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Rapid Diagnosis of Invasive Aspergillosis and Active Hepatitis-B Virus Co-Infection in a HIV-1 Infected Patient Using Cell Free DNA Sequencing

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Abstract

Multiple simultaneous opportunistic infections in Human Immunodeficiency virus-1/Acquired immunodeficiency syndrome (HIV-1/AIDS) is a known and dreaded occurrence that often leads to poor outcomes. We present a case of disseminated aspergillosis and active Hepatitis-B virus (HBV) infection in such a host, where cell free DNA (cfDNA) next generation sequencing (NGS) of plasma was used to expedite diagnosis. Bronchoscopy was avoided and treatment was started expeditiously. In this case report we discuss the interpretation of the cfDNA NGS, and its potential role for early diagnosis and avoidance of invasive testing.

Keywords

HIV/AIDS, cell free DNA sequencing, hepatitis B infection, invasive aspergillosis

What Do We Already Know About the Topic?

Based on previously published literature, use of cfDNA NGS of plasma has been validated for pathogen identification in addition to traditional method of blood and tissue culture.

How Does Your Research Contribute to the Field?

This work demonstrates that cell-free NGS of plasma can be very helpful in rapid diagnosis of life-threatening opportunistic infections such as invasive fungal infections in the HIV/AIDS patients and can avoid the need of invasive diagnostic procedures.

What Are Your Research Implications Toward Theory, Practice or Policy?

Cell free NGS of plasma, when utilized within an appropriate timeframe, can be a rapid diagnostic tool to improve the speed and accuracy of diagnosis for invasive aspergillosis and other opportunistic infections in immunocompromised hosts.

Introduction

NGS of cfDNA in plasma can be used to detect the presence of various microbes including bacteria, DNA viruses, protozoa and fungi.[1] Bacterial DNA can be detected in plasma even when corresponding blood cultures are negative or patients have already received antimicrobial treatment. However, interpretation of this test should be done in context of clinical data such as risk factors, physical examination findings, imaging, and other laboratory data. Reports of real-life application of this test in the scope of invasive fungal infection in immunocompromised hosts are still limited at this time. Our report here describes such a case.

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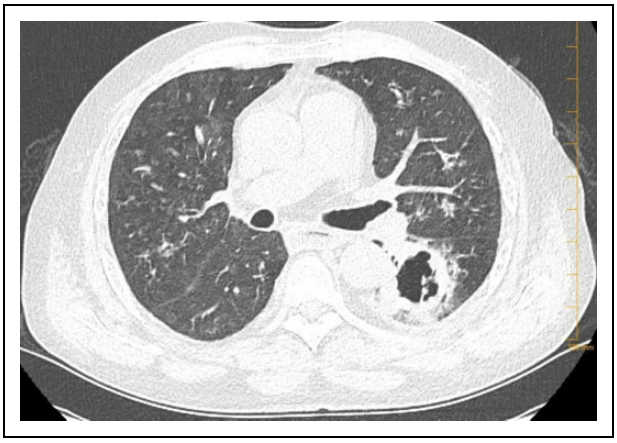

Figure 1. CT chest showing thickened irregular walls, shaggy inner linings and septations, ground glass halo and pericavitary irregular nodules and consolidations.

Case Presentation

A 54-year-old Hispanic man with a history of AIDS and poor adherence to antiretroviral therapy (ART), presented with progressive cough, wheezing, shortness of breath, and intermittent chills for 1 month. When these symptoms began, he was evaluated in the Emergency Department and was given trimethoprim-sulfamethoxazole for possible bronchitis. His symptoms did not improve, and presented again after 1 week at which time he was tested positive for influenza A. When symptoms still persisted, he was then started on prednisone and levofloxacin 1 week later. He had some relief from his symptoms but soon after his fevers recurred with productive cough and shortness of breath. He was therefore admitted and noted to have a fever of 102F. Piperacillin-tazobactam and vancomycin were started in addition to trimethoprim-sulfamethoxazole for broad empiric treatment. He left against medical advice on day 3, only to be readmitted to the hospital after cfDNA testing of plasma ("Karius Test," Karius, Inc., Redwood City, California, USA) returned positive for Aspergillus fumigatus, HBV, and Cytomegalovirus (CMV) within 72 hours of specimen collection.

The patient had a history of vaccination against HBV and had positive surface antibody titers noted since 2009. He reported stopping his ART for approximately 4 months prior to presentation due to nausea, but he had restarted again two months prior to admission. Social history was significant for smoking. The patient worked as a nurse in a prison facility for several years. He was in a monogamous relationship with 1 male partner. He had 4 dogs and 1 pet cat.

