad and clinical suspicion for infection is high [32]. Metagenomic NGS (mNGS) testing may be considered for syndromes where the yield of conventional microbiological testing (CMT) is expected to be low (e.g. sepsis, meningitis/encephalitis, and opportunistic pneumonia) [33▪,34–36]. Currently, mNGS is available through several reference laboratories [e.g., Karius, Redwood City, California, USA for plasma; the University of California San Francisco, California, USA for cerebrospinal fluid (CSF)] and may be performed in-house in some larger academic centers [1]. Additionally, off-the-shelf kits with associated bioinformatic tools such as the Respiratory Pathogen ID/AMR Enrichment Kit (RPIP, Illumina, San Diego, California, USA) are commercially available [32].

The Karius test has been used to help diagnose undifferentiated febrile illness, pneumonia, and culture-negative endovascular infection, or to provide an alternative diagnostic method when invasive sampling is contraindicated [36–39]. The Karius test detects microbial cell-free DNA (mcfDNA) in plasma. mcfDNA represents circulating short fragments of DNA, which can originate from an endovascular infection, deep-seated focal infection, or translocation of commensal microorganisms. Quantitative results are provided as molecules per microliter, with microorganisms reported if the plasma mcfDNA exceeds an organism-specific threshold [40]. Pertinent mcfDNA studies that include immunocompromised patients are summarized in Table 2. Overall, the clinical impact of Karius testing has varied widely across studies (range 7–80%) [7,41▪,42,43▪,44–47], which may be partly related to the absence of standardized impact definitions.

Table 2. Karius studies assessing clinical impact in immunocompromised patients 'ORIGINAL'

Study	Patient population	Study design	Collection details	Approval required	Primary outcome	Summary
Vissichelli *et al.* (2023) [41▪]	Adult patients with a suspected infection (92% IC)	Retrospective chart review	N/A, at clinician discretion	Yes	Clinical impact as defined by the study authors	Positive impact (52.8%), negative impact (2.8%), no impact (44.4%) 58% of KT detected 1–5 organisms
Benamu *et al.* (2022) [42]	Adult patients with acute leukemia and neutropenia. Enrolled during their first febrile neutropenia episode	Prospective observational	Blood drawn within 24 h of fever and every 2–3 days until resolution of neutropenia	N/A, enrolled by meeting study inclusion criteria	Comparison to composite reference standard (clinical, CMT, radiographic)	Potential to optimize antimicrobials in 47% of patients Sensitivity 85%, specificity 100%
Shishido *et al.* (2022) [43▪]	Adult patients with a suspected infection (56% IC)	Retrospective chart review	N/A, at clinician discretion	Yes	Clinical impact as defined by the study authors	Positive impact (42.5%), negative impact (2.5%), no impact (55.0%) Positive impact when sent in SOT recipients (71.4%)
Niles *et al.* (2022) [44]	Pediatric patients with a suspected infection (76% IC)	Retrospective chart review	N/A, at clinician discretion	No	Clinical impact as defined by the study authors	Positive impact (12.4%), negative impact (5.3%), no impact (82.2%) A plausible pathogen was identified more often in IC patients (56 vs. 30%; $P=0.006$)
Hogan *et al.* (2021) [45]	All patients with a suspected infection (65% IC)	Retrospective chart review	Within 1 week of CMT	Variable across the different institutions and time periods	Clinical impact as defined by the clinical team	Positive impact (7.3%), negative impact (3.7%), no impact (86.6%)
Yu *et al.* (2021) [46]	Adult patients with hematologic malignancy or HSCT	Retrospective chart review	N/A, at clinician discretion	Yes	Clinical impact as defined by the clinical team	Positive impact in 59% of patients (28% escalation, 31% de-escalation)
Goggin *et al.* (2020) [47]	<25 years old with relapsed or refractory cancer	Prospective cohort study	Within 1 week of bacteremia	N/A, enrolled by meeting study inclusion criteria	Comparison to blood cultures	In the 3 days prior to bacteremia, KT had 75% sensitivity In the week prior to or after bacteremia, KT had 82% specificity
Rossoff *et al.* (2019) [48]	Pediatric patients with suspected infection (76% IC)	Retrospective chart review	N/A, at clinician discretion	94% ordered by ID service	Comparison to CMT and clinically relevant pathogens detected	80% of KT were clinically relevant Among the immunocompromised: sensitivity 93%, specificity 59%

CMT, conventional microbiological tests; HSCT, hematopoietic stem cell transplant; IC, immunocompromised; ID, infectious disease; KT, Karius tests; N/A, not available.

