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Aspergillus Galactomannan Enzyme-Linked Immunosorbent Assay Cross-Reactivity Caused by Invasive *Geotrichum capitatum*

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We report three cases of invasive *Geotrichum capitatum* infection in patients with acute leukemia for which an enzyme-linked immunosorbent assay (ELISA) for *Aspergillus* galactomannan was positive, with no evidence of aspergillosis. Supernatants obtained from suspensions of 17 *G. capitatum* strains gave positive reactions with the *Aspergillus* galactomannan ELISA. These clinical and laboratory data seem to suggest that *G. capitatum* produces a soluble antigen that is cross-reactive with *Aspergillus* galactomannan.

Aspergillus galactomannan detection by a sandwich enzyme-linked immunosorbent assay (ELISA) is widely used throughout the world in diagnosing invasive aspergillosis, and it has been introduced among the international microbiological criteria for the diagnosis of this fungal infection in immunocompromised hosts (2, 11). A major problem with the detection of circulating galactomannan is the occurrence of false-positive results, which, in some cases, have been shown to be related to cross-reactivity with other opportunistic fungi (11). We report cases of three acute-leukemia patients who developed disseminated infection by *Geotrichum capitatum* for which ELISA for *Aspergillus* galactomannan was positive, with no evidence of aspergillosis.

Patient 1 was a 7-year-old child with acute lymphoblastic leukemia in second relapse who underwent salvage chemotherapy in February 2004. Ten days after this treatment, the patient developed fever and periorbital edema; broad-spectrum antibiotics and liposomal amphotericin B were administered. A week later, several serum samples were positive for galactomannan antigen and caspofungin was added. A computed-tomography scan showed an abscess of the cerebral base starting from the ethmoidal plan and involving the hypophyseal region. Therefore, a surgical debridement was performed; *G. capitatum* was isolated from samples of pus, liquor, and bone (identification was performed with the VITEK system [bioMerieux Italia, Rome, Italy]). Galactomannan antigen was detected in the pus. Histology of a bone fragment disclosed necrotic tissue in which mycotic invasion was evident. On periodic acid-Schiff- and Grocott-stained sections, the fungi consisted of septate hyphae, slightly bent with parallel disposition and only occasional branching at a wide acute angle or budding at a right angle. A few spores and fragmentation of the mycelium in arthroconidia were also observed. Caspofungin was replaced with voriconazole. Within a few days, the patient's clinical condition improved and galactomannan antigen, for which tests were previously constantly positive, disappeared from the blood. Two months after surgery, while under voriconazole treatment, the child underwent two consecutive aploidentical bone marrow transplants from his father and his mother, but after a few weeks, the child died from sepsis of an unknown origin. Serum galactomannan antigen was no longer detected.

Patient 2 was a 9-year-old girl with a diagnosis of myelodysplasia secondary to acute lymphoblastic leukemia and who underwent an allogeneic bone marrow transplant, receiving bone marrow from her HLA-compatible sister. Seven months later, she developed acute myeloblastic leukemia (AML) and started intensive chemotherapy. Hematological remission was not obtained, and 3 months after AML diagnosis (April 2005), the girl underwent salvage cytotoxic treatment that resulted in a very severe and prolonged aplasia, and antifungal prophylaxis with caspofungin was started. After a week, she developed fever, a cough, and papular skin lesions. Serum galactomannan antigen was repeatedly detected at increasing concentrations, and after a week, the girl died despite broad-spectrum antibiotic and antifungal treatment with caspofungin and liposomal amphotericin B. The same day, *G. capitatum* was isolated from multiple blood cultures performed during the febrile period.

Patient 3 was a 49-year-old man diagnosed with AML in February 2006. After induction remission chemotherapy, he developed fever and neutropenic and antibiotic treatment with piperacillin-tazobactam was started. The fever disappeared after 48 h. Five days later, the patient was newly febrile, and a computed-tomography scan showed a pulmonary infiltrate on the left basal region associated with numerous micronodular lesions without evidence of a halo sign. Antibiotic therapy with piperacillin-tazobactam was replaced with treatment with a combination of meropenem, teicoplanin, and amikacin. Three days later, based on the persistence of fever and the appearance of multiple little papular skin lesions highly suggestive of candida infection, treatment with liposomal amphotericin B (5 mg/kg/day) was started. A serum sample was positive for galactomannan antigen, and cultures from blood and from a skin lesion needle aspirate yielded a yeast morphologically compatible with *G. capitatum*. Based on the previous experience with the two above-described cases of *G. capitatum* infection, lipo-

