Cell-free DNA next-generation sequencing successfully detects infectious pathogens in pediatric oncology and hematopoietic stem cell transplant patients at risk for invasive fungal disease

Amy E. Armstrong1 | Jenna Rossoff1 | Desiree Hollemon2 | David K. Hong2 | William J. Muller3,4 | Sonali Chaudhury1,4

1Division of Hematology, Oncology and Transplantation, Ann & Robert H. Lurie Children’s Hospital of Chicago, Chicago, Illinois
2Karius, Inc., Redwood City, California
3Division of Infectious Diseases, Ann & Robert H. Lurie Children’s Hospital of Chicago, Chicago, Illinois
4Department of Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, Illinois

Correspondence
Sonali Chaudhury, Division of Hematology, Oncology and Transplantation, Ann & Robert H. Lurie Children’s Hospital of Chicago, 225 E. Chicago Avenue, Box #30, Chicago, IL 60611. Email: SoChaudhury@luriechildrens.org

Abstract
Background: We sought to determine if next-generation sequencing (NGS) of microbial cell-free DNA (cfDNA) in plasma would detect pathogens in pediatric patients at risk for invasive fungal disease (IFD).

Procedures: Pediatric hematologic, oncologic, and stem cell transplant patients deemed at risk for new IFD had blood samples drawn at three time-points separated by 1-month intervals. The primary outcome measure was detection of fungal pathogens compared to standard clinical testing. Secondary outcomes included identification of other infectious pathogens, relationship to European Organization for Research and Treatment of Cancer’s Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases’ Mycoses Study Group (EORTC/MSG) guidelines, and assessment of antifungal therapy.

Results: NGS identified fungal pathogens in seven of 40 at-risk patients for IFD and results were identical in four of six proven cases, including Aspergillus fumigatus by lung biopsy, Candida albicans by blood or pancreatic pseudocyst cultures, and Rhizopus delemar by skin biopsy. Rhizopus oryzae identified on skin biopsy and A. fumigatus isolated on day 27 of 28 of culture from lung biopsy were not detected by cfDNA NGS, possibly due to lack of bloodstream penetration and questionable pathogenicity, respectively. Numerous DNA viruses were detected in patients with prolonged febrile neutropenia or abnormal imaging. Extended antifungal therapy was used in 73% of patients. Follow-up cfDNA sequencing in patients who were positive at enrollment was negative at 1 and 2 months.

Conclusions: cfDNA NGS detected fungal pathogens from blood confirming its potential to guide treatment decisions in pediatric patients at risk for IFD and limit excessive empiric antifungal use. Future studies are needed to better understand the sensitivity and specificity of this approach.

KEYWORDS
cell-free DNA, fungal disease, pediatric oncology, pediatric stem cell transplantation

1 INTRODUCTION

Invasive fungal disease (IFD) poses a significant risk of morbidity and mortality to immunocompromised pediatric patients.1–4 Children and adolescents who receive chemotherapy, undergo hematopoietic stem-cell transplantation (HSCT), or have bone marrow failure are at increased risk for IFD due to their prolonged neutropenic states. Prompt diagnosis of IFD with administration of aggressive and appropriate antifungal therapy is crucial to increase survival and reduce morbidities.5,6

Abbreviations: ALL, acute lymphoblastic leukemia; BG, 1,3-β-D-glucan; cfDNA, cell-free DNA; CMV, cytomegalovirus; CT, computed tomography; FN, fever and neutropenia; GM, galactomannan; HSCT, hematopoietic stem cell transplantation; IFD, invasive fungal disease; MRI, magnetic resonance imaging; NGS, next-generation sequencing.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2019 The Authors. Pediatric Blood & Cancer Published by Wiley Periodicals, Inc.