Factors associated with positive clinical impact included immunocompromised status, infectious diseases/stewardship-led approval and support for interpreting results [49]. Prospective studies are needed to generate evidence to move the field forward, including recent efforts such as the PICKUP study, which was performed in immunocompromised patients with pneumonia [50].

The clinical utility of mNGS for diagnosing CNS infections has also been assessed. In one of the largest multicenter studies performed to date, Wilson *et al.* [51] enrolled 204 adult and pediatric patients with suspected CNS infection. CSF mNGS testing detected 32 infections (62.5% viruses, 18.8% bacteria). Overall, 8 of 204 (3.9%) of total tests and 8 of 13 (61.5%) of tests that detected a pathogen exclusively by NGS had a clinical impact. Furthermore, 26 infections were diagnosed with CMT but missed by NGS. These were categorized as: diagnoses made by serologic testing rather than direct evidence of the pathogen (e.g. West Nile virus), diagnoses by testing sites other than the CSF, compartmentalized brain abscess, or low pathogen concentration in the CSF [51].

mNGS is also an attractive option for pneumonia diagnosis. Interpreting mNGS results from respiratory specimens, however, is complicated by the complexity of the pulmonary microbiome [52].

Assessments of microbiome diversity may aid in separating pathogens from colonizers of the lung. For example, Zinter *et al.* [53] evaluated 34 immunocompromised children and found that pathogenic bacteria were more likely than commensal bacteria to have a higher abundance and decreased alpha-diversity. Among 30 immunocompromised patients who underwent a bronchoalveolar lavage (BAL), mNGS testing using the RPIP provided a microbiologic diagnosis in 58% compared with 35% for CMT [54]. Under hypothetical assumptions, increased detections would have led to a probable change in antimicrobials in 3%, possible change in 27%, and de-escalation of antimicrobials in 43% of patients [54].

Exactly where mNGS fits in current diagnostic algorithms remains an area of debate. Considering the cost of testing, it is reasonable to store collected samples for mNGS early in the evaluation until the results of CMT – including multiplex PCRs and/or 16S rRNA testing – are available. However, this strategy may delay diagnosis for some patients, and earlier testing could be considered for the critically ill. Additionally, as the positive predictive and negative predictive values of mNGS have yet to be fully characterized, results must be interpreted in the context of clinical, radiographic, and CMT findings.

WHOLE GENOME SEQUENCING

Multidrug-resistant (MDR) organisms are a common cause of healthcare-associated infection that may disproportionately affect immunocompromised hosts [55,56]. For example, MDR Enterobacterales (MDR-E) colonization is particularly problematic in liver transplant patients, where the infection rate is greater than 20% [57,58]. The most prevalent resistance genes, including extended-spectrum β-lactamases (ESBLs) and *Klebsiella pneumoniae* carbapenemase (*KPC*) gene, are spread by horizontal transfer, which can lead to nosocomial transmission [59,60]. Geographic transmission history can be extrapolated using WGS data with higher resolution than is possible with other methods, and WGS-based surveillance has been investigated with promising results. One study documented cryptic transmission of new MDR-E lineage using weekly perirectal swab surveillance cultures [59]. In another study, routine WGS on bacterial culture isolates (*Staphylococcus aureus*, enterococci, *Acinetobacter baumannii*, Enterobacterales) from hospitalized patients could identify the interhospital spread of drug resistance genes, as well as the sequence types that arose in the community [61]. Detailed transmission information could then help inform infection control measures and the need for continued surveillance.