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TABLE 1. Reactivities of soluble antigens from suspensions of clinical and reference strains of *G. capitatum* and other fungi in the Platelia *Aspergillus* assay

Species and strain	Site of isolation[b]	Index of reactivity in the Platelia *Aspergillus* assay
A. fumigatus Rom	BAL	>5
A. fumigatus Mor	Sputum	>5
A. niger Tri	Ear	>5
C. albicans ATCC 90028	Reference strain	<0.5
C. parapsilosis ATCC 22019	Reference strain	<0.5
C. neoformans		
Sob	CSF	3.4
Imp	CSF	2.9
G. capitatum		
469	Urine	1.2
448	Blood	1.8
E202	Blood	2.2
PE2	Blood	1.9
BG2	Blood	1.8
Pisa	Respiratory tract	3.2
116A	Respiratory tract	1.9
434	Blood	1.2
114A	Blood	1.5
Monica	Respiratory tract	1.8
Palermo Dr	Blood	1.9
Palermo Ma	Blood	2.5
E299[a]	Blood	>5
ATCC 62963	Reference strain	2.3
ATCC 62964	Reference strain	1.9
ATCC 200927	Reference strain	2.7
ATCC 200925	Reference strain	1.7

[a] Isolate from patient 3.
[b] BAL, bronchoalveolar lavage fluid; CSF, cerebrospinal fluid.

somal amphotericin B was replaced with voriconazole. Two days later, identification of the *G. capitatum* yeast was confirmed with the VITEK system and a second serum sample was positive for galactomannan antigen. After a week of voriconazole therapy, the patient developed a respiratory failure and died despite broad-spectrum antibiotic and antifungal treatment. A bronchoalveolar lavage sample was negative for fungi and mycobacteria but yielded *Stenotrophomonas maltophilia*. Two further serum samples were negative for galactomannan antigen.

Despite the positive Platelia *Aspergillus* assay results for multiple samples from the three patients, the histopathological, microbiological, and clinical findings suggested that coinfection by *G. capitatum* and *Aspergillus* spp. was unlikely. However, the possibility of an undetected *Aspergillus* infection cannot be formally excluded because the patients did not undergo autoptic examination and *Aspergillus* PCR was not performed on the available specimens. At the time of serum sample collection, the patients did not receive antibiotics, such as piperacillin-tazobactam or amoxicillin-clavulanate, known to cause false positivity in the Platelia *Aspergillus* assay (11). Therefore, we hypothesized that Platelia *Aspergillus* positivity may have been due to a cross-reaction with antigens released by *G. capitatum*. Consequently, we tested 17 clinical strains of *G. capitatum* and other control fungi for their reactivity in the ELISA assay, according to a previously described procedure (4) (Table 1). Cultures were plated on Sabouraud dextrose agar and incubated at 30°C for 5 days. For each strain, 5 to 10 colonies were suspended in 1 ml distilled water. After vigorous agitation, the suspensions were centrifuged for 5 min at 10,000 × *g*, and the supernatants were heated and tested with Platelia *Aspergillus*. Each experiment included several controls, i.e., distilled water and negative, positive, and standard (1-ng/ml) serum samples provided by the manufacturer. The ratio between the optical density of the serum sample and that of the control standard serum was calculated for each sample. According to the recently suggested 0.5 cutoff value for the test, values of ≥0.5 were considered relevant (10). The 17 *G. capitatum* strains, including the isolate from patient 3 (isolates from patients 1 and 2 were no longer available), showed various positive indexes of reactivity (range, 1.2 to >5). The three *Aspergillus* strains and the two strains of *Cryptococcus neoformans* showed high and intermediate indexes of reactivity, respectively, in the Platelia *Aspergillus* assay. The two reference *Candida* strains did not show any reactivity.

