CLINICAL AND VACCINE IMMUNOLOGY, Jan. 2009, p. 132–133 Vol. 16, No. 1
1556-6811/09/$08.00+0 doi:10.1128/CVI.00310-08

NOTES

Cross-Reactivity of *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, and *Cryptococcus* Species in the Commercial Platelia *Aspergillus* Enzyme Immunoassay▽

Melissa O. Xavier,[1] Alessandro C. Pasqualotto,[1,2,3]* Isabel Cristina E. Cardoso,[4] and Luiz Carlos Severo[1,3,4]

Postgraduation Program in Pulmonary Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil[1]; Infection Control Department, Santa Casa-Complexo Hospitalar, Porto Alegre, Brazil[2]; Conselho Nacional de Desenvolvimento e Pesquisa (CNPq), Brasilia, Brazil[3]; and Mycology Laboratory, Santa Casa-Complexo Hospitalar, Porto Alegre, RS, Brazil[4]

Received 21 August 2008/Returned for modification 3 November 2008/Accepted 13 November 2008

Cross-reactivity in the Platelia *Aspergillus* enzyme immunoassay was evaluated using 120 sera from patients with paracoccidioidomycosis, histoplasmosis, and cryptococcosis. At a cutoff value of 0.5, positivity rates were 50%, 67%, and 50%, respectively. The implications for these findings are discussed.

The sandwich Platelia *Aspergillus* enzyme immunoassay (EIA) (Bio-Rad, France) is a commercial test that has been used extensively for the diagnosis of invasive aspergillosis (IA). The assay detects galactofuranose-containing side chains of galactomannan, an antigen released from *Aspergillus* hyphae during growth in the host (1). In addition to varied sensitivity, the test is limited by false-positive results, mostly due to the use of antibiotics. Like *Aspergillus* species, many other fungi have been shown to produce galactomannan (1, 2, 4–6, 9). Here we evaluate the cross-reactivity of four important pulmonary fungal pathogens—*Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, and *Cryptococcus gattii*—in the commercial galactomannan test.

(This study was partially presented at the 48th Interscience Conference on Antimicrobial Agents and Chemotherapy [ICAAC abstract M-1720], Washington, DC, 2008.)

A total of 120 serum samples from 102 patients were evaluated. These included 30 samples from patients with each of the following: paracoccidioidomycosis (30 patients), histoplasmosis ($n = 26$), cryptococcosis due to *C. neoformans* ($n = 28$), and *C. gattii* ($n = 18$). Sera from patients not suspected to have fungal disease have been tested in parallel as negative controls ($n = 12$). Platelia *Aspergillus* EIA testing was performed according to the manufacturer's specifications, using a cutoff value of 0.5. Samples were obtained from the serum bank at the Santa Casa-Complexo Hospitalar mycology laboratory in Brazil. Most samples had been stored at −20°C for <5 years. A few samples were in the freezer for more than 10 years, including a 17-year-old serum sample.

All patients with paracoccidioidomycosis had positive serology (immunodiffusion) results for *P. brasiliensis*. The infection was diagnosed by microscopy in 27 cases and by culture in Sabouraud agar for five patients. Histoplasmosis was confirmed by immunodiffusion, microscopy, and culture in 30, 11, and 6 cases, respectively. Cryptococcosis was diagnosed by the Latex-Crypto antigen detection system (Immuno Mycologics, Inc.). *C. neoformans* and *C. gattii* were differentiated after culture in canavanine-glycine-bromothymol blue agar.

The results of this study are summarized in Table 1. All sera from patients not suspected to have fungal disease tested negative for the galactomannan test, with optical density indexes ranging between 0.16 and 0.41. Positive results for the galactomannan test occurred for 50%, 67%, 63%, and 37% of the samples from patients with *P. brasiliensis*, *H. capsulatum*, *C. neoformans*, and *C. gattii* infection, respectively. Lower galactomannan indexes were observed for *C. gattii* than for *C. neoformans* ($P = 0.017$). No correlation was observed between the galactomannan optical density index and the Latex titer for *Cryptococcus* species ($R^2 = 0.003$; $P = 0.689$).

