CLINICAL AND VACCINE IMMUNOLOGY, Oct. 2011, p. 1781–1782 Vol. 18, No. 10
1556-6811/11/$12.00 doi:10.1128/CVI.05017-11

Cross-Reactivity in the *Histoplasma* Antigen Enzyme Immunoassay Caused by Sporotrichosis▽

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Received 8 March 2011/Returned for modification 7 April 2011/Accepted 26 July 2011

Several endemic mycoses cause cross-reactions in the *Histoplasma* antigen enzyme immunoassay. Herein, a positive *Histoplasma* antigen result has been recognized in a patient with sporotrichosis.

Sporotrichosis is considered a "global health problem" in immunocompromised patients (7). Although *Sporothrix schenckii* is the main human pathogen, genetic sequencing indicates that the *S. schenckii* complex is comprised of several species: *S. albicans*, *S. brasiliensis*, *S. globosa*, *S. luriei*, *S. mexicana*, and *S. schencki* (7, 8).

The diagnosis of sporotrichosis can be suspected by demonstration of small yeast cells (3 to 5 μm) with multiple buds, some of which are cigar-shaped or show large spherical bodies (Fig. 1A). While serological diagnosis by detection of antibodies to mycelial antigens has been described, cross-reactions occurred in about one-quarter of patients with histoplasmosis (1). Antigen detection has proven useful for diagnosis of other fungal infections but has not been reported in sporotrichosis. Cross-reactivity in the MVista *Histoplasma* antigen enzyme immunoassay (EIA) occurs in 70 to 90% of patients with blastomycosis, paracoccidioidomycosis, and penicilliosis marneffei (2) and 59% of patients with coccidioidomycosis (5). Herein, cross-reactivity is reported in a patient with sporotrichosis.

A 34-year-old African-American male from Amarillo, Texas, with untreated AIDS was admitted with a fluctuating of level of consciousness. He had complained of dysphagia, but additional information, including epidemiological history, was not obtainable because of impaired mental status. Examination showed extensive ulcers and nodules on the oral mucosa, face, hands, and chest and impaired mental status. The CD4 count was 11 cells/ml, and the HIV viral load was 110,911 copies/ml. Tests for anti-*Blastomyces* and anti-*Coccidioides* antibodies and cryptococcal antigenemia were negative, but *Histoplasma* antigenuria was detected at 1.7 U (reference range, below 1.0 U). Skin biopsy showed yeast cells, some with multiple buds and spherical bodies (Fig. 1B), and *Sporothrix* was isolated by culture from the skin and blood. Identification was based upon using lactophenol cotton blue staining of colonies showing narrow hyphae with slender tapering conidiophores at right angles to the hyphae and tear-shaped conidia arranged in rosette-like clusters at the apex of the conidiophores, as well as conversion of the mold to yeast with typical cigar bodies at 37°C. The patient was treated with amphotericin B based upon the skin biopsy findings, but developed multiorgan failure and died after 6 days of treatment. Repeat blood cultures were negative after 24 h of antifungal therapy.