Investigations

At the time of initial diagnosis of HIV-1 approximately 25 years prior, his CD4 count was 12 cells/mm^3. During this admission, he was noted to have a CD4 count of 76 cells/mm^3 with undetectable HIV viral load. Four months prior, his CD4 count was 379 cells/mm^3. Computed tomography (CT) of the chest was done which showed a left lower lobe 4 centimeter necrotic cavitary mass-like consolidation with thickened irregular walls, septations, ground glass halo, as well as pericavitary irregular nodules and consolidations as shown in Figure 1. The appearance of the cavity was consistent with angioinvasive aspergillosis.

cfDNA NGS was sent and revealed the presence of Aspergillus fumigatus, HBV, and cytomegalovirus (CMV). Aspergillus fumigatus was detected at 242 DNA molecules per microliter (MPM;reference level <10) and CMV level was 378 MPM. DNA sequencing detected HBV, but MPM was not reported as there is no environmental control for this virus. A sputum fungal culture eventually returned positive for heavy Aspergillus fumigatus after approximately 2 weeks, with a voriconazole MIC of 0.5 ug/mL. An Aspergillus antigen level of 0.660 (ref range <0.5) and Beta-D-glucan value of >500 (ref range <80 pg/ml) were also noted. Histoplasma urine antigen testing was negative. Serum CMV PCR testing showed a low-level viremia of 256 IU/ml. Repeat HBV testing demonstrated positive surface antigen, loss of surface antibody, positive core antibody, and positive E antigen. HBV DNA level was found to be >9.00 log IU/ml, which along with elevated liver enzymes (AST of 55 U/L; ALT of 73 U/L) suggested active infection.

An MRI of the brain was done due to concern for central nervous system involvement. It showed 8, scattered, small nodular rim-enhancing lesions in bilateral cerebral and cerebellar hemisphere as shown in Figure 2. Lumbar puncture was performed that showed total nucleated cell count of 2 with 88% neutrophils on differential. CSF glucose was 42 mg/dl, protein level was 28.9 mg/dl, and no growth of fungus was noted from CSF culture. Toxoplasma IgG and IgM were negative. Given the overall clinical picture, these brain lesions were consistent with brain abscesses due to disseminated aspergillosis from the lungs.

Treatment

Given the result of cfDNA NGS, along with imaging and history suggestive of invasive aspergillosis of the lungs and brain, the patient's treatment was changed to voriconazole given as a loading dose of 6 mg/kg every 12 hours intravenously (IV) and then continued at 4 mg/kg every 12 hours after the initial 2 loading doses. Micafungin was added for synergy with voriconazole. He was switched to oral voriconazole 200 mg twice daily after 2 weeks of IV treatment with plans to continue treatment for at least 12 months. The patient was also started on emtricitabine-tenofovir treatment for HBV infection. Antiretroviral therapy was resumed with maraviroc, dolutegravir, darunavir, and ritonavir. CMV infection was assessed to be a result of reactivation in the setting of immunodeficiency, and not likely a contributing cause of lung or brain infection.

Outcome and Follow-Up

A repeat chest x-ray 2 weeks after discharge showed improvement in bilateral consolidations. Repeat serum aspergillus antigen level in 1 month was undetectable. HBV DNA PCR

repeated after 4 months was 5.75 log IU/ml, and the CD4 count improved to 265 cells/mm3. CT scan of the chest was done 6 months after starting treatment and showed regression of the irregular cavitary consolidation within the superior segment of the left lung. Additional follow-up labs are shown in Table 1.

Discussion

Invasive aspergillosis is a major cause of morbidity and mortality in the HIV-1 population and Aspergillus fumigatus is the most common species found to cause infection.[2] In recent years, newer diagnostic tools such as PCR based testing have improved our ability to make early diagnoses in these cases. As per prior practice guidelines by Infectious Disease Society of America (IDSA) in 2008, the definitive diagnosis of aspergillosis requires documentation of infection with positive culture results from a sterile sample.[3] With advancement in PCR testing, the IDSA has changed the requirements needed to confirm diagnosis in 2016, stating that a positive culture is not a requirement. However there is still no agreement in the equivalency of PCR detection over direct culture sampling, and research continues in this area.[4]

Other tests commonly used for diagnosis of aspergillus infection include serum and bronchoalveolar lavage (BAL) galactomannan measurement. These tests generally are less specific than PCR or direct culture. Beta-d-glucan measurement is also inadequately specific for aspergillus, nor is it highly sensitive. Marr et al reported that use of antifungal treatment empirically or prophylactically can further decrease the sensitivity of these tests.[5] When serum PCR testing is used, it is recommended to be used in conjunction with other test results, imaging findings and more invasive tissue sampling on a case-to-case basis. CT scan of chest is recommended whenever there is clinical suspicion for invasive aspergillosis and follow-up CT scan is recommended to evaluate response to treatment. The IDSA still recommends performing bronchoscopy and obtaining BAL.[4] However, in cases of severe infection where patients may have complications such as severe hypoxemia or platelet dysfunction, invasive procedures such as bronchoscopy may not be easily performed, causing further delay. Thus, non-invasive testing could play an important role in rapid diagnosis in such cases.

