CLINICAL VIGNETTE

Curious Case of *Cunninghamella* Diagnosed Utilizing Metagenomic Next-Generation Sequencing

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Introduction

Disseminated fungal infections are associated with high morbidity and mortality in the immunocompromised patient population. Metagenomic next-generation sequencing (mNGS) has allowed the identification of pathogenic DNA within a greater library of bacteria, fungi, and parasites leading to increased sensitivity in diagnosing atypical or difficult to diagnose cases. We present the case of a kidney transplant recipient who developed dark papule lesions on his distal extremities associated with pulmonary cavitary lesions and was found to have disseminated *Cunninghamella* infection using metagenomic next-generation sequencing.

Case Presentation

A 66-year-old male with a history of end stage renal disease status post kidney transplantation presented with progressive, dark, painful plaque-like lesions on his left 4th distal finger and left 2nd distal toe concerning for potential embolic events. He initially developed the left finger lesion three months prior and had been admitted previously for evaluation of this lesion (Figure 1). Other than the painful finger lesion, he was asymptomatic with an extensive negative review of systems. Given the wide differential of skin lesions in an immunocompromised patient, several services were consulted throughout this first admission including dermatology, transplant nephrology, transplant infectious disease, and vascular surgery. There was highest suspicion for digital ischemia given the degree of pain and eschar-like appearance, however doppler ultrasounds performed revealed normal arterial flow. Infectious evaluation included negative blood and fungal cultures. Ultimately, patient underwent skin biopsy that revealed fibrin-rich deposition thought to be secondary due to an atheroembolic event. He underwent a transthoracic echocardiogram that was negative for vegetations or source for cardioembolic event and discharged with plan for outpatient transesophageal echocardiogram and hypercoagulable testing.

Subsequently, the patient developed a new painful plaque-like lesions on his 2nd left toe that were similar to his prior finger lesion (Figure 2). He was urgently readmitted for expedited transesophageal echocardiogram to evaluate for vegetations versus other source of atheroembolic event. A CT angiogram was obtained to observe for potential intraluminal sources of emboli, and he was incidentally found to have several pulmonary nodules with a right upper lobe cavitation concerning for potential disseminated fungal infection versus septic emboli (Figure 3). Transplant infectious disease was consulted and recommended a broad evaluation including routine bacterial and fungal cultures, fungal antibodies, mycobacterial cultures, as well as the Karius test - a commercially available metagenomic next-generation sequencing (mNGS) test. He received empiric broad antimicrobial coverage with vancomycin, ceftriaxone, azithromycin, and posaconazole. Vasculitis evaluation was significant for negative ANA, ANCA antibodies, and normal inflammatory markers. By hospital day 6, infectious cultures and antibodies remained negative. Pulmonology performed bronchoscopy with bronchoalveolar lavage cultures but these also returned negative. The Karius test sent on admission then resulted positive for *Cunninghamella*, a member of the zygomycetes family. The patient treatment was narrowed to posaconazole and he was discharged and had not developed subsequent lesions.

Discussion

Candida species, *Aspergillus* species, and *Cryptococcus neoformans* are the most common disseminated fungal infections in immunocompromised cases.[1] Unfortunately, there are many other potential pathogens, but only a small portion are able to be easily detected using traditional culture and antibody testing. With the rapid morbidity in immunocompromised population, early recognition and diagnosis of the underlying pathogen remains paramount in prompt treatment and management. This case highlights the diagnostic difficulty in correctly diagnosing the causative agent as he underwent substantial evaluation that was negative including TTE, TEE, bronchoscopy, skin biopsy, and traditional infectious work-up with cultures and antibody testing.

The Karius test allowed us to find the causative organism, *Cunninghamella,* which is a genus of fungi found in soil which be inoculated to patients via skin abrasions or inhalation.[2] Most infections have been described in those with hematologic malignancies and prolonged neutropenia.[2-4] Our patient represents one of the few cases reported in solid organ transplant recipients. Presentations vary but have included pulmonary, cutaneous, gastrointestinal, and rhino-orbital symptoms. Mortality is high with 77% mortality in a series of 22 patients with short median survival of 2-3 weeks. Those with pulmonary involvement had higher mortality.[3] Given the few reported

cases of disseminated *Cunninghamella* infections, there is little evidence to guide anti-fungal therapy. Treatment has varied with case reports documenting successful treatment seen with amphotericin B and posaconazole although many patients required additional debridement of affected tissues.[4]

The Karius test is a commercially available non-invasive blood test (referred to as a 'liquid biopsy for infectious diseases') that detects microbial cell-free DNA through metagenomic next generation sequencing (mNGS).[5,6] The test allows for identification of >1200 clinically relevant pathogens including bacteria, fungi, mold, and viruses by identifying the infective agent's DNA circulating in the patient's blood.[5] Testing has been validated with clinical trials and reported to have higher sensitivity in identifying causative organisms compared to typical culture mediums.[5] It has been found to be highly useful in diagnosing infections in immunocompromised patients including patients with hematological malignancies or solid organ transplant recipients, often associated with difficulty to identify an underlying pathogen. For example, in cases of febrile neutropenia, no identifiable pathogen is found in up to 40% of cases.[7]

The clinical utility of mNGS such as the Karius test is not absolute as the test only identifies cell-free DNA - a positive test does not definitively indicate whether the organism is pathogenic in nature and requires correlation with the clinical scenario. Unlike with our case, a retrospective cohort review of 82 immunocompromised patients who received Karius testing found a limited impact on clinical care to date with no change in management based on the Karius test result in 71 of these patients.[8] However, in health systems that see a large immunocompromised patient population, the real world impact may be much larger especially with the difficult to diagnose cases such as ours above.