https://doi.org/10.1002/pbc.27734
Conventional diagnostic techniques for fungal infection are invasive, slow to produce a result, and often lack sensitivity and species-level identification. Direct evidence of fungi through evaluation of sterile specimens is the gold standard to prove IFD, but requires potentially invasive procedures to obtain infected tissue. Alternatively, noninvasive options that can suggest IFD include testing for galactomannan (GM), a cell wall component of Aspergillus species, and the glucose polymer β-D-glucan (BG), a major cell wall component of most fungal species (with the exception of Mucorales and Cryptococcus). While these components may be released in blood and tissues in the course of invasive fungal infections, GM and BG do not detect all fungal pathogens and when studied in pediatric patients as a diagnostic tool, these have variable performance. Specifically, the GM test has a positive predictive value of 0–100% and negative predictive value of 70–100%, and the BG test has a positive predictive value of 17–49% and negative predictive value of 84–96%. Radiographic findings are used to aid diagnosis of fungal pulmonary pathology in the setting of fever and neutropenia (FN), but are notably nonspecific, result in overdiagnosis, and cannot accurately detect a specific fungal pathogen to best guide treatment. PCR-based technologies are a promising approach to compliment current methods, but false-positive and -negative results continue to impede widespread applicability. Targeted fungal sequencing (either 18S or ITS2) of biopsy tissue has reasonable performance with one institution showing sensitivity and specificity of 96.6% and 98.2% in patients with known diagnoses. However, this approach requires an invasive procedure to obtain adequate specimen for testing.

Next-generation sequencing (NGS) technologies are being investigated for noninvasive diagnosis and monitoring of infectious diseases, including fungal pathogens. Sequencing of cell-free DNA (cfDNA) in the bloodstream has previously demonstrated clinical utility in the detection of fetal abnormalities, transplanted organ rejection, and malignant tumors, although results are disappointing for several major tumor types. Similar approaches have been used to sequence circulating cfDNA, identify nonhuman sequences, and compare them with known genomic databases of bacterial, viral, and fungal pathogens. While NGS technology is newer with a smaller number of samples studied, inhibiting comprehensive evaluation of its performance, this method has been effective for diagnosing bacterial infections in a real-time setting as well as noninvasively identifying molds in patients with invasive fungal infection. In this pilot study (ClinicalTrials.gov identifier: NCT03262584), we hypothesized that cfDNA NGS could accurately and noninvasively identify fungal pathogens in pediatric hematology, oncology, and HSCT patients at risk for new IFD.

2 PATIENTS AND METHODS

2.1 Eligibility

Pediatric hematology, oncology, and HSCT patients at our institution and deemed at risk for IFD were eligible. Enrollment criteria included at least one of the following: (1) prolonged (≥96 h) FN despite broad-spectrum antibiotic therapy, (2) recrudescence FN, or (3) findings that triggered consideration of a new fungal infection (i.e., abnormal imaging or laboratory results). FN was defined as an absolute neutrophil count <500 cells/μL and axillary or oral temperature ≥38.5°C or two temperatures at least 1 h apart in a 24-h period ≥38°C. Recrudescence FN was defined as a second febrile episode after the first one resolved with antimicrobial treatment, on or after day 4 of empirical antibiotic therapy. Patients on prophylactic antifungal therapy were eligible for participation. Those patients without concern for fungal infection or those on >4 days of treatment-level antifungal therapy were excluded from the study given potential clearance of cfDNA from circulation on treatment-level therapy.

2.2 Trial design

The pilot study was an Institutional Review Board approved single center, prospective study conducted from May 2017 through May 2018 to determine if cfDNA NGS could identify fungal pathogens. Depending on patient age, eligible patients were contacted for consent (age ≥18 years), assent (age ≥12 years), and/or parental permission (age <18 years). Patients could only participate in the study once. Of the 41 enrolled patients, initial blood samples were obtained from 40 patients with plans to collect a total of three samples (enrollment, follow-up 1, follow-up 2) during routine laboratory draws separated by approximately 1-month intervals. Follow-up samples were not collected from patients who were discharged from the institution or who passed away before study completion (n = 3), who did not have routine blood draws scheduled around time of follow-up (n = 4), or for whom follow-up was missed (n = 3). Clinical data, antimicrobial usage, and results from testing ordered by treating providers as part of standard care were obtained via electronic chart review; no study-specific testing was required for enrolled patients. Results from cfDNA NGS were not made available in real-time and did not direct decision making.

2.3 cfDNA NGS of infectious pathogens

Six milliliters of whole blood was collected in BD Vacutainer™ K2EDTA blood collection tubes (Becton Dickinson and Company, NJ) and plasma was separated within 24 h by centrifugation (1500 rpm for 10 min at room temperature). Two 1.5 cc aliquots were stored in sterile cryovials and frozen at −80°C until transport to the Karius CLIA/CAP laboratory (Redwood City, CA) for processing. cfDNA was extracted from plasma, NGS libraries were prepared, and sequencing was performed on an Illumina NextSeq® 500. Sequencing reads identified as human were removed, and remaining sequences were aligned to a curated pathogen database. Any of over 1000 organisms in the Karius clinical reportable range found to be present above a predefined statistical threshold were reported as previously described. Those interpreting the NGS results were blinded to clinical information. For detailed methods, see Supporting Information Appendix.

