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Short communication

False-positive *Aspergillus* galactomannan assay in solid organ transplant recipients with histoplasmosis

P. Vergidis, R.C. Walker, D.R. Kaul, C.A. Kauffman, A.G. Freifeld, D.C. Slagle, A.B. Kressel, L.J. Wheat. False-positive *Aspergillus* galactomannan assay in solid organ transplant recipients with histoplasmosis.
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Abstract: Post-transplantation histoplasmosis may be acquired via inhalation, may result from endogenous reactivation, or may be derived from the allograft. The *Histoplasma* and *Aspergillus* enzyme-linked immunoassays are increasingly being relied upon for rapid diagnosis of fungal infections, especially in immunocompromised patients. We describe 4 cases of solid organ transplant recipients who had histoplasmosis and a falsely positive *Aspergillus* galactomannan (GM) obtained from the serum or bronchoalveolar lavage (BAL) fluid. We also report our experience, testing for *Histoplasma* antigen (Ag) in specimens positive for *Aspergillus* GM. From January 2007 through December 2010, of 2432 unique patients who had positive *Aspergillus* GM tests, 514 (21%) were tested for *Histoplasma* Ag, and 27 were found to be positive. Most specimens that tested positive for both *Aspergillus* and *Histoplasma* were obtained by BAL. False-positive tests for *Aspergillus* GM can occur in immunosuppressed patients who have histoplasmosis, and may obscure the correct diagnosis.

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Histoplasmosis is a disease endemic in the Mississippi and Ohio River valleys, Central America, several countries in South America, and parts of Asia and Africa. Post-transplantation histoplasmosis is relatively uncommon even in these endemic areas (1). Infection may be acquired via inhalation from the environment, may result from endogenous reactivation in the setting of immunosuppression, or may be derived from the allograft. The diagnosis is established by microbiologic or histopathologic evidence of fungal infection. The *Histoplasma* enzyme-linked immunoassay (EIA) can detect antigen (Ag) in serum, urine, bronchoalveolar lavage (BAL) fluid, or cerebrospinal fluid, and has become a useful diagnostic tool, especially in immunosuppressed patients (2).

Galactomannan (GM), a heat-stable polysaccharide present in the cell wall of most fungi, is released during fungal growth in the host. The Platelia *Aspergillus* EIA detects a circulating GM Ag produced by *Aspergillus* species and a few other fungi. False-positive results of the assay have been reported after exposure to semisynthetic penicillins, such as amoxicillin and piperacillin, derived from molds of the genus *Penicillium*. Cross-reactivity between the *Aspergillus* GM and

Histoplasma Ag has also been observed (3). Herein, we describe 4 patients with histoplasmosis, initially misdiagnosed as aspergillosis, because of a falsely positive *Aspergillus* GM assay. We also review whether testing for *Histoplasma* Ag was requested on specimens sent to MiraVista Diagnostics that were positive for the *Aspergillus* GM.

Case reports

Case 1

A 60-year-old woman from Minnesota with autoimmune hepatitis and cirrhosis underwent deceased-donor liver transplantation in 2009. She developed diarrhea followed by fevers and hypoxia, 1 month after transplantation. Chest x-ray (CXR) showed bilateral interstitial infiltrates. She progressed to respiratory failure, requiring mechanical ventilation. *Histoplasma capsulatum* was recovered from blood cultures and BAL specimens; BAL fluid did not yield *Aspergillus* species. Urine *Histoplasma* Ag was markedly elevated (>39 ng/mL). Serum *Aspergillus* GM was positive at an optical density index of 2.6. The patient responded to treatment with liposomal amphotericin B (L-AmB), which was later changed to itraconazole.

