Rapid, Non-Invasive Detection of Invasive Mucormycosis Caused by *Syncephalastrum monosporum* using Next-Generation Sequencing of Circulating Microbial Cell-Free DNA in Plasma

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BUILT TO BEAT CANCER

Abstract

Background
Improving diagnostics have led to newly identified causes of invasive fungal infection (IFI) in immunocompromised hosts. *Syncephalastrum spp.* are Zygomycetes more commonly associated with skin infections and have only rarely been implicated as a cause of IFI[1]. Next generation sequencing (NGS) for circulating microbial cell-free DNA (mcfDNA) in plasma offers a unique tool to diagnose rare causes of IFI[2,3].

Methods
Karius results were reviewed for *Syncephalastrum* detections with two identified at the same institution. The Karius Test (KT) was developed and validated in Karius' CLIA certified/CAP accredited lab in Redwood City, CA and detects mcfDNA in blood plasma. McfDNA is extracted, NGS performed, human sequences removed and remaining sequences are aligned to a curated database of >1400 pathogens. Organisms present above a statistical threshold are reported and quantified in molecules per microliter (MPM). Chart review was performed for clinical correlation.

Results
An adult male one month out of induction therapy for acute myeloblastic leukemia (AML) developed pneumonia. Although BAL was negative for mold and despite empiric antifungals, plasma NGS for mcfDNA showed *S. monosporum* at 562 MPM; the reference range is 0 MPM. Amphotericin was added to empiric posaconazole. The patient was discharged 10 days later and serial CT scans showed improvement. Repeat NGS mcfDNA 11 days later was negative. He underwent stem cell transplant (SCT) 4 months later.

In a second case, an adult female with acute prolymphocytic leukemia was admitted for fever with neutropenia. A CT chest showed new multifocal, bilateral, nodular opacities. Despite negative BAL fungal culture and pretreatment with fluconazole, plasma NGS mcfDNA revealed *S. monosporum* at 575 MPM. She was treated with micafungin, amphotericin, and posaconazole with clinical improvement. Repeat NGS mcfDNA 8 weeks later was negative. Serial CT scans showed improvement over 5 months. She proceeded to SCT.

Conclusion
Plasma-based NGS for mcfDNA enabled rapid, non-invasive detection of pulmonary mucormycosis caused by *S. monosporum* despite antifungal pretreatment and unrevealing invasive procedures in two patients with leukemia. The rapid identification of the specific etiology of IFI enabled targeted anti-fungal therapy and resumption of definitive oncological care including SCT.

References
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2. Hong, DK., et al. 2018. "Liquid Biopsy for Infectious Diseases: Sequencing of Cell-Free Plasma to Detect Pathogen DNA in Patients with Invasive Fungal Disease." Diagnostic Microbiology and Infectious Disease 92 (3): 210–13.3.
3. Rossoff J, Chaudhury S, Soneji M, Patel SJ, Kwon S, Armstrong A, et al. Noninvasive Diagnosis of Infection Using Plasma Next-Generation Sequencing: A Single-Center Experience. Open Forum Infect Dis. 2019;6.

Two Cases of *Syncephalastrum monosporum* Infection Detected by The Karius® Test

	Patient 1	Patient 2
Age	Adult (60-70 years of age)	Adult (60-70 years of age)
Sex	Male	Female
Underlying illness	AML	B cell prolymphocytic leukemia
Chemotherapy (within prior 30 days)	FLAG-IDA induction chemotherapy	Rituximab, Ibrutinib, Bendamustine, Venetoclax, dexamethasone
Clinical manifestations	Productive cough, hypoxemia	Fever, dyspnea
Initial CT chest	Bilateral pulmonary ground-glass opacities	Multifocal, bilateral, irregular nodular opacities
Follow up CT chest	Nodular/mass-like opacities	Worsening pneumonia with multiple areas of cavitation
BAL results	Culture: *Rothia mucilaginosa* Fungal culture: Negative Path: No fungal elements	PCR: *Aspergillus fumigatus*, HHV6 Culture: *Prevotella*, *Streptomyces* Fungal culture: Negative Path: No fungal elements
Karius Test	*Syncephalastrum monosporum* 562 MPM (RR < 10 MPM)*	*Syncephalastrum monosporum* 575 MPM (RR < 10 MPM)**
Treatment	Posaconazole, Amphotericin liposomal	Posaconazole, Micafungin, Amphotericin liposomal
Outcome	Survival, stable CT imaging, repeat KT negative, underwent haplo-HSCT	Survival, improvement in CT imaging, repeat KT negative, underwent allogeneic PBSCT

RR = reference range based on the 97.5% of a cohort of 684 healthy individuals.
**Rothia mucilaginosa* reads were present in the raw data but did not reach the required statistical significance for the commercial threshold
***Aspergillus* and *Prevotella* reads were present in the raw data but did not reach the required statistical significance for the commercial threshold; HHV6 reads were not present