Cross-reactivity of *Aspergillus* galactomannan in an HIV-infected patient with histoplasmosis

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ABSTRACT

Non-invasive assays are increasingly being used in patients with suspected fungal infections. Limitations to these tests include limited sensitivity, specificity, and cross reactivity with other fungal pathogens. Herein we report a case of disseminated histoplasmosis producing a false positive serum and bronchoalveolar lavage (BAL) *Aspergillus* galactomannan assays. This test may have a role in the evaluation of patients with suspected histoplasmosis in settings where *Histoplasma* antigen testing is not widely available.

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1. Introduction

Invasive fungal infections are an important cause of morbidity and mortality in patients with immunocompromised conditions, such as transplant recipients, HIV-infected patients, and recipients of corticosteroids. Histoplasmosis remains an important endemic mycosis, especially in patients with advanced HIV/AIDS. Often, more than one opportunistic infection may be present in those advanced HIV/AIDS patients, underscoring the importance of thorough clinical and laboratory evaluations [1,4]. In recent years, new diagnostic assays have become available to evaluate patients suspected of having invasive fungal infections, but with variable sensitivity and specificity. *Histoplasma* antigen and *Aspergillus* galactomannan (GM) testing are two widely used fungal diagnostic tests available in the United States.

The *Histoplasma* antigen enzyme-linked immunoassay (EIA) can be performed from samples in serum, urine, bronchoalveolar lavage (BAL) fluid or cerebrospinal fluid [2]. The Platelia® *Aspergillus* GM EIA has been commercially available in Europe since the mid-1990s and was approved by the US Food and Drug Administration in 2003. It is used to detect GM in serum or BAL fluid for diagnosis of invasive aspergillosis. GM antigen positivity has been incorporated into the microbiological diagnostic criteria proposed by European Organization for Research and Treatment of Cancer (EORTC) and Mycoses Study Group (MSG) for diagnosis of invasive aspergillosis [3,20]. Herein we describe a patient with advanced HIV infection and disseminated histoplasmosis who had a strongly positive *Aspergillus* GM test due to cross-reactivity between histoplasmosis infection and the *Aspergillus* GM assay and we briefly review literature for similar cases.

2. Clinical presentation

A 49-year-old man from the southeastern United States, diagnosed with HIV infection more than twenty years prior, presented with fever, dry cough, dyspnea, and left hip pain for four weeks. Two weeks prior to admission he noticed a skin rash that started in his left leg and spread sparsely to other parts of the body. He reported considerable amount of weight loss over the past 3 months but denied nausea, diarrhea or abdominal pain. He admitted that he had not been taking anti-retroviral therapy for the past year.

On examination, temperature was 102 °F, blood pressure was 92/54 mmHg, pulse was 130 beats per minute and respiratory rate was 18 breaths per minute. His oxygen saturation was 95% on room air, and 89% with minimal activity. Lungs were clear to auscultation. There were multiple punched out hemorrhagic ulcers over his left leg (Fig. 1). Similar skin lesions were scattered over face, scalp, behind the left ear (Fig. 2), upper back, chest and

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Fig. 1. Multiple punched out hemorrhagic skin ulcers over the left leg.

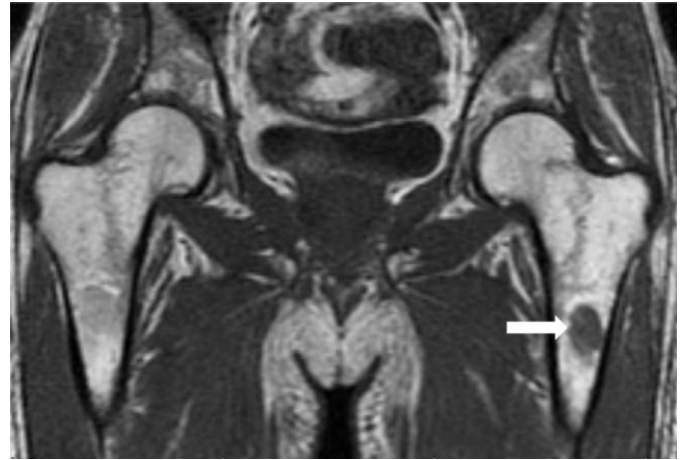**Fig. 2.** A hemorrhagic skin rash over the posterior part of the left ear.