We looked for other variables that could result in false-positive results in the galactomannan test. Only two patients were on hemodialysis, and none had received piperacillin-tazobactam or amoxicillin-clavulanate. False-positive results usually do not occur with other antimicrobial drugs, even when serum concentrations are high (8). Although the influence of freezing and thawing on the galactomannan antigen assay is not clear, it is usually believed that long-term storage may actually decrease galactomannan levels (1). The impact of serum storage at different freezing temperatures, from −20°C to −80°C, is also unknown.

When more than one sample was obtained from the same patient, concordant results were usually observed. Discordant results occurred for one individual with histoplasmosis and for another one with *C. neoformans* infection. In both cases, a positive sample was followed by a negative test after antifungal therapy was started, a very well-known phenomenon (1). Sera from seven patients with *C. gattii* infection were tested more

* Corresponding author. Mailing address: Av Independência 75, Hospital Dom Vicente Scherer, Serviço de Controle de Infecção Hospitalar, Porto Alegre 90035-075, Brazil. Phone: 55 51 99951614. Fax: 55 51 32148629. E-mail: pasqualotto@santacasa.tche.br.

▽ Published ahead of print on 19 November 2008.

TABLE 1. Results of serum galactomannan testing from patients infected with *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, and *Cryptococcus gattii*

Agent[b]	Serum galactomannan optical density index[a]			No. (%) of samples with optical density index of:			
	Range	Mean value	95% Confidence interval	>2.0	>1.0 to 2.0	≥0.5 to 1.0	<0.5
Paracoccidioides brasiliensis	0.23–5.47	0.99	0.53–1.44	3 (10.0)	5 (16.6)	7 (23.3)	15 (50.0)
Histoplasma capsulatum	0.24–5.38	1.43	0.88–1.98	7 (23.3)	7 (23.3)	6 (20.0)	10 (33.3)
Cryptococcus neoformans	0.18–>10	1.95	0.95–2.94	9 (30.0)	5 (16.6)	5 (16.6)	11 (36.6)
Cryptococcus gattii	0.18–>9	1.03	0.38–1.68	4 (13.3)	3 (10.0)	4 (13.3)	19 (63.3)

[a] Optical density indexes higher than or equal to 0.5 are considered positive. High indexes (i.e., >1.0) increase the test specificity in the diagnosis of invasive aspergillosis (1).
[b] $n = 30$ (samples) for each group.

than once. For six of these patients, the same serum sample was run in duplicate, and one patient was tested six times. Again, the most discordant results were seen after antifungal therapy was started. Due to the retrospective character of our study, we were not able to obtain additional clinical information for our patients. Thus, any assumption about reduced galactomannan indexes in patients started on antifungal therapy is speculative at most. Moreover, the impact of the mycosis clinical presentation (e.g., fungemia versus nondisseminated disease) on galactomannan testing could not be evaluated.

Previous studies have described that cross-reactivity might occur with several fungi in the commercial galactomannan test. These include *Geotrichum capitatum* (4) and *Penicillium marneffei* (5), molds that contain galactomannan in the cell wall. The cross-reactivity of *H. capsulatum* and *C. neoformans* has also been described (2, 5, 6, 9). The mechanism of *H. capsulatum* cross-reactivity is not yet elucidated. Although samples from patients with histoplasmosis may give positive results for the Platelia *Aspergillus* EIA, samples from patients with aspergillosis are negative for the *Histoplasma* antigen EIA (9). The *C. neoformans* cell wall contains galactoxylomannan, an antigen similar to galactomannan, which may result in a cross-reaction in the galactomannan test (2). However, this was not confirmed in a recent study (3). The hypothesis that genotypic variations could explain the differences observed among studies with *C. neoformans* remains to be tested.

To the best of our knowledge, this is the first study in which reactivity with antibody used for the Platelia assay was tested for patients infected with *P. brasiliensis* and *C. gattii*. These are important pulmonary pathogens, affecting mostly nonimmunocompromised hosts. Galactomannan is a cell wall component for both the yeast and the mycelial forms of *P. brasiliensis*. In addition, *H. capsulatum* and *P. brasiliensis* are phylogenetically closely related fungi (7). Similarly for *C. neoformans*, the presence of an epitope causing cross-reactivity may also occur for *C. gattii*. Actually a previous investigation showed that soluble antigens from one reference strain of *C. gattii* tested positive in the Platelia *Aspergillus* EIA (2).

