Serial Microbial Cell-Free DNA Next Generation Sequencing (NGS) As A Means of Diagnosis and Monitoring of Clinical Response To Treatment Of Invasive Fungal Infections (IFI) In Immunocompromised Pediatric Patients

Negar Ashouri MD, Delma Nieves MD, Jasjit Singh MD, Antonio Arrieta MD

Division of Infectious Diseases, CHOC Children's Hospital, Orange, Ca.

Contact: Nashouri@choc.org
Poster number: 1342

Abstract

Background: IFIs are a leading cause of morbidity and mortality in immunocompromised pediatric patients. Early diagnosis and initiation of therapy is critical for treatment success but is often hindered by difficulty obtaining tissue samples, lack of growth on culture and time required for culture positivity. Non-culture methods such as aspergillus galactomannan and 1-3 B-D glucan lack sensitivity and specificity. NGS has shown promise in identifying a wide array of pathogens . The Karius ® test is a NGS which can identify viral, bacterial, and fungal infections by detecting cell free pathogen DNA in peripheral blood with a rapid turnaround time. Over the past few years, we have used the Karius test as an adjunct to our standard of care in the workup and treatment of IFI in our oncology population.

Methods: We report our experience in using serial NGS testing for diagnosis of IFI as well as a means for monitoring clinical response to treatment in our pediatric hematological malignancy population

Results: Between 12/1/20-12/31/21, 5 patients had serial NGS for IFI. Most common indication for testing was prolonged fever and neutropenia with abnormal imaging. Initial diagnosis was made by NGS in all 5 patients. All had tissue biopsies with fungal elements seen on path in 4 of 5 patients. Culture was positive in 3 of 5 patients (2 days, 10 days and 12 days post NGS testing result). One patient with persistent positive NGS testing was found to have resistance to treatment drug on susceptibility testing and subsequently had negative NGS testing once appropriate antifungals were started. A second patient had sterilization of cultures with concomitant negative NGS results but developed increasing fungal copies on NGS testing followed by positive culture when chemotherapy was resumed and subsequently died due to his infection. 4 of 5 patients had serial negative follow up NGS and continue to do well.

Conclusion- In our small series of patients NGS testing allowed for rapid diagnosis of IFI and corresponded to clinical response to treatment. Further prospective controlled studies will be needed to further evaluate the use of NGS in this patient population.

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Background

- Invasive fungal infections are a leading cause of morbidity and mortality in the pediatric transplant and hematological malignancy patient population.
- Diagnosis of fungal infections in this vulnerable population remains difficult
  - Non-invasive test modalities such as *Aspergillus* galactomannan and 1,3 B-D glucan lack sensitivity and specificity.
- Invasive diagnostic modalities such as bronchoscopy and biopsy are based on histology until culture result is available.
  - Specific fungal identification by histology is difficult and culture is often negative.
- Recent developments using NGS to detect microbial cell free DNA offer an alternative means of diagnosis
- The Karius® test is a NGS test in plasma that detects microbial cell free DNA (cfDNA).
- After cfDNA is extracted and NGS performed, human reads are removed and remaining sequences are aligned to a curated database of > 1400 organisms.
  - Organisms present above a statistical threshold are reported.

We report our experience in using this technology to not only diagnose but monitor response to treatment in a small series of patients with hematological malignancies.

Methods

We conducted a retrospective chart review of patients with hematological malignancies and/or transplant between Dec 1, 2020 until Dec 31, 2021 in whom the Karius® test was used for diagnosis and serial monitoring was done for follow up .

Dates of testing, culture results, pathology results as well as any Karius® guided changes in treatment regimen were recorded.

Results

- 5 patients were identified during study period, all had underlying hematological malignancies
- All five patients had their infection identified initially by the Karius® test
- 2 patients had fungal elements seen on pathology but their cultures remained negative.
- In the 3 patients with positive cultures, the Karius® test preceded the positive culture results by 1-19 days
- Repeat Karius® testing was done at various intervals ranging from 2-8 weeks.
- Patient # 3 had persistent elevated readings (mpm) on repeat Karius® testing and susceptibility of organism revealed resistance to the antifungal used. Repeat testing after change in antifungal treatment showed improvement in Karius® readings (mpm)
- Patient #2 had initial improvement in Karius® reading (mpm) which coincided with clinical improvement then a rise in Karius® DNA particle count which also coincided with breakthrough infection and subsequent death of the patient.

Patient	Age	Sex	Diagnosis	Organism	Date of Positive Karius te	Date Culture Positive	Date Final ID of Fungus
1	13y	M	T cell ALL-delayed intensification	Rhizomucor miehi	4/21/2021	none	N/A
2	5y	M	HR-ALL Induction	Lomentospora prolificans	3/14/2021	3/15/2021	3/18/2021
3	12y	M	HR-ALL s/p transplant	Rhizopus oryzae	7/21/2021	8/9/2021	8/10/2021
4	20y	M	T cell lymphoma- Induction	Aspergillus fumigatus	2/27/2021	none	N/A
5	15y	M	HR-ALL induction	Lichtheimia corymbifera	12/6/2020	12/8/2020	12/23/2020

Patient	Organism	Karius Result (mpm)	Pathology	Culture	Clinical Cure 1 year
1	Rhizomucor meihei	#1-784 #2 Negative #3 Negative #4 Negative	Fungal elements seen in lung tissue	Negative	Yes
2	Lomentospora prolificans	#1 1972 #2 14 #3 Negative #4 22 #5 Negative #6 14 #7 669	No fungal elements seen	Positive	No
3	Rhizopus oryzae	#1 11,045 #2 11,869* #3 1,375 #4 1375** #5 110 #6 Negative #7 Negative #8 Negative #9 Negative #10 Negative	Fungal elements seen in lung biopsy Fungal elements seen later in bone biopsy, Karius and culture negative	Positive Negative	Yes
4	Aspergillus fumigatus	#1 95 #2 Negative #3 Negative #4 Negative	Fungal elements seen in brain thrombus	Negative	Yes
5	Lichtheimia Corymbifera	#1 20,178 #2 Negative #3 Negative #4 Negative	Fungal elements seen skin and muscle biopsy	Positive	Yes

*Increase antifungal dose above maximum recommended
** Changed antifungal agent from azole to liposomal amphotericin B after susceptibility available

Conclusions

- In our small series of patients, the use of the Karius® test led to a rapid diagnosis of IFI and in 2/5 of our patients served as the only means to identify the causative pathogen.
- Use of NGS technology allowed for rapid identification of the infection and allowed for initiation of targeted therapy days to weeks before final identification was made on culture results.
- Results of the Karius® test mirrored clinical response or relapse of infection with most patients having a negative Karius® test reading once on effective treatment.
- In our experience, the use of NGS as an adjuvant to traditional diagnostic methods such as biopsy and culture allowed for rapid diagnosis and the ability to monitor clinical response to treatment