2.4 Definitions of IFD

We classified patients as having proven, probable, possible, or no evidence of invasive fungal infection according to the criteria of...
TABLE 1 Baseline characteristics and eligibility criteria for patients with enrollment samples analyzed (n = 40)

| Age (year)          | Median (range)   | 11.0 years (1.2-24.2 years) |
|---------------------|------------------|-----------------------------|
| Sex, No. (%)        | Male             | 26 (65%)                    |
|                     | Female           | 14 (35%)                    |
| Race, No. (%)       | Caucasian/non-Hispanic | 16 (40%)           |
|                     | Other            | 24 (60%)                    |
| Disease classification, No. (%) | Leukemia/lymphoma | 22 (55%)               |
|                     | Solid/CNS tumor  | 7 (18%)                     |
|                     | Stem cell transplant | 9 (23%)             |
|                     | Other            | 2 (5%)                      |
| Criteria for enrollment, No. | Fever and neutropenia | 22                    |
|                     | Recrudescence fever and neutropenia | 6        |
|                     | Chest CT concerning for fungal infection | 8        |
|                     | Clinical/laboratory concern for fungal infection | 7        |

Abbreviation: CT, computed tomography.

| Disease classification, No. (%) | Leukemia/lymphoma | 22 (55%) |
|--------------------------------|--------------------|----------|
|                                 | Solid/CNS tumor    | 7 (18%)  |
|                                 | Stem cell transplant | 9 (23%)  |
|                                 | Other              | 2 (5%)   |
| Criteria for enrollment, No.   | Fever and neutropenia | 22      |
|                                 | Recrudescence fever and neutropenia | 6      |
|                                 | Chest CT concerning for fungal infection | 8    |
|                                 | Clinical/laboratory concern for fungal infection | 7 |

Abbreviation: CT, computed tomography.

3.1 Patient characteristics

Baseline patient characteristics and eligibility criteria are shown in Table 1. Forty patients with a median age of 11 years had at least an enrollment sample collected. The majority (55%) had an underlying diagnosis of leukemia or lymphoma and nine patients were HSCT recipients. Study patients were considered at risk for IFD mainly due to FN. Seven patients had clinical or laboratory findings that increased suspicion for fungal infection including four patients with yeast growing in urine, blood, pancreatic pseudocyst fluid, or wound culture and three patients with skin lesions or a diffuse rash thought to be consistent with disseminated fungal infection. Three patients met two eligibility criteria. Thirty-six patients (90%) were started on treatment-dosing antifungal therapy and twenty-nine (73%) remained on treatment for ≥1 week.

3.2 Fungal classifications

Of the 40 patients who had enrollment samples drawn for cfDNA NGS, findings from chart review confirmed that six patients had proven IFD (Table 2), one had possible IFD, 11 had possible IFD, one had an elevated BG but no clinical findings, and 21 (>50%) met no criteria for IFD according to EORTC/MSG definitions.8

3.3 cfDNA NGS detects six fungal pathogens in patients with proven and possible IFD

cfDNA sequencing identified the same pathogen in four of the six proven IFD cases with fungi detected by invasive biopsy (skin, lung, pseudocyst fluid drainage) and blood culture (Table 2). One proven IFD case with negative cfDNA NGS involved a 15-month old female with acute lymphoblastic leukemia (ALL) who had a skin lesion with central eschar, which revealed Rhizopus oryzae on fungal culture and 18S rDNA targeted sequencing on sterile biopsy. The other discrepant case was documented in a 21-year-old male with ALL with persistent fevers, cough, hypoxia, radiologic evidence of extensive bilateral nodular opacities with regions of cavitation, and an elevated BG at >500 pg/mL. Aspergillus fumigatus grew on day 27 of 28 of lung biopsy culture, while 18S rDNA and fungal stains were negative. cfDNA NGS did not detect Aspergillus, but did report Pneumocystis jirovecii.