The antigen detected in specimens from patients with histo-

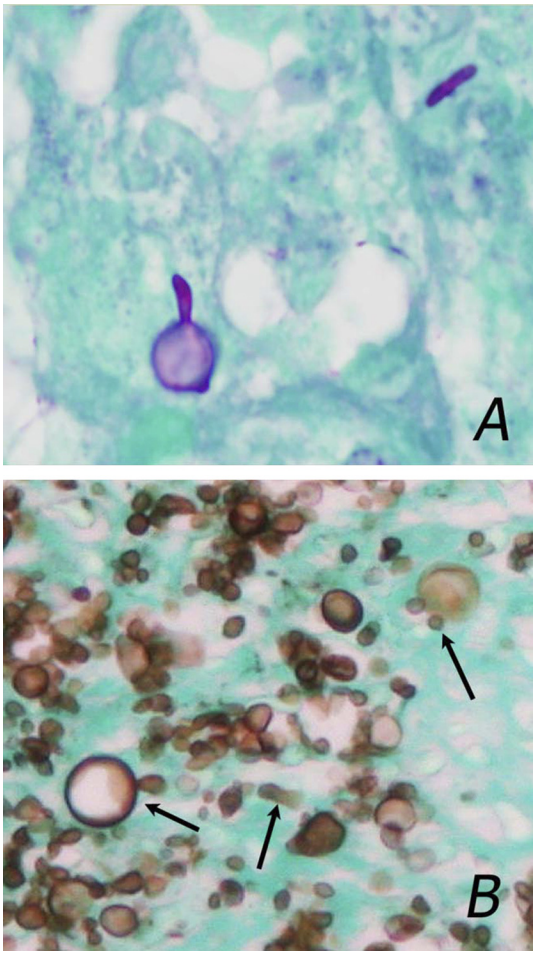

FIG. 1. (A) Photomicrograph of tissue using periodic acid-Schiff stain with a light green counterstain, showing typical features, including cigar-shaped cells and large spherical bodies. (Shown is a representative image that was not from this case.) (B) Photomicrograph of a skin biopsy specimen from the patient using Gomori methenamine silver stain, showing large numbers of small yeast cells, some demonstrating multiple buds, the cigar shape, and spherical bodies. Arrows point to cells showing these features.

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▽ Published ahead of print on 3 August 2011.

TABLE 1. *Aspergillus* galactomannan and *Histoplasma* antigen results in culture supernatants from three isolates of *S. schenckii* grown in *Histoplasma* macrophage medium

Specimen	Result by:	
	Platelia *Aspergillus* EIA (index units)[a]	MVista *Histoplasma* antigen EIA (U)[b]
Isolate 1		
Undiluted	6.6	Negative
1:10 dilution	3.0	Negative
Isolate 2		
Undiluted	6.5	Negative
1:10 dilution	3.2	Negative
Isolate 3		
Undiluted	5.2	Negative
1:10 dilution	1.4	Negative
Isolate 4		
Undiluted	11.2	4.1
1:10 dilution	2.0	1.2
Positive control	4.9	36.8
Histoplasma macrophage medium	0.1	Negative

[a] Results of 0.5 U or higher are positive.
[b] Results of 1.0 U or higher are positive.

plasmosis in the MVista *Histoplasma* antigen EIA is a galactomannan that has (1→6)-α-D-galactofuranosyl side chains (2), which contain the epitopes detected in the antigen assay. A galactomannan with (1→5)-β-D-galactofuranosyl side chains has been described in *S. schenckii*, but its immunoreactivity was not reported (9). The epitope detected in the Platelia *Aspergillus* EIA is also (1→5)-β-galactofuranose (6), and low-level cross-reactivity in the *Histoplasma* antigen EIA has been reported in 8% of patients with aspergillosis (4). *Aspergillus* antigen testing was not performed for this patient. Four isolates of *S. schenckii* were incubated at 37°C for 7 days in *Histoplasma* macrophage medium (12), and culture supernatants were tested in the Platelia *Aspergillus* EIA and the *Histoplasma* antigen EIA. *Aspergillus* galactomannan was detected in the culture supernatants from all four isolates, and *Histoplasma* antigen was detected in one (Table 1), suggesting weak cross-reactivity, as observed with *Aspergillus* galactomannan (11).

False-positive *Histoplasma* antigen results in patients without fungal infections and cross-reactions in aspergillosis are usually at low concentrations: 1 to 4 U in the second generation (10) and 2 ng/ml or less in the quantitative assay (2). In this case, the fungal burden was high, considering the extensive mucocutaneous involvement, large number of yeast cells in the skin lesions, fungemia, multiorgan failure, and rapid death. In patients with severe histoplasmosis, the antigen concentration is low in only 5% of cases (3). Thus, a low-positive *Histoplasma* antigen result, as in this case, in a severely ill patient suggests an etiology other than histoplasmosis. Raising the cutoff would reduce false-positive results and cross-reactions caused by fungi with weakly related epitopes, but at the cost of lower sensitivity for diagnosis of histoplasmosis: low-positive results occur in 15% of disseminated histoplasmosis (3) and should not be disregarded. Instead, false-positive results and cross-reactivity caused by fungi with weakly cross-reactive antigens, including *Sporothrix* spp., should be suspected in severely ill patients with low-positive results. The magnitude of cross-reactivity in the *Histoplasma* antigen assay and the occurrence of cross-reactivity in the Platelia *Aspergillus* EIA in specimens from patients with sporotrichosis must be determined by testing a larger number of cases.

We thank Nancy Kountz and Thomas R. Kluzak, Via Christi Hospitals and University of Kansas School of Medicine, and Stephen D. Allen, Indiana University School of Medicine, for providing photomicrographs and Melinda Smedema and Samantha Swartzentruber, MiraVista Diagnostics, for testing culture supernatants of *Sporothrix* spp. with the Platelia *Aspergillus* EIA and *Histoplasma* antigen EIA.

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