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BRIEF REPORT

Noninvasive Diagnosis of Infection Using Plasma Next-Generation Sequencing: A Single-Center Experience

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Pediatric patients frequently present with illnesses strongly suggesting infection, but without a clearly identified etiology. Our center has recently added a commercially available plasma metagenomic sequencing assay to its available diagnostic testing. Our experience with the first 100 tests suggests that this technology has good clinical performance with >90% sensitivity.

Keywords. bacterial infection; metagenomic next-generation sequencing; viral infection.

Next-generation sequencing (NGS) technology has revolutionized genomic research throughout the biological sciences [1], with the potential to impact patient care through improved diagnostic sensitivity and precise therapeutic targeting [2]. Since the first bacterial genome was sequenced in 1995, molecular diagnostic approaches have increasingly been used in the diagnosis and surveillance of infectious diseases [3, 4]. Conventional diagnostic techniques in microbiology are limited by the time required and difficulty of growing certain organisms in culture and the need for invasive procedures when an infection is confined to a particular anatomic location [5]. In contrast, plasma NGS generates sequences of circulating cell-free DNA, which can be compared with known genomic databases of bacterial, viral, fungal, and parasitic pathogens for expedited and noninvasive identification [6]. Given the limitations of conventional diagnostic techniques and potential for multiple concurrent infections in immunocompromised (IC) patients, there is a need for noninvasive testing methods that rapidly and accurately identify pathogen(s) to allow for implementation of appropriate antimicrobial therapy. Plasma NGS for the diagnosis of infectious pathogens became available for clinical use in our institution in 2016 and has been applied in selected patients with infections of unknown etiology. The purpose of this study was to examine the diagnostic capabilities of plasma NGS vs conventional techniques for pediatric infections.

METHODS

This study was approved by the Institutional Review Board of Ann & Robert H. Lurie Children's Hospital of Chicago. We retrospectively reviewed data from patients for whom NGS testing of plasma for infectious pathogens was sent for clinical purposes at our institution from December 2016 through August 2018. Plasma samples were analyzed using a commercially available NGS assay at a CLIA-certified laboratory (Karius, Redwood City, CA), which sequences cell-free DNA, reporting bacteria, fungi, DNA viruses, and parasites present at levels greater than a predefined threshold after removal of human sequences [7]. Plasma NGS testing was ordered at the request of infectious diseases specialists in 94% of cases, typically when conventional testing had not identified a specific pathogen or with concern for concurrent infections. Although there were no institutional restrictions to ordering the test, an infectious diseases consultant was involved in interpretation in all cases. We reviewed the indication for NGS testing, turnaround time for NGS results, other infectious diagnostic tests utilized, and physician interpretation of results. Patients were classified as IC if they were receiving any immunosuppressive therapy, were post-transplant, or had an underlying medical condition that compromised their immune system.

Any organism identified by either NGS or conventional infectious diagnostic testing was retrospectively classified as "clinically relevant" if the treating physician made management decisions based on the result; for cases in which this was unclear from the medical records, clinical relevance was determined by expert opinions from 2 pediatric infectious diseases (ID) physicians not directly involved in the patient's care (M.S. and S.J.P.), with the opinion of a third ID physician (W.J.M.) used to resolve any discrepant opinions. Patients were classified as positive for an infectious disease if conventional and/or NGS testing revealed a clinically relevant pathogen. Sensitivity and specificity were then calculated for NGS and conventional testing methods, comparing the clinical relevance of test results with the patient classification of positive (or not) for a specific infection; for example, a test was considered a true positive when it identified the "clinically relevant" infectious pathogen or a false

Received 8 May 2019; editorial decision 9 July 2019; accepted 11 July 2019.

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DOI: 10.1093/ofid/ofz327

negative if it did not identify a pathogen that was deemed "clinically relevant" based on another test. An NGS test could therefore be classified as a true positive even if it identified other (not clinically relevant) pathogens at the same time as a clinically relevant one, and all conventional tests were grouped together in the analysis so that if 1 were negative but another positive for a clinically relevant pathogen, the group of tests would be considered a true positive. This method of evaluating test characteristics is similar to a method used in a prior study involving NGS testing [8]. The significance level was set at 0.05. All analyses were conducted using SAS 9.4 (Cary, NC).

RESULTS

One hundred NGS tests were sent on 79 patients between December 2016 and August 2018. The patients had a median age (range) of 11 (0.5–24) years, with 51% females and 76% IC. The most common underlying diagnoses among IC patients were hematologic malignancies (27% of the IC patients), primary immune deficiencies (22%), and post-hematopoietic cell transplant (HCT; 17%); most non-IC patients had no underlying diagnosis. Concern for a fungal (or other atypical) infection was the most common indication for testing (55% of all tests), followed by acute fever/sepsis (22%), prolonged/recurrent fever (18%), recurrent lymphadenopathy (4%), and evaluation for stopping antifungals (1%). Repeat tests were sent on 21 occasions involving 15 patients. Results of NGS testing were available within 48 hours of sample receipt at the testing laboratory 86% of the time.