Probable IFD was determined in one patient: a 9-year-old male with late relapse of infant ALL who presented with fevers and respiratory distress. He had CT findings of diffuse alveolar opacities associated with mediastinal and hilar adenopathy and an elevated BG at 389 pg/mL. Bronchoalveolar lavage testing was unrevealing, whereas cfDNA NGS detected P. jirovecii.

3.4 cfDNA NGS results in patients with possible or no evidence of IFD

Eleven patients had possible IFD due to abnormal CT chest findings (Table S1). No fungal pathogens were detected by cfDNA NGS for these patients, although two had cytomegalovirus (CMV) infections and one had BK polyomavirus infection by clinical testing and cfDNA NGS. Twenty-one patients met no clinical or mycologic criterion to support a classification of IFD. Candida glabrata was identified in one of these patients by cfDNA NGS: a 2-year-old male child receiving an autologous HSCT for neuroblastoma who had prolonged FN. This was not confirmed by microbiology testing.
3.5 | cfDNA NGS negative at subsequent evaluations

Thirty-seven patients had follow-up samples drawn 1-month after their enrollment sample, and 30 patients had samples drawn at 2 months. Of the seven patients with fungal positivity on initial cfDNA NGS testing, including five pathogens defined in Table 2 along with P. jirovecii and C. glabrata, follow-up testing did not detect these organisms; all of these patients received at least a brief course of appropriate antifungal therapy. Overall, there were no fungi detected on follow-up testing for any patient.

3.6 | cfDNA NGS detects infectious pathogens in patients with prolonged FN

Twenty-two patients were enrolled with prolonged FN despite broad-spectrum antibiotic therapy and of these patients, two had proven IFD and six had possible IFD. While cfDNA NGS detected the same fungal pathogen in two patients with proven IFD and also detected C. glabrata in a patient otherwise not meeting any criteria for IFD, testing also revealed a variety of viral and bacterial pathogens in this population (Table 3). The majority detected are commensal organisms found in the human oral, gastrointestinal tract, and skin and may represent translocation of microbial DNA. These organisms were not confirmed by conventional microbiology; however, it is difficult to ascertain their contribution to the patient’s clinical status since these organisms would have been treated with the empiric antibiotics being given.

3.7 | cfDNA NGS results correlate to viral and bacterial findings

Viral pathogens were detected by cfDNA NGS in 10 enrollment samples, including CMV in four of four patients with positive blood PCRs (range: detected but <137 to 3647 IU/mL) and BK polyomavirus in two of two patients with 20,000 and 22,000 IU/mL. cfDNA NGS detected varicella zoster in one patient with a rash characteristic of this virus but for whom clinical testing was not sent. Additionally, CMV was detected by cfDNA sequencing in two other patients, one where CMV PCR was not ordered for clinical care and a second where CMV PCR was sent 2 days after the study sample was drawn and returned negative. Epstein-Barr virus was found by cfDNA sequencing in one patient who did not undergo other directed testing. Escherichia coli was confirmed by cfDNA NGS in a patient with E. coli bacteremia determined from a blood culture obtained one day prior to the enrollment sample being drawn. All other patients with positive blood cultures prior to study enrollment had enrollment study samples drawn 3 or more days after the study sample was drawn and returned negative. Epstein-Barr virus was found by cfDNA sequencing in one patient who did not undergo other directed testing.

3.8 | High anti-fungal use in patients with no fungal pathogen detected on cfDNA NGS

cfDNA NGS did not detect fungal pathogens in 33 of the 40 initial enrollment samples drawn, however routine clinical testing revealed one of these 33 patients had proven IFD (R. oryzae) for which she received appropriate antifungal therapy. In assessing the 32 patients with no fungal pathogen detected by NGS and otherwise no proven IFD, 23 patients (72%) received treatment-dosing antifungal therapy for 1 week or longer, namely micafungin or liposomal amphotericin B. Overall, in the 21 patients who met no mycologic or clinical criterion of IFD, 19 were started on antifungal treatment and 13 continued micafungin, amphotericin B liposomal, or voriconazole for at least 1 week.
TABLE 3  Pathogens detected by microbial cell-free DNA sequencing in 22 patients with prolonged fever and neutropenia