Conclusion

Immunocompromised patients are at elevated risk for developing disseminated fungal infections which are often difficult to diagnose using typical culture and antibody testing. Our case represents a successful utilization of the Karius test, a commercially available metagenomic next-generation sequencing test to detect a rare case of disseminated *Cunninghamella* infection in a solid organ transplant recipient. With increasing interest in bioinformatics, metagenomic next-generation sequencing may become an important and common tool in the detection and prompt treatment of these infections.

Figures

Figure 1: Initial presentation of skin lesion on left 4th distal phalanx 3 months prior to admission

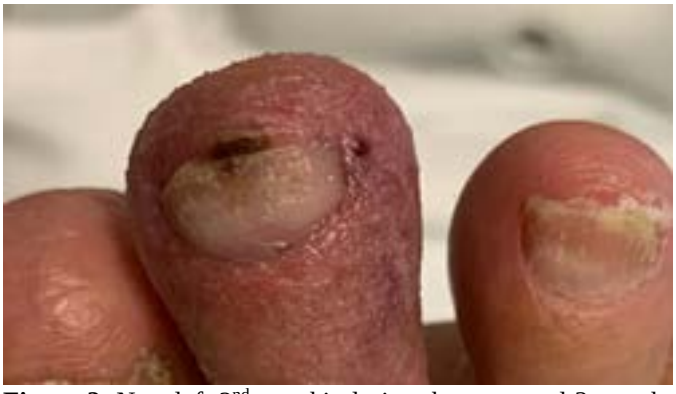

Figure 2: New left 2nd toe skin lesion that occurred 3 months after initial finger lesion

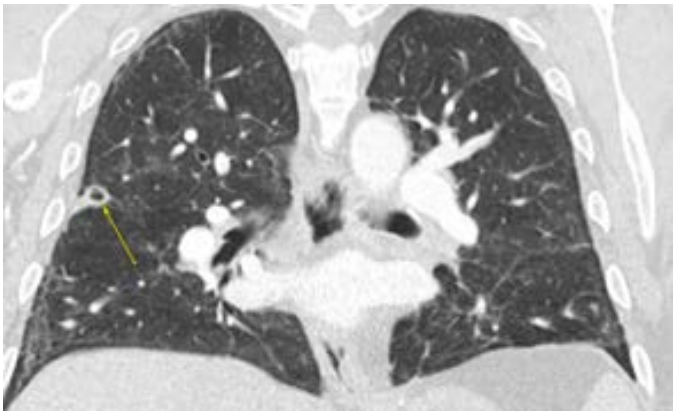

Figure 3. Computer tomography (CT) coronal view of right upper lobe cavitary lesion with several bilateral pulmonary nodules seen

REFERENCES

1. **Azoulay E, Russell L, Van de Louw A, Metaxa V, Bauer P, Povoa P, Montero JG, Loeches IM, Mehta S, Puxty K, Schellongowski P, Rello J, Mokart D, Lemiale V, Mirouse A; Nine-i Investigators.** Diagnosis of severe

respiratory infections in immunocompromised patients. *Intensive Care Med*. 2020 Feb;46(2):298-314. doi: 10.1007/s00134-019-05906-5. Epub 2020 Feb 7. PMID: 32034433; PMCID: PMC7080052.

2. **Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisova TA, Schaufele RL, Sein M, Sein T, Chiou CC, Chu JH, Kontoyiannis DP, Walsh TJ**. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. *Clin Infect Dis*. 2005 Sep 1;41(5):634-53. doi: 10.1086/432579. Epub 2005 Jul 29. PMID: 16080086.
3. **Righi E, Giacomazzi CG, Lindstrom V, Albarello A, Soro O, Miglino M, Perotti M, Varnier OE, Gobbi M, Viscoli C, Bassetti M**. A case of Cunninghamella bertholettiae rhino-cerebral infection in a leukaemic patient and review of recent published studies. *Mycopathologia*. 2008 Jun;165(6):407-10. doi: 10.1007/s11046-008-9098-z. Epub 2008 Mar 14. PMID: 18340546.
4. **Navanukroh O, Jitmuang A, Chayakulkeeree M, Ngamskulrungroj P**. Disseminated Cunninghamella bertholletiae infection with spinal epidural abscess in a kidney transplant patient: case report and literature review. *Transpl Infect Dis*. 2014 Aug;16(4):658-65. doi: 10.1111/tid.12251. Epub 2014 Jun 24. PMID: 24962170.
5. **Morales M**. The Next Big Thing? Next-Generation Sequencing of Microbial Cell-Free DNA Using the Karius Test. *Clin Microbiology Newsletter*. 2021 May 1; 43(9): 69-79. https://doi.org/10.1016/j.clinmicnews.2021.04.003. Epub 2021 April 23. PMID
6. **Han D, Li R, Shi J, Tan P, Zhang R, Li J**. Liquid biopsy for infectious diseases: a focus on microbial cell-free DNA sequencing. *Theranostics*. 2020;10(12):5501-5513. Published 2020 Apr 7. doi:10.7150/thno.45554
7. **Schmidt-Hieber M, Teschner D, Maschmeyer G, Schalk E**. Management of febrile neutropenia in the perspective of antimicrobial de-escalation and discontinuation. *Expert Rev Anti Infect Ther*. 2019 Dec; 17(12):983-995. doi: 10.1080/14787210.2019.1573670. Epub 2019 Mar 11. PMID: 30686067.
8. **Hogan CA, Yang S, Garner OB, Green DA, Gomez CA, Dien Bard J, Pinsky BA, Banaei N**. 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 Jan 27;72(2):239-245. doi: 10.1093/cid/ciaa035. PMID: 31942944.