NGS of DNA to detect genomic material from pathogens in plasma has been shown to be helpful in the diagnosis of invasive fungal infections.[6] Its advantages include rapid turn-around time, a potential decrease in procedure related morbidity, and an overall reduction in healthcare cost. It could also lead to a decrease in unnecessary exposure to empiric broad-spectrum antibiotic treatment for immunocompromised patients while waiting for traditional culture results.

cfDNA was initially defined by Mandel and Metais in 1948, and NGS of cfDNA continues to be studied for use in detecting infection as well as detecting fetal chromosomal defects and solid organ rejection.[6-9] The utility of plasma NGS in diagnosing invasive fungal infection was first reported by Hong et al, where results of cfDNA sequencing was compared to biopsy sampling.[6] 7 out of 9 cases showed the same results as proven by biopsy. Imbert et al demonstrated that Aspergillus DNA in serum remains relatively stable for the first week after treatment.[10] Therefore, the use of NGS or PCR, when applied early within 1 week of initiating antifungal therapy, may have the best yield to detect invasive aspergillosis.[10] They also reported that clearance of detectable cfDNA by 2-3 weeks was associated with a favorable outcome. However, more extensive research is needed to study the stability of pathogen genomic

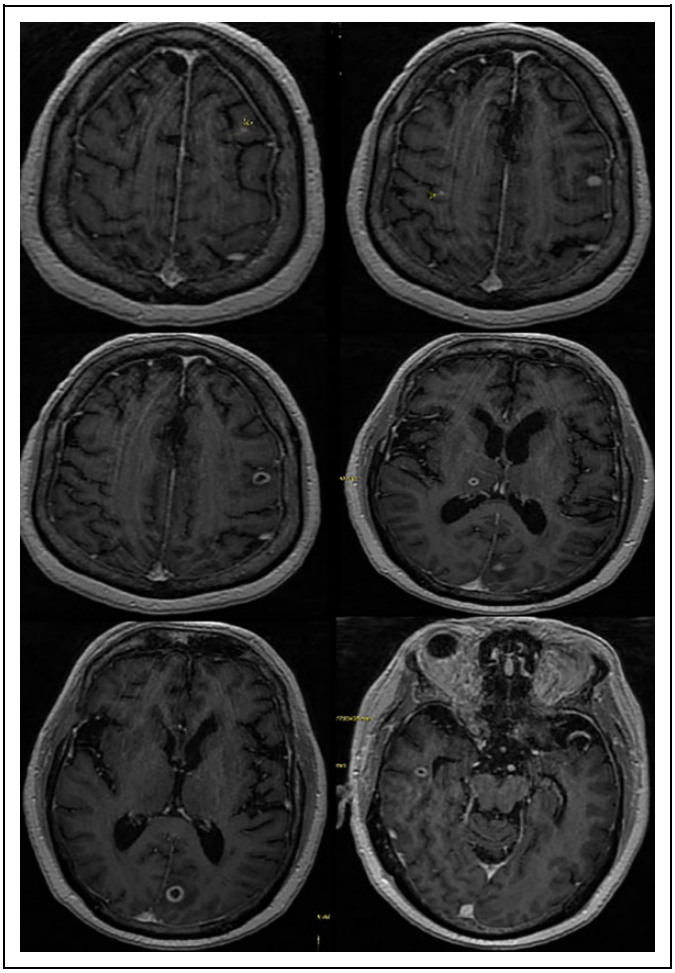

Figure 2. MRI brain images showing scattered brain abscesses with small nodular rim-enhancing lesions in bilateral cerebral and cerebellar hemisphere.

Table 1. Levels of Serum Tests Performed on Initial Presentation and on Follow Up.

	Presentation	Four months later
Aspergillus Galactomannan (index)	0.660	<0.50
Beta-d Glucan (pg/ml)	>500	63
Hep B DNA(IU/ml)	>1000,000,000	541000
ALT (U/l)	73	49

DNA in serum. Abril et al illustrated the usefulness of NGS to detect fastidious organisms such as Capnocytophaga canimorsus. In this study a patient with septic shock after reported dog bites demonstrated Gram negative rods within neutrophils, but cultures did not show growth of any pathogens. NGS performed after 4 days of antibiotic therapy detected the presence of this fastidious pathogen.[11]

NGS of cfDNA has another advantage of detecting co-infections. Our patient, who reported prior immunization to HBV with documented protective titers, tested positive for this virus as an incidental finding. Subsequently, HBV DNA detected in the blood by PCR and elevated liver transaminases confirmed active infection. The patient's HBV infection could be explained by the loss of immune response from initial vaccination with subsequent exposure in the setting of lowered CD4 count off of ART. This case highlights that the HBV co-infection could have been missed if the focus was on obtaining bronchoscopy-guided tissue sampling due to high suspicion of invasive aspergillosis on presentation. There is, therefore, potential utility of using NGS of cfDNA to rapidly diagnose multiple co-infections in severely immunocompromised patients without the need for invasive diagnostic procedures. In this case, the patient benefited from early Hepatitis B treatment because of this test.