WGS shows promise for use at the individual level as well. Recently, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has exposed the increased risk of severe disease in immunocompromised hosts [62,63]. Such individuals may shed detectable viruses for prolonged periods, complicating the distinction between chronic infection and reinfection. WGS performed on longitudinal samples from the same individual can resolve this, thus helping to inform therapeutic decision-making [64]. In addition to individual-level applications, WGS-based surveillance to monitor for the emergence of new mutations and variants of concern can directly inform public health efforts for SARS-CoV-2 prevention and control.

HOST RESPONSE AND TRANSCRIPTOMICS

Pathogen-directed molecular diagnostics have greatly enhanced organism detection rates for infectious diseases. However, targeted approaches may not detect all potential pathogens, and 'shotgun' strategies may identify organisms of uncertain clinical significance. Furthermore, none of these approaches reliably separates invasive organisms from colonizers, especially when testing from non-sterile sites. Transcriptomic profiling of the host immune response is a complementary method that may assist in identifying patients with an inflammatory response because of infection (vs. a non-infectious process), and when performed in conjunction with organism identification, could help separate commensals from true pathogens. The premise is that host gene expression is predictably conserved and unique to different types of infection.

Studies have been performed assessing the accuracy of whole blood transcriptomic signatures to differentiate viral vs. bacterial infections as well to help diagnose active tuberculosis [65,66]. The accuracy of these signatures has varied widely due in part to heterogeneity in the number of genes included in the signature, differences in the population from which the signature was derived, and/or the reference method used for comparison. In general, viral infection is easier to differentiate than bacterial infection (overall accuracy of 84 vs. 79%, respectively), and host gene expression classifiers may perform better in adults than younger children for both bacterial and viral infections [overall accuracy of 73 vs. 82% ($P = 0.001$] and 80 vs. 88%, respectively ($P = 0.001$)] [65].

There is theoretical concern that immunosuppression could limit the discriminatory power of immune response profiling. Although relatively few studies performed to date included immunocompromised hosts, performance for the detection of bacterial infection was lower in an immunocompromised vs. nonimmunocompromised cohort [overall accuracy of 73.9 vs. 84.6% ($P = 0.4$), respectively] [67], and the blood transcriptome was not suitable for determining the cause of febrile neutropenia in children because of too few circulating immune cells for reliable gene expression analysis [68]. In contrast, HIV infection does not appear to reduce the sensitivity of TB signatures [69].

Simultaneous characterization of pathogens, microbial diversity, and the host transcriptome from the same sample may be the way of the future. Early proof-of-concept studies suggest that integrated host and microbe mNGS profiling using BAL, for example, improves diagnostic predictive value for lower respiratory tract infection beyond what is possible with CMT [70], and this will require expanded investigation across other clinical syndromes.

CONCLUSION

Sequence-based testing is an important diagnostic adjunct to consider for immunocompromised patients, especially when CMT is negative. When NGS testing is considered, it should be done in collaboration with the microbiology laboratory and infectious disease consultation [71]. In the future, it is expected that tNGS and WGS will become less expensive, more automated, and show improved analytic performance, which will facilitate their more widespread adoption in clinical laboratories. Additional research is needed to determine the positive predictive and negative predictive value of mNGS testing, and to understand the highest yield clinical syndromes, optimal timing of mNGS and its added value relative to CMT alone [72]. Prospective studies that include significant numbers of immunocompromised should be designed to measure patient-level outcomes as assessed by standardized criteria, including the impact of adjunctive NGS on antimicrobial use, potential for averting the need for invasive sampling, length-of-hospital stay, and mortality, as recently suggested [73]. Robust assessments of test performance and clinical utility will be key to justify widespread adoption outside of reference, academic, and public health laboratories [74].