The patient was found to have moderate to severe acute cellular rejection of the allograft, 7 months after transplantation, which was refractory to corticosteroids. Therefore, the patient received anti-thymocyte globulin. She went on to develop chronic allograft rejection with progressive hepatic failure and worsening renal function. Chest computed tomography (CT) scan demonstrated a 2.5 cm rounded nodule and an adjacent nodular infiltrate. In BAL fluid, the *Aspergillus* GM index was positive at 0.55 (cutoff for positivity: 0.5) and this was concerning for aspergillosis. The *Histoplasma* Ag was markedly elevated at 29.63 ng/mL. Small pleomorphic yeasts were seen on fungal smear, but *H. capsulatum* did not grow in culture. The serum itraconazole level was undetectable. Antifungal treatment was changed to L-AmB, which she received for 6 weeks. She underwent successful combined liver and kidney transplantation, 10 months after the first transplant. Treatment was eventually stepped down to itraconazole in liquid form.

Case 2

A 25-year-old woman from Michigan with renal failure precipitated by meningococcal meningitis underwent a living-donor renal transplantation in 2005. She developed fever associated with frontal headache, 2 years after transplantation. Chest CT scan showed a small nodule in the right upper lobe surrounded by ground-glass opacities. After initiating empirical antimicrobial treatment, she developed increasing dyspnea, and CXR showed diffuse pulmonary infiltrates. She eventually required mechanical ventilation. Serum *Aspergillus* GM index was 3.03, and she was started on voriconazole and micafungin for possible aspergillosis. However, urine *Histoplasma* Ag was >39 ng/mL, and later blood and BAL cultures yielded *H. capsulatum*; BAL fluid did not grow *Aspergillus* species. L-AmB was started, and the patient was able to be extubated on hospital day 11. Antifungal treatment was changed to itraconazole. The patient completed 18 months of treatment without recurrence of disease.

Case 3

A 40-year-old woman from Nebraska with a history of alcoholic cirrhosis underwent living-donor liver transplantation in 2009. The patient presented with fevers, tachycardia, shortness of breath, and a right-sided pleural effusion, 3 weeks post transplant. Chest CT scan showed multifocal nodular consolidation throughout the lungs. Pleural fluid grew viridans *Streptococcus* and *Candida albicans*. The patient remained febrile. The BAL *Aspergillus* GM index was 4.49, with a negative serum GM. The BAL *Histoplasma* Ag was >39 ng/mL. The BAL fluid grew *H. capsulatum* and never showed *Aspergillus*. The patient was treated with voriconazole for 12 months because of concern for possible concomitant invasive pulmonary aspergillosis, and her disease resolved.

Case 4

A 60-year-old man from Illinois with end-stage renal disease due to focal segmental glomerulosclerosis underwent living-donor renal transplantation in 2008. This was his second renal transplant. He presented with a febrile illness 2 years later. Physical exam was notable for new symmetric polyarthritis. Chest CT scan showed diffuse bilateral interstitial infiltrates. Serum *Aspergillus* GM index was 8.61. As the patient had recently received high-dose steroids, the diagnosis of invasive aspergillosis was considered likely, and voriconazole was begun. The BAL fluid yielded *H. capsulatum*, but no *Aspergillus* species. The urine

Histoplasma Ag was 25 ng/mL and the serum Ag was 19.4 ng/mL. The patient initially responded to voriconazole, but then had recurrent fever, and therapy was changed to L-AmB for 4 weeks, followed by itraconazole.

Methods

The cases described had clinical signs and symptoms attributed to fungal infection and positive culture for *H. capsulatum*. Specimens stored at −20°C at MiraVista Diagnostics in Indianapolis were tested with the Platelia *Aspergillus* GM EIA and the *Histoplasma* Ag EIA (4). As of February 2010, for both assays, serum specimens were pretreated with EDTA at 100°C for 6 min. The BAL specimens were pretreated for the *Aspergillus* GM EIA only. Urine specimens were not pretreated. Results of *Aspergillus* GM testing from January 1, 2007 through December 31, 2010 were reviewed to determine if testing for *Histoplasma* Ag was requested by the ordering practitioners on specimens that were positive for *Aspergillus* GM. We then identified the proportion of specimens containing *Aspergillus* GM that were also positive for *Histoplasma* Ag.