| Patient | Pathogen detected |
|---------|-------------------|
| IFI-001 | Granulicatella adiacens |
| IFI-002 | Abiotrophia defectiva, human herpesvirus 6A, Veillonella parvula |
| IFI-003 | BK polyomavirus |
| IFI-005 | Enterococcus faecalis |
| IFI-008 | Candida glabrata |
| IFI-010 | None |
| IFI-011 | Candida albicans |
| IFI-015 | Cytomegalovirus, Prevotella melaninogenica |
| IFI-017 | Lactobacillus rhamnosus, Prevotella melaninogenica, Prevotella oris, Veillonella parvula |
| IFI-019 | Rhizopus delemar, Acinetobacter pittii, Enterobacter cloacae complex, Pseudomonas fluorescens, Pseudomonas luteola |
| IFI-020 | Fusobacterium nucleatum, Ureaplasma parvum |
| IFI-022 | Varicella zoster virus, Staphylococcus warneri |
| IFI-025 | Escherichia coli |
| IFI-027 | Escherichia coli |
| IFI-031 | None |
| IFI-032 | Fusobacterium nucleatum, Helicobacter pylori, Cytomegalovirus |
| IFI-035 | Cytomegalovirus, Prevotella melaninogenica |
| IFI-036 | None |
| IFI-038 | None |
| IFI-039 | Pseudomonas pseudoalcaligenes |
| IFI-040 | Cytomegalovirus |
| IFI-041 | Actinomyces oris, Enterococcus faecalis, Prevotella melaninogenica, Streptococcus mitis, Veillonella parvula |

Note. Fungal pathogens are given in bold.

4 | DISCUSSION

Forty pediatric oncology, hematology, and HSCT patients considered at risk for IFD had cfDNA NGS of plasma samples. Sequencing of circulating cfDNA detected fungal pathogens in five of seven cases with proven and probable IFD, none of the cases with possible IFD, and one of 21 cases that did not satisfy criteria for IFD by EORTC/MSG guidelines. This methodology correlated with fungi isolated by invasive testing from lung tissue, pancreatic pseudocyst fluid, and a scalp wound, demonstrating the ability of a blood test to detect fungal pathogens at a species level from various sites of infection. When cfDNA NGS is performed for clinical purposes in real time, results can become available within 24 h and provide a quick noninvasive method to diagnose IFD and direct therapy. Delays in diagnosis contribute to the significant mortality and morbidity from IFD despite advances in antifungal therapy.

Furthermore, in one patient R. oryzae was detected by skin biopsy but not from plasma cfDNA NGS. As Rhizopus delemar was successfully detected by both skin biopsy and plasma cfDNA NGS in another patient, this raises the question of the degree of invasion and dissemination for the patient with R. oryzae growing from a cheek lesion; imaging with full body CT and brain and face MRI showed no extension of infection. Mucormycosis (including infections from Rhizopus spp., Mucor spp., and Lichtheimia) is an important emerging fungal infection associated with a near 50% mortality rate, where delay in amphotericin B-based therapy significantly increases mortality. One study in children, however, showed no mortality from localized cutaneous mucor infection but dissemination of infection increased the risk of death by sevenfold. The ability to differentiate between localized versus disseminated disease may provide a better understanding of prognosis and guide duration of therapy. While cfDNA sequencing done in isolation would have missed the diagnosis of R. oryzae in this patient, the negative result combined with a positive superficial skin biopsy likely supports localized infection.

A second discrepant finding in this study involved the identification of A. fumigatus from lung tissue on day 27 of 28 of culture growth, which was not detected by plasma cfDNA NGS. Aspergillus fumigatus is a ubiquitous fungal pathogen that releases thousands of spores into the environment that are cleared by macrophages and neutrophils in healthy individuals, but in immunocompromised hosts can cause disease. A clear diagnosis of invasive pulmonary aspergillosis was difficult in this patient who had other findings inconsistent with the diagnosis, including a negative serum GM, negative 18S rDNA, and negative fungal stains on biopsy. However, the isolation of Aspergillus in culture led to antifungal treatment. Plasma cfDNA NGS testing detected P. jirovecii, which could explain the abnormal CT chest findings and elevated serum BG in a patient with self-reported poor adherence to trimethoprim-sulfamethoxazole prophylaxis and could represent a coinfection. This case highlights the fact that results from this test, like all diagnostic tests, need to be interpreted in the context of all data available to determine the relevance of pathogen detection.