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Chiu CY, Miller SA. Clinical metagenomics. Nat Rev Genet 2019; 20:341–355.
2. Heather JM, Chain B. The sequence of sequencers: the history of sequencing DNA. Genomics 2016; 107:1–8.
3. ■■ Hilt EE, Ferrieri P. Next generation and other sequencing technologies in diagnostic microbiology and infectious diseases. Genes (Basel) 2022; 13:1566. Provides a foundation for understanding the evolution of sequencing and the differences between sequencing generations. This also provides an overview about Sanger sequencing, targeted NGS, metagenomic NGS, and WGS. Delves into the clinical applications for each sequencing method.
4. Wright WF, Simner PJ, Carroll KC, Auwaerter PG. Progress report: next-generation sequencing, multiplex polymerase chain reaction, and broad-range molecular assays as diagnostic tools for fever of unknown origin investigations in adults. Clin Infect Dis 2022; 74:924–932.
5. Hu T, Chitnis N, Monos D, Dinh A. Next-generation sequencing technologies: an overview. Hum Immunol 2021; 82:801–811.
6. Petersen LM, Martin IW, Moschetti WE, *et al.* Third-generation sequencing in the clinical laboratory: exploring the advantages and challenges of nanopore sequencing. J Clin Microbiol 2019; 58:e01315–e1319.
7. Simar SR, Hanson BM, Arias CA. Techniques in bacterial strain typing: past, present, and future. Curr Opin Infect Dis 2021; 34:339–345.
8. Chen L, Cai Y, Zhou G, *et al.* Rapid sanger sequencing of the 16S rRNA gene for identification of some common pathogens. PLoS One 2014; 9:e88886.
9. Arteche-López A, Ávila-Fernández A, Romero R, *et al.* Sanger sequencing is no longer always necessary based on a single-center validation of 1109 NGS variants in 825 clinical exomes. Sci Rep 2021; 11:5697.
10. Woese CR. Bacterial evolution. Microbiol Rev 1987; 51:221–271.
11. Clarridge JE. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clin Microbiol Rev 2004; 17:840–862.
12. Wang X, Jordan IK, Mayer LW. Chapter 29 - a phylogenetic perspective on molecular epidemiology. In: Tang YW, Sussman M, Liu D, Poxton I, Schwartzman J, editors. Mol Med Microbiol, 2nd ed Boston: Academic Press; 2015. pp. 517–536.
13. Vargas-Albores F, Ortiz-Suárez LE, Villalpando-Canchola E, Martínez-Porchas M. Size-variable zone in V3 region of 16S rRNA. RNA Biol 2017; 14:1514–1521.
14. Del Chierico F, Ancora M, Marcacci M, *et al.* Bacterial pangenomics. 1st ed. New York: Springer; 2014.
15. Raina V, Nayak T, Ray L, *et al.* Chapter 9 - a polyphasic taxonomic approach for designation and description of novel microbial species. In: Das S, Dash HR, editors. Microbial diversity in the genomic era. Boston: Academic Press; 2019. pp. 137–152.
16. Chakravorty S, Helb D, Burday M, *et al.* A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. J Microbiol Methods 2007; 69:330–339.
17. Fida M, Khalil S, Abu Saleh O, *et al.* Diagnostic value of 16S ribosomal RNA gene polymerase chain reaction/sanger sequencing in clinical practice. Clin Infect Dis 2021; 73:961–968.
18. Fenollar F, Raoult D. Molecular genetic methods for the diagnosis of fastidious microorganisms. APMIS 2004; 112:785–807.
19. Imrit K, Goldfischer M, Wang J, *et al.* Identification of bacteria in formalin-fixed, paraffin-embedded heart valve tissue via 16S rRNA gene nucleotide sequencing. J Clin Microbiol 2006; 44:2609–2611.
20. Peker N, Garcia-Croes S, Dijkhuizen B, *et al.* A comparison of three different bioinformatics analyses of the 16S-23S rRNA encoding region for bacterial identification. Front Microbiol 2019; 10:620.
21. Santibáñez P, García-García C, Portillo A, *et al.* What does 16S rRNA gene-targeted next generation sequencing contribute to the study of infective endocarditis in heart-valve tissue? Pathogens 2021; 11:34.
22. Singh RR. Target enrichment approaches for next-generation sequencing applications in oncology. Diagnostics 2022; 12:1539.
23. Flurin L, Wolf MJ, Mutchler MM, *et al.* Targeted metagenomic sequencing-based approach applied to 2146 tissue and body fluid samples in routine clinical practice. Clin Infect Dis 2022; 75:1800–1808.