Fig. 3. MRI of the pelvis showing oval-shaped infiltrated mass (white arrow) in the left proximal femoral shaft.
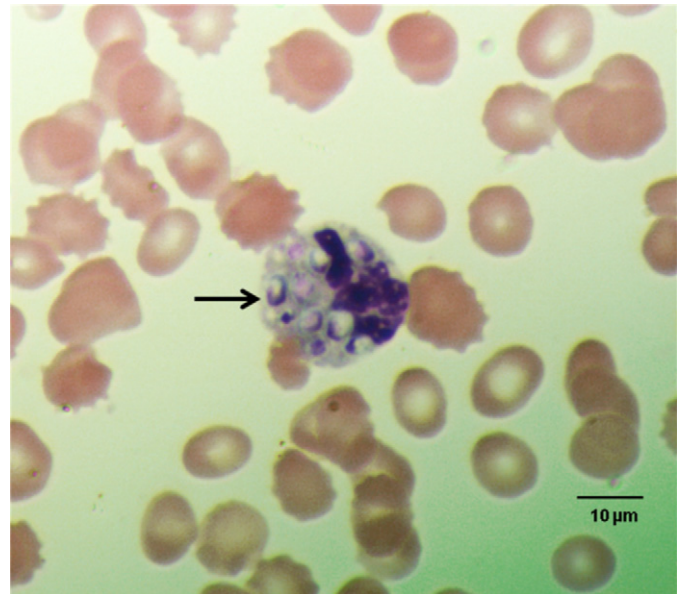

Fig. 4. Peripheral blood smear illustrating the intracellular yeast (arrow) (Wright s stain, 1000 x magnification).

the both upper extremities. He had limited range of motion on left hip examination, especially on adduction, and required assistance with cane on walking. His oropharynx was clear and remainder of the physical examination was unremarkable.

Laboratory studies revealed a white blood cell count of 900/mm^3 (reference range 4000–11,000) with 92% neutrophils, hemoglobin level was 9 g/dL (12–15) and platelet count was 15,000/mm^3 (150,000–400,000). Peripheral blood smear on admission showed no evidence of intracellular inclusions. Basic metabolic panel was within normal range. Liver function tests revealed alanine amino-transferase 200 U/L (10–44), aspartate aminotransferase 61 U/L (14–40), total bilirubin 1.1 mg/dL (0.4–1.4), albumin 2.3 g/dL (3.4–5.0), lactate dehydrogenase 266 U/L (120–240), with normal alkaline phosphatase level and international normalization ratio (INR). His CD4 count was 12 cells/μL (380–1500) and HIV RNA-PCR was 6.7 million copies/mL. Computed tomography of the chest and abdomen showed multiple pulmonary micro- and macro-nodules with right hilar lymphadenopathy and hepatosplenomegaly. Magnetic resonance imaging (MRI) of the pelvis noted an infiltrative mass over the proximal part of the left femur shaft (Fig. 3) without evidence of osteomyelitis or avascular necrosis of the femoral head.

Blood cultures (bacterial, fungal and mycobacterial) were obtained and empiric intravenous vancomycin and piperacillin–tazobactam were initiated on admission. Biopsies were obtained from skin ulcers and left hip lesion. Anti-retroviral therapy with

tenofovir, emtricitabine, ritonavir and atazanavir was started. Serum and urine *Histoplasma* antigen, and serum *Aspergillus* GM assays were performed on the day of admission. On day 4 of admission, the patient underwent bronchoscopy, BAL and trans-bronchial tissue biopsy with bacterial, fungal and mycobacterial cultures. BAL fluid for *Aspergillus* GM and *Histoplasma* antigen were sent as well.

On day 5 of hospitalization, peripheral blood smear showed intracellular inclusions suggestive of *Histoplasma capsulatum* (Fig. 4). Amphotericin B lipid complex (5 mg/kg daily) was started with significant improvement of symptoms, pancytopenia and transaminitis within 3 days. On day 6, intravenous vancomycin and piperacillin–tazobactam were discontinued after bacterial blood cultures returned negative and resolution of neutropenia. Fungal blood cultures were positive for *H. capsulatum* a week after collection (on day 7 of admission). *H. capsulatum* also grew from fungal cultures of skin ulcers and left femur bone tissue samples.