We further evaluated cfDNA NGS results beyond patients with proven IFD and confirmed findings of CMV, BK polyomavirus, and E. coli as detected on routine clinical blood tests. There were no other positive viral or bacterial results that cfDNA NGS did not also subsequently detect in the same timeframe. Interestingly, of the 11 patients with possible IFD due to abnormal chest CT results, none had fungi detected by cfDNA NGS and nine (82%) of these patients received antifungal therapy for >1 week. While certain radiographic findings can be suggestive of IFD, an image-guided diagnostic approach is often nonspecific and can lead to overdiagnosis of IFD in febrile neutropenic patients. Comparing abnormal imaging results to a noninvasive reliable blood test might help establish fungal pathogens as the causal agents. In three of our patients with abnormal imaging and no further evidence of fungal disease, CMV and BK polyomavirus were detected and could explain the radiographic findings.

Ultimately, proving a diagnosis of IFD is difficult and routine practice promotes initiation of therapy based on concerning clinical or imaging findings, especially in high-risk neutropenic patients with persistent or recurrent fevers despite broad-spectrum antibiotics. In accordance with this practice, 21 of 28 (75%) of enrolled patients at risk for IFD due to prolonged or recrudescent FN were placed on >1 week of treatment dosing antifungal therapy by their primary providers. While other clinical or mycological findings may have
supported this use, we determined prolonged antifungal therapy was used in 72% of patients who had no proven IFD and for whom cfDNA NGS testing was negative. If the negative predictive value of cfDNA NGS testing can be established, this could lead to decreased antifungal drug use since these drugs can have associated toxicities, lead to growth of resistant species, and increase medical costs. For patients with high-risk FN, using a diagnostic test-guided preemptive strategy instead of an empirical approach to antifungal therapy may decrease overall antifungal use without increasing mortality. A diagnostic non-invasive test like cfDNA NGS could help guide treatment decisions; in our patients with prolonged FN, isolated viral pathogens from cfDNA sequencing such as BK polyomavirus, varicella zoster virus, CMV, and human herpesvirus 6A may represent the primary infectious source. The currently validated version of the test offers qualitative reporting on all pathogens detected, which can aid clinicians in determining which organisms are more likely to cause true disease.

In conclusion, sequencing of circulating cfDNA revealed multiple infectious pathogens in immunocompromised paediatric patients at risk for IFD. Fungal pathogens were detected in seven patients and correlated to four of six proven cases; the absence of detection in two cases may be explained by lack of bloodstream penetration in a cutaneous infection and by a questionably pathogenic fungi isolated in lung parenchyma. In those patients who had positive fungal cfDNA at enrollment, follow-up samples at 1 month were negative, suggesting a more rapid clearance of fungal cfDNA from the bloodstream and need for future studies to test shorter time intervals. Our data support the emerging promise of plasma cfDNA NGS to aid in diagnosis of IFD in at-risk patients. Studies designed to evaluate the performance characteristics of this test in a larger population of at-risk patients are recommended.

CONFLICT OF INTERESTS
This is an investigator-initiated study with support provided by Karius, Inc. for testing and supplies. The authors Desiree Hollemon and David K. Hong are both employed by Karius, Inc.

ORCID

Amy E. Armstrong https://orcid.org/0000-0002-7784-0476
Jenna Rossoff https://orcid.org/0000-0003-0471-9883
Desiree Hollemon https://orcid.org/0000-0003-4971-9334