24. Azad MA, Wolf MJ, Strasburg AP, *et al.* Comparison of the BioFire joint infection panel to 16S ribosomal RNA gene-based targeted metagenomic sequencing for testing synovial fluid from patients with knee arthroplasty failure. J Clin Microbiol 2022; 60:e0112622.
25. Flurin L, Wolf MJ, Fisher CR, *et al.* Pathogen detection in infective endocarditis using targeted metagenomics on whole blood and plasma: a prospective pilot study. J Clin Microbiol 2022; 60:e0062122.
26. Marsch G, Orszag P, Mashaqi B, *et al.* Antibiotic therapy following polymerase chain reaction diagnosis of infective endocarditis: a single centre experience. Interact Cardiovasc Thorac Surg 2015; 20:589–593.
27. Bravo MS, Tilloy V, Plault N, *et al.* Assessment of UL56 mutations before letermovir therapy in refractory cytomegalovirus transplant recipients. Microbiol Spectr 2022; 10:e0019122.
28. Hall Sedlak R, Castor J, Butler-Wu SM, *et al.* Rapid detection of human cytomegalovirus UL97 and UL54 mutations directly from patient samples. J Clin Microbiol 2013; 51:2354–2359.
29. Chou S. Advances in the genotypic diagnosis of cytomegalovirus antiviral drug resistance. Antiviral Res 2020; 176:104711.
30. Sahoo MK, Lefterova MI, Yamamoto F, *et al.* Detection of cytomegalovirus drug resistance mutations by next-generation sequencing. J Clin Microbiol 2013; 51:3700–3710.
31. Chorlton SD, Ritchie G, Lawson T, *et al.* Next-generation sequencing for cytomegalovirus antiviral resistance genotyping in a clinical virology laboratory. Antiviral Res 2021; 192:105123.
32. Gaston DC, Miller HB, Fissel JA, *et al.* Evaluation of metagenomic and targeted next-generation sequencing workflows for detection of respiratory pathogens from bronchoalveolar lavage fluid specimens. J Clin Microbiol 2022; 60:e00526–e622.
33. ■ Wilson MR, Tyler KL. The current status of next-generation sequencing for diagnosis of central nervous system infections. JAMA Neurol 2022; 79:1095.
This viewpoint describes the available data for the application of metagenomic NGS on CSF. It provides a strong framework for understanding the benefits and limitations of this approach for diagnosing CNS infections.
34. Ramachandran PS, Wilson MR. Metagenomics for neurological infections — expanding our imagination. Nat Rev Neurol 2020; 16:547–556.
35. Graff K, Dominguez SR, Messacar K. Metagenomic next-generation sequencing for diagnosis of pediatric meningitis and encephalitis: a review. J Pediatr Infect Dis Soc 2021; 10(Suppl 4):S78–S87.
36. Hill JA, Dalai SC, Hong DK, *et al.* Liquid biopsy for invasive mold infections in hematopoietic cell transplant recipients with pneumonia through next-generation sequencing of microbial cell-free DNA in plasma. Clin Infect Dis 2021; 73:e3876–e3883.
37. Ranganath N, Khodadadi RB, Abu Saleh OM. Karius with a Q: role for microbial cell-free DNA next-generation sequencing in diagnosis of acute Q fever. Open Forum Infect Dis 2023; 10:ofac666.
38. Shah P, Ruffin F, Seng H, *et al.* 156. Direct detection and quantification of bacterial cell-free DNA in patients with infective endocarditis (IE) using the Karius Plasma Next Generation Sequencing (NGS) test. Open Forum Infect Dis 2018; 5(Suppl 1):S12–S112.
39. To RK, Ramchandar N, Gupta A, *et al.* Use of plasma metagenomic next-generation sequencing for pathogen identification in pediatric endocarditis. Pediatr Infect Dis J 2021; 40:486–488.
40. Morales M. The next big thing? Next-generation sequencing of microbial cell-free DNA using the Karius Test. Clin Microbiol Newslett 2021; 43:69–79.