Seventy of the 100 NGS tests sent were positive for at least 1 organism above the assay threshold (Supplementary Table 1); 37 tests identified a single organism, 15 identified 2 organisms, 5 identified 3 organisms, and 4 or more organisms were identified in 13 samples. Bacterial organisms accounted for the majority of positive results, with 52 tests identifying at least 1 bacterium, 25 at least 1 virus, and 18 at least 1 fungus. Fifty-six (80%) of the positive NGS tests were assessed to be clinically relevant, 14 of which identified clinically relevant organism(s) when conventional testing modalities were nondiagnostic. The remaining 14 (20%) positive NGS tests showed organisms thought to be not clinically relevant. The blood stream and respiratory tract were the most common clinical sites of infection; however, plasma NGS was also able to identify infections clinically isolated to the skin, bone, internal hardware, urinary tract, and cerebrospinal fluid. There were 6 cases in which conventional infectious testing was diagnostic when NGS was not.

To assess the diagnostic capabilities of NGS vs conventional microbiological testing, we compared results from each testing modality to the patient's infectious disease classification (as described above). The sensitivity and specificity of plasma NGS testing for diagnosis of a clinically relevant infection was 92% and 64%, respectively, vs 77% and 89%, respectively, for conventional microbiological testing ($P < .01$ for both comparisons) (Table 1). Among IC patients, the sensitivity and specificity of NGS testing were 93% and 59%, respectively, vs 76% and 92%, respectively, for conventional testing ($P < .01$ for both comparisons).

Fifty-four invasive procedures were performed in 42 patients to identify causative infectious pathogens. These procedures included bronchoalveolar lavage (8), pleurocentesis (4), paracentesis (3), biopsy of lung (14), skin (6), bone (4), lymph node (5), spleen (4), liver (1), colon (1), parotid gland (1), or parapharyngeal mass (1), or aspirate of gall bladder (1) or sinus (1). Fifteen procedures were performed in patients ultimately not diagnosed with a specific infection. From the 39 procedures performed in patients with a diagnosed infection, a microbiological diagnosis was made by NGS testing in 87% vs 67% with conventional testing on invasively obtained specimens.

DISCUSSION

Our single-center experience with the use of plasma NGS testing for detecting infectious pathogens in a diverse cohort of

Table 1. NGS or Conventional Microbiologic Testing Results vs Infectious Disease Diagnosis (Positive or Negative)

All Patients[a]					
NGS Testing	Infectious Disease +	Infectious Disease -	Conventional Testing	Infectious Disease +	Infectious Disease -
Test +	56	14	Test +	48	4
Test -	5	25	Test -	14	34
Immunocompromised Patients[a]					
NGS Testing	Infectious Disease +	Infectious Disease -	Conventional Testing	Infectious Disease +	Infectious Disease -
Test +	49	11	Test +	41	2
Test -	4	16	Test -	13	24
Immunocompetent Patients					
NGS Testing	Infectious Disease +	Infectious Disease -	Conventional Testing	Infectious Disease +	Infectious Disease -
Test +	7	3	Test +	7	2
Test -	1	9	Test -	1	10

Abbreviation: NGS, next-generation sequencing.

[a]The discrepancy in number of patients classified as having an infectious disease when evaluating NGS vs conventional testing is related to 1 patient's NGS testing results showing only non–clinically relevant organisms (false positive) vs a clinically relevant pathogen seen on conventional testing (true positive).

pediatric patients found a high sensitivity, with only 6% of tests sent failing to detect a clinically relevant organism identified by a conventional assay. NGS testing had a higher yield in IC patients, in whom 61% of tests sent were positive for a clinically relevant pathogen vs 35% of those sent in non-IC patients; this may relate to the higher likelihood of infection in this population. Importantly, results were available in a timely manner to treating teams.

Plasma NGS testing can detect pathogen DNA in the bloodstream even when an infection is confined to a particular anatomic location. In our cohort, 34 invasive procedures might have been avoided based on positive NGS results. Additionally, the diagnostic yield of plasma NGS testing was higher than that of invasively obtained specimens. Given the associated risks with invasive diagnostic procedures, which are often magnified in the setting of acute illness, the ability to diagnose an infection by noninvasive means has potential to significantly improve patient outcomes.

There are some limitations of plasma NGS testing. It provides a lower specificity than that of conventional testing, with false positives likely resulting from disrupted mucosal barrier function or reflecting lymphocyte immunosuppression, which is particularly true in oncology and transplant patients, who are often at the highest risk for infection. This contributes to a degree of subjectivity in determining if results represent true infection. Although the majority of NGS tests identified 2 or fewer organisms, allowing for straightforward clinical interpretation, there was a minority of cases in which NGS testing reported multiple organisms that are a part of the normal gastrointestinal or oral microbiome. These were typically interpreted as reflecting disrupted barrier function and not true infection; however, there were a few instances where infectious diseases specialists felt compelled to treat these organisms given the gravity of the patient's illness. Additionally, NGS provides no data on antimicrobial sensitivity and resistance. These limitations suggest that infectious disease expertise is important in interpretation of NGS testing results and in guiding management based on these results.

Effective clinical implementation of plasma NGS testing will likely benefit from institutional guidelines that maximize the likelihood of clinically relevant results and minimize false-positive results, which could lead to inappropriate treatment. This study is an initial view of the clinical use of this test, though given its retrospective nature and the overrepresentation of immunocompromised patients, firm conclusions are difficult to draw about patient characteristics that would inform such guidelines. Further data on real-world use of the test in specific patient populations under evaluation for clearly defined clinical presentations are needed to better delineate the test characteristics in these specific clinical settings.

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.

Acknowledgments

Author contributions. J.R., S.C., A.A., and W.J.M. designed the study. J.R., S.C., and W.J.M. prepared the initial draft. J.R., S.C., W.J.M., M.S., S.J.P., and S.K. analyzed the data. All authors contributed to manuscript revisions.

Potential conflicts of interest. All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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