REFERENCES

1. Mor M, Gilad G, Kornreich L, et al. Invasive fungal infections in pediatric oncology. Pediatr Blood Cancer. 2011;56:1092–1097.
2. Rosen GP, Nielsen K, Glenn S, et al. Invasive fungal infections in pediatric oncology patients: 11-year experience at a single institution. J Pediatr Hematol Oncol. 2005;27:135–140.
3. Castagnola E, Cesaro S, Giacchino M, et al. Fungal infections in children with cancer: a prospective, multicenter surveillance study. Pediatr Infect Dis J. 2006;25:634–639.
4. Groll AH, Castagnola E, Cesaro S, et al. Fourth European Conference on Infections in Leukaemia (ECIL-4): guidelines for diagnosis, prevention, and treatment of invasive fungal diseases in paediatric patients with cancer or allogeneic haemopoietic stem-cell transplantation. Lancet Oncol. 2014;15:e327-e340.
5. Maschmeyer G. Invasive fungal disease: better survival through early diagnosis and therapeutic intervention. Expert Rev Anti Infect Ther. 2011;9:279–281.
6. Hahn-Ast C, Glasmacher A, Mückter S, et al. Overall survival and fungal infection-related mortality in patients with invasive fungal infection and neutropenia after myelosuppressive chemotherapy in a tertiary care centre from 1995 to 2006. J Antimicrob Chemother. 2010;65:761–768.
7. Lass-Florl C. Current challenges in the diagnosis of fungal infections. Methods Mol Biol. 2017;1508:3-15.
8. de Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis. 2008;46:1813–1821.
9. Lehrnbecher T, Robinson PD, Fisher BT, et al. Galactomannan, β-D-glucan, and polymerase chain reaction-based assays for the diagnosis of invasive fungal disease in pediatric cancer and hematopoietic stem cell transplantation: a systematic review and meta-analysis. Clin Infect Dis. 2016;63:1340-1348.
10. Lass-Florl C, Resch G, Nachbaur D, et al. The value of computed tomography-guided percutaneous lung biopsy for diagnosis of invasive fungal infection in immunocompromised patients. Clin Infect Dis. 2007;45:e101-e104.
11. Vallipuram J, Dhalla S, Bell CM, et al. Chest CT scans are frequently abnormal in asymptomatic patients with newly diagnosed acute myeloid leukemia. Leuk Lymphoma. 2017;58:834-841.
12. Chamilos G, Marom EM, Lewis RE, et al. Predictors of pulmonary zygomycosis versus invasive pulmonary aspergillosis in patients with cancer. Clin Infect Dis. 2005;41:60–66.
13. Khot PD, Fredricks DN. PCR-based diagnosis of human fungal infections. Expert Rev Anti Infect Ther. 2009;7:1201–1221.
14. Gomez CA, Budvytiene I, Zemek AJ, et al. Performance of targeted fungal sequencing for culture-independent diagnosis of invasive fungal disease. Clin Infect Dis. 2017;65:2035-2041.
15. Lefterova MI, Suarez CJ, Banaei N, et al. Next-generation sequencing for infectious disease diagnosis and management: a report of the association for molecular pathology. J Mol Diagn. 2015;17:623–634.
16. Hong DK, Blauwkamp TA, Kertesz M, et al. Liquid biopsy for infectious diseases: sequencing of cell-free plasma to detect pathogen DNA in patients with invasive fungal disease. Diagn Microbiol Infect Dis. 2018;92:210-213.
17. Stokowski R, Wang E, White K, et al. Clinical performance of non-invasive prenatal testing (NIPT) using targeted cell-free DNA analysis in maternal plasma with microarrays or next generation sequencing (NGS) is consistent across multiple controlled clinical studies. Prenat Diagn. 2015;35:1243–1246.
18. De Vlaminkx I, Martin L, Kertesz M, et al. Noninvasive monitoring of infection and rejection after lung transplantation. Proc Natl Acad Sci USA. 2015;112:13336–13341.
19. Aravanis AM, Lee M, Klausner RD. Next-generation sequencing of circulating tumor DNA for early cancer detection. Cell. 2017;168:571–574.
20. Demosthenes EZ, Kyrochristos ID, Lykoudis EG, et al. Early solid tumor diagnosis through next-generation sequencing of cell-free DNA. Biomark Med. 2018;12:1197-1201.
21. De Vlaminck I, Khush KK, Strehl C, et al. Temporal response of the human virome to immunosuppression and antiviral therapy. Cell. 2013;155:1178–1187.

22. Abril MK, Barnett AS, Wegermann K, et al. Diagnosis of capnocytophaga canimorsus sepsis by whole-genome next-generation sequencing. Open Forum Infect Dis. 2016;3. 10.1093/ofid/ofw144

23. Blauwkamp TA, Thair S, Rosen MJ, et al. Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease. Nat Microbiol. 2019;4:663–674.

24. Fung M, Zompi S, Seng H, et al. Plasma cell-free DNA next-generation sequencing to diagnose and monitor infections in allogeneic hematopoietic stem cell transplant patients. Open Forum Infect Dis. 2018;5. https://doi.org/10.1093/ofid/ofy301

25. Wagner K, Springer B, Pires VP, et al. Molecular detection of fungal pathogens in clinical specimens by 18S rDNA high-throughput screening in comparison to ITS PCR and culture. Sci Rep. 2018;8:6964.