41. ■ Vissichelli NC, Morales MK, Kolipakkam B, *et al.* Cell-free next-generation sequencing impacts diagnosis and antimicrobial therapy in immunocompromised hosts: A retrospective study. Transplant Infect Dis 2023; 25:e13954.
In this article, 92% of patients were immunocompromised. Every plasma mcfDNA test was approved and interpreted by the Infectious Diseases service to maximize actionable results. The criteria for positive, negative, and absence of clinical impact are clearly defined.
42. Benamu E, Gajurel K, Anderson JN, *et al.* Plasma microbial cell-free DNA next-generation sequencing in the diagnosis and management of febrile neutropenia. Clin Infect Dis 2022; 74:1659–1668.
43. ■ Shishido AA, Noe M, Saharia K, Luethy P. Clinical impact of a metagenomic microbial plasma cell-free DNA next-generation sequencing assay on treatment decisions: a single-center retrospective study. BMC Infect Dis 2022; 22:372.
This article analyzed multiple variables for an association with positive clinical impact. 71.4% of tests sent for solid organ transplant recipients have a positive clinical impact, compared with 43% overall.
44. Niles DT, Revell PA, Ruderfer D, *et al.* Clinical impact of plasma metagenomic next-generation sequencing in a large pediatric cohort. Pediatr Infect Dis J 2022; 41:166–171.
45. 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; 72:239–245.
46. Yu J, Diaz JD, Goldstein SC, *et al.* Impact of next-generation sequencing cell-free pathogen DNA test on antimicrobial management in adults with hematological malignancies and transplant recipients with suspected infections. Transplant Cell Ther 2021; 27:500.e1–500.e6.
47. Goggin KP, Gonzalez-Pena V, Inaba Y, *et al.* Evaluation of plasma microbial cell-free DNA sequencing to predict bloodstream infection in pediatric patients with relapsed or refractory cancer. JAMA Oncol 2020; 6:552.
48. Rossoff J, Chaudhury S, Soneji M, *et al.* Noninvasive diagnosis of infection using plasma next-generation sequencing: a single-center experience. Open Forum Infect Dis 2019; 6:ofz327.
49. Parrish NF, Gaston DC. Metagenomics in infectious disease diagnostics: toward best-use practices to optimize actionable results. Transplant Infectious Dis 2023; 25:e13959.
50. Bergin SP, Chemaly RF, Duttagupta R, *et al.* 544. PICKUP: pneumonia in the immunocompromised – use of the Karius test for detection of undiagnosed pathogens. Open Forum Infect Dis 2022; 9(Suppl_2):ofac492.597.
51. Wilson MR, Sample HA, Zorn KC, *et al.* Clinical metagenomic sequencing for diagnosis of meningitis and encephalitis. N Engl J Med 2019; 380:2327–2340.
52. Kitsios GD. Translating lung microbiome profiles into the next-generation diagnostic gold standard for pneumonia: a clinical investigator's perspective. mSystems 2018; 3:e00153–e217.
53. Zinter MS, Dvorak CC, Mayday MY, *et al.* Pulmonary metagenomic sequencing suggests missed infections in immunocompromised children. Clin Infect Dis 2019; 68:1847–1855.
54. Azar MM, Schlaberg R, Malinis MF, *et al.* Added diagnostic utility of clinical metagenomics for the diagnosis of pneumonia in immunocompromised adults. Chest 2021; 159:1356–1371.
55. Jernigan JA, Hatfield KM, Wolford H, *et al.* Multidrug-resistant bacterial infections in U.S. hospitalized patients, 2012–2017. New Engl J Med 2020; 382:1309–1319.
56. Sikora A, Zahra F. Nosocomial infections. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022.