Results

Demographic characteristics, Ag levels, and culture results of the reported cases are summarized in Table 1. As shown in Table 2, of 2432 unique patients who had positive *Aspergillus* GM tests from January 2007 through December 2010, 514 (21%) were tested for *Histoplasma* Ag, and 27 were found to be positive. Sixteen of the positive samples were BAL specimens and 11 were serum specimens. A follow-up serum specimen for *Aspergillus* GM was submitted within 4 days of the initial serum specimen in 1 patient and was also positive: initial, 7.7 index units; follow-up, 6.5 index units. Among patients with negative *Histoplasma* Ag, the median *Aspergillus* GM index was 1.4 units (range, 0.5–11.9). In those with positive *Histoplasma* Ag, the median serum *Aspergillus* GM index was 3.2 units (range, 0.7–10.9) ($P = 0.096$).

The number of patients who had tests performed in both assays, increased from 15% in 2007 to 25% in 2010. More specifically, in 2010, 10 patients, separate from those reported herein, had positive results for both assays. Nine had a positive BAL *Aspergillus* GM and 1 had a positive serum GM. Urine *Histoplasma* Ag was positive in 7 specimens, serum Ag in 6 specimens, and BAL Ag in 5 specimens. Three patients were stated to have histoplasmosis and did not have aspergillosis, 1 had dual infection with both *Histoplasma* and *Aspergillus*, and 1 had blastomycosis. In the 5 other cases, further clinical data were unavailable.

Discussion

Our report illustrates that false-positive results for *Aspergillus* GM in serum or BAL fluid can lead to a mistaken diagnosis of aspergillosis in solid organ transplant recipients who have histoplasmosis. No other causes of false positivity, such as exposure to amoxicillin or piperacillin, were identified in the reported cases. False-positive reactions for *Aspergillus* GM have been shown to occur twice as often in specimens that have *Histoplasma* Ag levels of >39 ng/mL (3), as was noted in 3 patients in this report. Major

Demographic characteristics, antigen levels, and culture results in the reported cases

Age, gender	*Histoplasma* Ag* (ng/mL), serum	*Histoplasma* Ag* (ng/mL), urine	*Histoplasma* Ag* (ng/mL), BAL	*Aspergillus* GM** (OD index), serum	*Aspergillus* GM** (OD index), BAL	Growth of *Histoplasma capsulatum* in culture
60 F	ND	>39	ND	2.60	ND	Blood, BAL
	<0.6	2.36	29.63	Negative	0.55	No growth
25 F	ND	>39	ND	3.03	6.20	Blood, BAL
40 F	Negative	18.75	>39	Negative	4.49	BAL
60 M	19.4	25.0	ND	8.61	ND	BAL

*For *Histoplasma* Ag: <0.6–3.9 ng/mL is low positive; 4.0–19.9 ng/mL is moderately positive; 20.0 to >39 ng/mL is highly positive.
**For *Aspergillus* GM: Cutoff for positivity is 0.5.
Ag, antigen; BAL, bronchoalveolar lavage fluid; GM, galactomannan; OD, optical density; ND, not done.

Table 1

Testing for *Histoplasma* antigen in samples that had a positive *Aspergillus* galactomannan assay

Year	2007	2008	2009	2010	Total
Number of patients with positive *Aspergillus* GM	246	433	729	1024	2432
Number of patients with positive *Aspergillus* GM tested for *Histoplasma* Ag	38 (15%)	80 (18%)	140 (19%)	256 (25%)	514 (21%)
Number of patients with positive *Aspergillus* GM and *Histoplasma* Ag	4 (11%)	5 (6%)	8 (6%)	10 (4%)	27 (5%)

GM, galactomannan; Ag, antigen.