The result of serum *Aspergillus* GM assay taken on admission was > 3.0 (normal < 0.5 Index), and the BAL *Aspergillus* GM assay showed a result of 4.72 (normal < 0.5 Index). Serum *Aspergillus*

GM assay was repeated 3 days after discontinuation of piperacillin–tazobactam antibiotic and it remained markedly elevated as > 3.0 (normal < 0.5 Index). However, BAL and transbronchial tissue biopsy cultures and microscopic examination were positive only for *H. capsulatum* (Fig. 5). Urine and BAL *Histoplasma* antigen tests were positive > 19 ng/mL (above the limit of quantification). The patient's condition continued to improve with amphotericin B lipid complex therapy and after 2 weeks he was transitioned to oral itraconazole. At 1-month follow-up, he was doing well with resolution of skin lesions and respiratory symptoms. He had minimal left hip pain and he was able to walk unassisted. His CD4 count had increased to 115 cells/μL and HIV RNA-PCR was reduced to 2000 copies/mL.

3. Discussion

Invasive fungal infections should be considered in immunocompromised hosts with proper clinical syndromes and the appropriate epidemiological exposures. Histoplasmosis is an endemic mycosis seen in the midwestern and southeastern United States (highly prevalent in the Mississippi and Ohio river valleys), and in many areas of Central and South America. In Europe, most patients of histoplasmosis have been reported from Italy, where the organism has been isolated from soil in the Po River valley. Other European countries, mainly United Kingdom, Spain, France, Germany and Belgium, have also published several cases of histoplasmosis [5–7]. C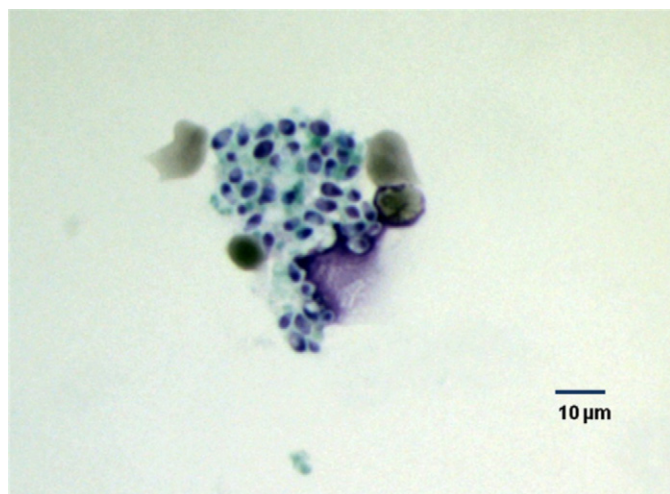ertain countries of Asia-Pacific region are endemic for histoplasmosis, such as Australia, India and Southeast Asia [8]. Histoplasmosis is also endemic in central and west areas of Africa as well as on the island of Madagascar [9]. Histoplasmosis can produce life-threatening manifestations in patients with HIV infection, especially with CD4 counts less than 100 cells/μL. Diagnosis is usually established by direct microscopy or cultures from respective specimens (blood, BAL, tissues, bone marrow, CSF) or by antigen detection (urine, blood, BAL or CSF). In patients with advanced HIV/AIDS, such as our patient, it is common to have more than one opportunistic infection, especially if the CD4 count is less than 50 cells/μL.

Invasive aspergillosis is an uncommon infection in patients with HIV infection. One of the studies analyzed 35,252 HIV-infected patients in the US showed the incidence of aspergillosis was as low as 3.5 cases per 1000 person-years [10]. An epidemiological survey conducted in France also demonstrated significant lower incidence (0.02–0.13% per annum) of aspergillosis in HIV patients compared to those in allogenic stem cell transplant recipients (12.8%), solid organ transplant patients (11.1%) and hematological malignancies (6.3–8%) [11]. However, invasive aspergillosis, although rare in HIV patients, is associated with poor survival. Risk factors for invasive aspergillosis in HIV patients include advanced HIV (CD4 count of less than 50 cells/μL), severe neutropenia (< 500 cells/mm^3) and recent corticosteroid use [12]. Co-infection with invasive aspergillosis in a patient with HIV/AIDS and disseminated histoplasmosis has been reported [4]. However, in our patient, there was no convincing clinical or radiological evidence for invasive aspergillosis.