26. Ostrosky-Zeichner L. Invasive mycoses: diagnostic challenges. Am J Med. 2012;125:514–524.

27. Skiaa A, Pagano L, Groll A, et al. Zygomycosis in Europe: analysis of 230 cases accrued by the registry of the European Confederation of Medical Mycology (ECMM) Working Group on Zygomycosis between 2005 and 2007. Clin Microbiol Infect. 2011;17:1859–1867.

28. Chamilos G, Lewis RE, Kontoyiannis DP. Delaying amphotericin B-based frontline therapy significantly increases mortality among patients with hematologic malignancy who have zygomycosis. Clin Infect Dis. 2008;47:503–509.

29. Zaat TE, Rolides E, Chiou CC, et al. Zygomycosis in children: a systematic review and analysis of reported cases. Pediatr Infect Dis J. 2007;26:723–727.

30. Schaffner A, Douglas H, Braude A. Selective protection against conidia by mononuclear and against mycelia by polymorphonuclear phagocytes in resistance to Aspergillus. Observations on these two lines of defense in vivo and in vitro with human and mouse phagocytes. J Clin Invest. 1982;69:617–631.

31. Marty FM, Koo S, Bryar J, et al. (1→3) beta-D-glucan assay positivity in patients with Pneumocystis carinii jiroveci pneumonia. Ann Intern Med. 2007;147:70–72.

32. Qin J, Xu J, Dong Y, et al. High-resolution CT findings of pulmonary infections after orthotopic liver transplantation in 453 patients. Br J Radiol. 2012;85:e959–e965.

33. Caillot D, Couaillier JF, Bernard A, et al. Increasing volume and changing characteristics of invasive pulmonary aspergillosis on sequential thoracic computed tomography scans in patients with neutropenia. J Clin Oncol. 2001;19:253–259.

34. Potente G. Computed tomography in invasive pulmonary aspergillosis. Acta Radiol. 1989;30:587–590.

35. Franquet T, Müller NL, Giménez A, et al. Infectious pulmonary nodules in immunocompromised patients: usefulness of computed tomography in predicting their etiology. J Comput Assist Tomogr. 2003;27:461–468.

36. Ho DY, Lin M, Schaeinman J, et al. Yield of diagnostic procedures for invasive fungal infections in neutropenic febrile patients with chest computed tomography abnormalities. Mycoses. 2011;54:59–70.

37. Kim M-C, Kim MY, Lee HJ, et al. CT findings in viral lower respiratory tract infections caused by parainfluenza virus, influenza virus and respiratory syncytial virus. Medicine (Baltimore). 2016;95:e4003.

38. Akazawa Y, Terada Y, Yamane T, et al. Fatal BK virus pneumonia following stem cell transplantation. Transpl Infect Dis. 2012;14:E142-E146.

39. Restrepo-Gualteros SM, Jaramillo-Barberi LE, Gonzalez-Santos M, et al. Characterization of cytomegalovirus lung infection in non-HIV infected children. Viruses. 2014;6:2038–2051.

40. Ruhnke M, Schwartz S. Recent developments in the management of invasive fungal infections in patients with oncohematological diseases. Ther Adv Hematol. 2016;7:345–359.

41. Walsh TJ, Anaissie EJ, Denning DW, et al. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis. 2008;46:327–360.

42. Freifeld AG, Bow EJ, Sepkowitz KA, et al. 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;52:427–431.

43. de Pauw BE. Between over- and undertreatment of invasive fungal disease. Clin Infect Dis. 2005;41:1251–1253.

44. Fung M, Kim J, Marty FM, et al. Meta-analysis and cost comparison of empirical versus pre-emptive antifungal strategies in hematologic malignancy patients with high-risk febrile neutropenia. PLoS One. 2015;10:e0140930.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Armstrong AE, Rossoff J, Hollemon D, Hong DK, Muller WJ, Chaudhury S. Cell-free DNA next-generation sequencing successfully detects infectious pathogens in pediatric oncology and hematopoietic stem cell transplant patients at risk for invasive fungal disease. Pediatr Blood Cancer. 2019;66:e27734. https://doi.org/10.1002/pbc.27734