This study reinforces that caution should be taken when considering a positive galactomannan test for a patient with respiratory infection. The diagnostic of IA using galactomannan may be tricky for patients coming from areas where paracoccidioidomycosis and histoplasmosis are endemic. Cryptococcosis also affects solid organ transplant recipients, a population at risk for IA, which may present with multiple pulmonary nodules. Moreover, these mycoses may differ in terms of response to antifungal drugs. For instance, echinocandins are not active against *Histoplasma* species, and experience using voriconazole for histoplasmosis remains limited (9). Both *Geotrichum* and *Cryptococcus* species are usually susceptible to amphotericin B and azoles but intrinsically resistant to echinocandins. Conversely, *P. brasiliensis* infection can be treated with sulfonamides. In order to properly interpret the meaning of a positive galactomannan test, clinical and epidemiological data should be taken into consideration.

We disclose no conflicts of interest in association with the manuscript.

This study was supported by a grant from the Conselho Nacional de Desenvolvimento Científico e Technológico (CNPq), Brazil.

REFERENCES

1. **Aquino, V. R., L. Z. Goldani, and A. C. Pasqualotto.** 2007. Update on the contribution of galactomannan for the diagnosis of invasive aspergillosis. Mycopathologia **163:**191–202.
2. **Dalle, F., P. E. Charles, K. Blanc, D. Caillot, P. Chavanet, F. Dromer, and A. Bonnin.** 2005. *Cryptococcus neoformans* galactoxylomannan contains an epitope(s) that is cross-reactive with *Aspergillus* galactomannan. J. Clin. Microbiol. **43:**2929–2931.
3. **De Jesus, M., E. Hackett, M. Durkin, P. Connolly, A. Casadevall, R. Petraitiene, T. J. Walsh, and L. J. Wheat.** 2007. Galactoxylomannan does not exhibit cross-reactivity in the Platelia *Aspergillus* enzyme immunoassay. Clin. Vaccine Immunol. **14:**624–627.
4. **Giacchino, M., N. Chiapello, S. Bezzio, F. Fagioli, P. Saracco, A. Alfarano, V. Martini, G. Cimino, P. Martino, and C. Girmenia.** 2006. *Aspergillus* galactomannan enzyme-linked immunosorbent assay cross-reactivity caused by invasive *Geotrichum capitatum*. J. Clin. Microbiol. **44:**3432–3434.
5. **Huang, Y., C. Hung, C. Liao, H. Sun, S. Chang, and Y. Chen.** 2007. Detection of circulating galactomannan in serum samples for diagnosis of *Penicillium marneffei* infection and cryptococcosis among patients infected with human immunodeficiency virus. J. Clin. Microbiol. **45:**2858–2862.
6. **Narreddy, S.** 2008. False-positive Aspergillus galactomannan (GM) assay in histoplasmosis. J. Infect. **56:**80–81.
7. **San-Blas, G., and G. Niño-Vega.** 2008. *Paracoccidioides brasiliensis*: chemical and molecular tools for research on cell walls, antifungals, diagnosis, taxonomy. Mycopathologia **165:**183–195.
8. **Singh, N., A. Obman, S. Husain, S. Aspinall, S. Mietzner, and J. E. Stout.** 2004. Reactivity of Platelia *Aspergillus* galactomannan antigen with piperacillin-tazobactam: clinical implications based on achievable concentrations in serum. Antimicrob. Agents Chemother. **48:**1989–1992.
9. **Wheat, L. J., E. Hackett, M. Durkin, P. Connolly, R. Petraitiene, T. J. Walsh, K. Knox, and C. Hage.** 2007. Histoplasmosis-associated cross-reactivity in the BioRad Platelia *Aspergillus* enzyme immunoassay. Clin. Vaccine Immunol. **14:**638–640.