57. Lübbert C, Becker-Rux D, Rodloff AC, *et al.* Colonization of liver transplant recipients with KPC-producing Klebsiella pneumoniae is associated with high infection rates and excess mortality: a case-control analysis. Infection 2014; 42:309–316.
58. Bert F, Larroque B, Paugam-Burtz C, *et al.* Pretransplant fecal carriage of extended-spectrum β-lactamase-producing enterobacteriaceae and infection after liver transplant, France. Emerg Infect Dis 2012; 18:908–916.
59. Hong Nguyen M, Shields RK, Chen L, *et al.* Molecular epidemiology, natural history, and long-term outcomes of multidrug-resistant enterobacterales colonization and infections among solid organ transplant recipients. ClinInfect Dis 2021; 74:395–406.
60. van Duin D, Doi Y. The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence 2016; 8:460–469.
61. Forde BM, Bergh H, Cuddihy T, *et al.* Clinical implementation of routine whole-genome sequencing for hospital infection control of multidrug resistant pathogens. Clin Infect Dis 2023; 76:e1277–e1284.
62. Fung M, Babik JM. COVID-19 in immunocompromised hosts: what we know so far. Clin Infect Dis 2021; 72:340–350.
63. Haidar G, Mellors JW. Improving the outcomes of immunocompromised patients with coronavirus disease 2019. Clin Infect Dis 2021; 73:e1397–e1401.
64. Snell LB, Alcolea-Medina A, Charalampous T, *et al.* Real-time whole genome sequencing to guide patient-tailored therapy of severe acute respiratory syndrome coronavirus 2 infection. Clin Infect Dis 2022; 76:ciac864.
65. Bodkin N, Ross M, McClain MT, *et al.* Systematic comparison of published host gene expression signatures for bacterial/viral discrimination. Genome Med 2022; 14:18.
66. Warsinske H, Vashisht R, Khatri P. Host-response-based gene signatures for tuberculosis diagnosis: a systematic comparison of 16 signatures. PLoS Med 2019; 16:e1002786.
67. Mahle RE, Suchindran S, Henao R, *et al.* Validation of a host gene expression test for bacterial/viral discrimination in immunocompromised hosts. Clin Infect Dis 2021; 73:605–613.
68. Wahlund M, Sinha I, Broliden K, *et al.* The feasibility of host transcriptome profiling as a diagnostic tool for microbial etiology in childhood cancer patients with febrile neutropenia. IJMS 2020; 21:5305.
69. ■ Mendelsohn SC, Mbandi SK, Fiore-Gartland A, *et al.* Prospective multicentre head-to-head validation of host blood transcriptomic biomarkers for pulmonary tuberculosis by real-time PCR. Commun Med (Lond) 2022; 2:26.
70. Langelier C, Kalantar KL, Moazed F, *et al.* Integrating host response and unbiased microbe detection for lower respiratory tract infection diagnosis in critically ill adults. Proc Natl Acad Sci USA 2018; 115:E12353–E12362.
71. Dickerson JA, Fletcher AH, Procop G, *et al.* Transforming laboratory utilization review into laboratory stewardship: guidelines by the PLUGS National Committee for Laboratory Stewardship. J Appl Labor Med 2017; 2:259–268.
72. Gaston DC. Clinical metagenomics for infectious diseases: progress toward operational value. J Clin Microbiol 2023; 61:e01267–e1322.
73. Hogan CA, Miller S, Piantadosi A, *et al.* Which trial do we need? plasma metagenomic next-generation sequencing to diagnose infections in patients with hematological malignancies and febrile neutropenia: proposal for a randomized-controlled trial. Clin Microbiol Infect 2023. S1198-743X(23)00248-3.
74. Lewandrowski K. Managing utilization of new diagnostic tests. Clin Leadersh Manag Rev 2003; 17:318–324.