Galactomannan is a polysaccharide cell wall component of *Aspergillus* species and the target of detection by the Platelia® *Aspergillus* GM enzyme-linked immunoassay (EIA). A Cochrane review reported the sensitivity of *Aspergillus* GM assay for invasive pulmonary aspergillosis as 70% (serum) and 85% (BAL) whereas its specificity was 89% (serum) and 95% (BAL) primarily in patients with hematological malignancies and stem cell transplantation [3]. Fewer data are available for solid organ transplant recipients. A study reported sensitivities of 25% in serum and 100% in BAL, with specificities of 97% in serum and 84% in BAL among patients with solid organ transplants [13]. No large studies are available for *Aspergillus* GM assay performance in HIV infected patients. Another drawback of this assay is its cross-reactivity with other fungal species other than *Aspergillus* in which GM is a component of their cell walls. These fungi include *Histoplasma*, *Penicillium*, *Blastomyces*, *Paracoccidioides*, *Cryptococcus*, *Nigrospora*, *Paecilomyces*, *Trichothecium*, *Lichtheimia ramose*, *Fusarium* and *Geotrichum* species [14–17]. Furthermore, false-positive *Aspergillus* GM tests have been reported in patients taking certain antibiotics (e.g. piperacillin–tazobactam and amoxicillin–clavulanate) [18]. In our patient, serum *Aspergillus* GM test was repeated 3 days after

Fig. 5. *Histoplasma capsulatum* yeasts on transbronchial tissue biopsy culture (Grocott's Methenamine Silver stain, 400 x magnification).

Table 1
Cross-reactivity of Platelia® *Aspergillus* Galactomannan (GM) antigen assay with culture-proven *Histoplasma* infections.

Hosts	Type of Histoplasma Infection	Serum Platelia® Aspergillus GM (OD Index)	BAL Platelia® Aspergillus GM (OD Index)
Solid organ transplants (4 Renal transplants, 2 Liver transplants) [20,22]	Disseminated (2 patients), Pulmonary (4 patients)	2.6–8.61	0.55–6.20
Autologous stem cell transplant [23]	Disseminated, Pulmonary	5.2	NA
Rheumatoid Arthritis [24]	Disseminated, Pulmonary	5.38	NA
HIV (6 patients) [20]	Disseminated	1.2–4.0	NA
NA (6 patients) [25][a]	Disseminated (4 patients), Pulmonary (2 patients)	1.5–7.8	NA
Advanced HIV/AIDS(Our index patient)	Disseminated (Blood, Cutaneous, Pulmonary, Bone, Bone Marrow, Liver)	> 3.0	4.72

NA—Not Available; GM—Galactomannan; BAL—bronchoalveolar lavage fluid; OD—Optical Density; Platelia® Serum & BAL *Aspergillus* GM (OD Index)—Negative if less than 0.5.

[a] Unable to elicit underlying host diseases on personal communication with the main corresponding study author.

stopping the antibiotics as to avoid cross-reactivity between the *Aspergillus* GM assay and piperacillin–tazobactam. The repeat serum *Aspergillus* GM assay level was still elevated significantly above the level of quantification which effectively rules out possibility of cross-reactivity with piperacillin–tazobactam [19].

The cross-reactivity of *Aspergillus* GM assay has been observed in the management of a patient with histoplasmosis and some authors have suggested the *Aspergillus* GM assay may have utility as an adjunctive test for the diagnosis of histoplasmosis when other non-invasive tests are unavailable [20,21]. Table 1 enlists reported evidence of cross-reactivity of Platelia® *Aspergillus* GM assay in immunocompromised patients with culture-proven histoplasmosis. It is important to differentiate these two fungal infections because voriconazole has limited efficacy in the treatment of histoplasmosis and itraconazole has not been established as a preferred therapy for invasive pulmonary aspergillosis [26,27]. Because of the cross-reactivity of *Aspergillus* GM assay in patients with histoplasmosis, a false-positive result could easily obscure the correct diagnosis and lead to inappropriate therapy and suboptimal outcomes. Of note, patients with invasive aspergillosis who have high level of *Aspergillus* GM do not have cross-reactivity with *Histoplasma* antigen assay as it seems to be just "one-way" cross-reactivity [25].

4. Conclusion

Patients with HIV infection can have multiple co-infections and the treating physician may perform a diagnostic evaluation that includes non-invasive fungal diagnostic assays. The clinician should be aware of the possibility of cross-reactivity of *Aspergillus* GM assay in patients with histoplasmosis, but the potential to use this assay as a diagnostic tool for histoplasmosis requires further studies. It is important to recognize the limitations of these assays and understand that cultures and histology remain the gold standard for diagnosis of *Aspergillus* infection, especially in HIV-infected patients.

Conflicts of interest

There are none.

Acknowledgments

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