Table 2

differences between the 2 fungi in the chemical structure of the galactofuranose side chains of the GM (5) explain why high concentrations of *Histoplasma* Ag are required for cross-reactivity. The *Aspergillus* GM index, however, was not significantly different in patients who had a negative *Histoplasma* Ag test when compared with those who had a positive *Histoplasma* Ag test.

Cross-reactivity between *Histoplasma* Ag and *Aspergillus* GM has been described in experimental animal models (3). Few reports have documented a positive reaction for *Aspergillus* GM in immunosuppressed patients with disseminated histoplasmosis (6–8). False positivity of the *Aspergillus* GM has also been described in blastomycosis (9), cryptococcosis (10), coccidioidomycosis (L.J. Wheat, unpublished data), paracoccidioidomycosis (11), and infection due to *Penicillium marneffei* (12) and *Geotrichum capitatum* (13).

Cross-reactivity in the *Histoplasma* Ag assay can occur in infections due to other endemic fungi, such as *Paracoccidioides brasiliensis*, *P. marneffei*, *Blastomyces dermatitidis* (14), and *Coccidioides immitis* (15). Notably, *Histoplasma* and *Blastomyces* Ags are immunologically identical. In this report, 1 patient with blastomycosis had a falsely positive *Histoplasma* Ag assay, as has been previously described (16). In most dual-tested cases, we do not have further data and some of these may have been caused by endemic mycoses other than histoplasmosis.

No false-positive *Histoplasma* Ag measured in urine or serum has been reported in patients who had invasive aspergillosis. Presumably, this is a result of the low level of circulating GM in these patients in comparison with the high level of circulating *Histoplasma* Ag in patients with disseminated histoplasmosis. However, in regard to BAL fluid, a stronger possibility of a falsely positive exists for *Histoplasma* Ag assay occurring in a patient who actually has invasive pulmonary aspergillosis. In a study of 60 BAL fluid specimens positive for *Aspergillus* GM, 5 were also positive in the *Histoplasma* Ag assay, but all at values <0.6 ng/mL (17). The sensitivity of the *Histoplasma* Ag assay in BAL fluid was estimated to be 93.5% and the specificity 97.8%.

H. capsulatum may take as long as 4 weeks to grow in the laboratory. Results of Ag testing are available within days after collection of the specimen. Increasingly, Ag testing is relied upon for rapid diagnosis of fungal infections. When cross-reactivity occurs in non-culture-based tests, this can create confusion, as occurred in the cases presented in this report. Although rare, dual infection with both *Histoplasma* and *Aspergillus* is possible and should be considered in the appropriate clinical setting. In all patients, culture and histopathologic evidence for specific fungal organisms remain important for definitive diagnosis.

Despite prior recognition of cross-reactivity and recommendations to exclude histoplasmosis in patients with positive results for *Aspergillus* GM (3), *Histoplasma* Ag testing was only performed for 21% of such cases. Notably, a modest year-to-year increase was observed in requests for *Histoplasma* Ag testing in specimens positive for *Aspergillus* GM (Table 2). This level of testing can be further improved as more practitioners become aware of the cross-reactivity between the 2 assays. Differentiating between the 2 disease entities has important treatment implications, especially when it comes to alternative therapeutic agents. Echinocandins, which can be used in the treatment of invasive aspergillosis, are not active against *H. capsulatum*, and the effectiveness of voriconazole has not been established for histoplasmosis (18).

In conclusion, these findings indicate that cross-reactivity of *Aspergillus* GM and *Histoplasma* Ag can lead to false-positive *Aspergillus* EIA in patients who actually have histoplasmosis. Especially in immunosuppressed patients with diffuse pulmonary infiltrates and a relevant epidemiologic history, it is important to test for *Histoplasma* Ag, as well as *Aspergillus* GM, in serum and BAL fluid.

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