Despite all its advantages mentioned above, there are limitations for the cfDNA sequencing of plasma. These include the lack of susceptibility data, inability to detect RNA viruses, and limited data on mycobacterial and parasitic infections. Frequently, this test can detect DNA viruses, such as CMV and herpes simplex virus 1, which could be present in the blood of immunocompromised hosts without clinical manifestations indicative of active disease to warrant directed treatment.[12] Our case reflected that as well; the patient improved with ART and did not need directed CMV treatment. It is also possible that infections in certain organ systems may result in lower levels of pathogen cfDNA, reducing the sensitivity of the test. Thus, the clinical utility of plasma cfDNA testing should be assessed for usefulness on a case-by-case basis, and needs to be interpreted cautiously within the clinical context.

Conclusion

Plasma NGS testing of cfDNA has been helpful in diagnosing invasive fungal infections, including Rhizopus, Scedosporium, and Aspergillus.[6] In order to maximize sensitivity, it is best if this test is sent early and prior to initiating antimicrobial therapy. The advantage of the test is that it is non-invasive and can potentially help with pathogen identification with results available within 72 hours. This may provide significant diagnostic value even if sent after initiation of empiric antimicrobial therapy. Another advantage is its capacity to detect co-infections, which is important in immunocompromised patients who can present with multiple opportunistic infections as well as reactivation of prior infections, as seen in our patient.

Several limitations of NGS should be kept in mind. At this time, more research is needed to determine the appropriate window period within which the diagnostic sensitivity will be the most optimal. This period may also vary depending on the type of pathogen, anatomical system involved in infection, stage of infection, and host immunity. This test can help identify pathogens but cannot offer susceptibility data. Lastly, cost and availability of the test may still be a concern for many patients and health-care providers.

Authors' Note

MR wrote in initial manuscript, performed data collection, data editing, image collection and compilation. RL conceptualized the manuscript and obtained patient consent. JK and RL performed manuscript editing. All authors reviewed and approved the final manuscript.

Declaration of Conflicting Interests

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Human and Animal Welfare Statement

Our study did not require an ethical board approval because it did not contain human or animal trials.

Patient Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

References

1. 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(4):663–674. doi:10.1038/s41564-018-0349-6
2. Perfect JR, Cox GM, Lee JY, et al. The impact of culture isolation of Aspergillus species: a hospital-based survey of aspergillosis. *Clin Infect Dis*. 2001;33(11):1824–1833. https://doi.org/10.1086/323900
3. 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(3):327–360. doi:10.1086/525258
4. Patterson TF, Thompson GR 3rd, Denning DW, et al Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2016;63(4):e1-e60. doi:10.1093/cid/ciw326
5. Marr KA, Balajee SA, McLaughlin L, Tabouret M, Bentsen C, Walsh TJ. Detection of galactomannan antigenemia by enzyme immunoassay for the diagnosis of invasive aspergillosis: variables that affect performance. *J Infect Dis*. 2004;190(3):641–649. doi:10.1086/422009

6. Hong DK, Blauwkamp TA, Kertesz M, Bercovici S, Truong C, Banaei N. 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(3):210–213. doi:10.1016/j.diagmicrobio.2018.06.009
7. Mandel P, Metais P. Les acides nucléiques du plasma sanguin chez l'homme. C R Seances *Soc Biol Fil*. 1948;142(3-4):241–243.
8. Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. *Proc Natl Acad Sci U S A*. 2008; 105(42):16266–16271. doi:10.1073/pnas.0808319105
9. Snyder TM, Khush KK, Valantine HA, Quake SR. Universal noninvasive detection of solid organ transplant rejection. *Proc Natl Acad Sci U S A*. 2011;108(15):6229–6234. doi:10.1073/pnas.1013924108
10. Imbert S, Gauthier L, Joly I, et al. Aspergillus PCR in serum for the diagnosis, follow-up and prognosis of invasive aspergillosis in neutropenic and nonneutropenic patients. *Clin Microbiol Infect*. 2016;22(6):562.e1–562.e5628. doi:10.1016/j.cmi.2016.01.027
11. 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(3):ofw144. Published 2016 Jul 12. doi:10.1093/ofid/ofw144
12. De Vlaminck I, Khush KK, Strehl C, et al. Temporal response of the human virome to immunosuppression and antiviral therapy. *Cell*. 2013;155(5):1178–1187. doi:10.1016/j.cell.2013.10.034