The monoclonal antibody EB-A2, used both in the Pastorex *Aspergillus* latex agglutination test and in the Platelia *Aspergillus* assay, showed cross-reactivity with other fungi, bacteria, drugs, foods, and cotton swabs (3, 8, 11–14). The occurrence of false-positive results with the *Aspergillus* galactomannan detection test for patients receiving antibiotics, in particular piperacillin and amoxicillin either alone or combined with β-lactamase inhibitors, is a major drawback of this technique; as such, results may lead to a false diagnosis of probable or possible aspergillosis and unjustified antifungal therapy (3, 11). This finding has forced some institutions to change their antibacterial protocols and the FDA to issue a warning (5, 7). False-positive reactivity in the Platelia *Aspergillus* assay has also been observed in the context of an invasive infection caused by other opportunistic fungal pathogens. The reactivities of the monoclonal antibody EB-A2 with the exoantigens of some filamentous fungi other than *Aspergillus* spp. were documented several years ago, and further fungal pathogens have been added to this series over the years (4, 8, 13, 14). Culture supernatants of *Trichophyton* species, *Botrytis* species, *Wallemia sebi*, *Cladosporium cladosporioides*, *Penicillium* species, *Paecilomyces variotii*, *Acremonium* species, *Alternaria* species, and *Fusarium* species showed weak to strong reactivities with the sandwich ELISA (8, 13, 14), and very recently, *Cryptococcus neoformans* galactoxylomannan was also shown to be cross-reactive with *Aspergillus* galactomannan (4). On the other hand, only a few cases of non-*Aspergillus* fungal infections in whom the test for galactomannan was positive have been reported to date: two human immunodeficiency virus-positive patients, one with disseminated *Penicillium marneffei* infection and one with cryptococcosis (4, 12), and one blood stem cell transplant patient with a Hickman intravenous line infection by *Penicillium* species (9).

Our three cases and in vitro studies show that *G. capitatum* exoantigens may be cross-reactive in the *Aspergillus* galactomannan assay. Although almost all of the yeast mannans do not contain other carbohydrate components except a trace of *N*-acetylglucosamine and glucose, some yeasts, including *Geotrichum* spp., are known to have galactomannan as a cell wall component (1), but until now, no data on detection of circulating galactomannan in patients with invasive infection due to this genus of yeasts have been published.

G. capitatum, formerly known as *Trichosporon capitatum* or *Blastoschizomyces capitatus*, is an uncommon, but frequently fatal, cause of invasive infections in immunocompromised patients, particularly those with hematological malignancies (6). In a recent retrospective multicenter study from Italy, the incidence of *G. capitatum* infection among patients with acute leukemia was 0.5%, with a 55.7% crude mortality rate (6). Early diagnosis is difficult to establish because clinical symptoms are not specific and frequently resemble those of invasive candidosis. Blood cultures, which are the "gold standard" for diagnosis of invasive *G. capitatum* infections, are negative in about 30% of cases and usually take several days to show detectable growth of yeast cells. Therefore, noncultural methods could be useful for the improvement of laboratory diagnosis, but experiments on *G. capitatum* antigens or DNA detection are not available to date. This false-positive reactivity in the Platelia *Aspergillus* assay does not seem to constitute an important problem for specificity in the diagnosis of invasive aspergillosis, as the incidence of *G. capitatum* infections is very low even in leukemic patients (6). On the contrary, this unexpected reactivity in the test could be considered a useful diagnostic and prognostic tool in the management of *G. capitatum* infections. A positive galactomannan assay result for a patient with an infective clinical picture suggestive of *Candida* infection could indicate the possibility of a *G. capitatum* infection, considering also that galactomannan serum detection may precede by several days the microbiological documentation of the infection (for patients 1, 2, and 3, serum galactomannan was detected 7, 9, and 2 days before identification of the pathogen from cultures, respectively). This is a crucial point not only for specific diagnosis but also for the choice of antifungal therapy. In fact, *G. capitatum* is susceptible to amphotericin B and azoles, in particular voriconazole, but is intrinsically resistant to echinocandins, which are drugs of choice for the treatment of invasive *Candida* and *Aspergillus* infections (6). Interestingly, for patient 1, infection improved when caspofungin was replaced by voriconazole; patient 2 developed a breakthrough *G. capitatum* infection while under antifungal prophylaxis with caspofungin; and for patient 3, the choice of voriconazole therapy was based on the observation of multiple maculopapular skin lesions suggestive of disseminated *Candida* infection, the positivity of the Platelia *Aspergillus* assay, and the experience with the previous two cases of *G. capitatum* infection.

In conclusion, the cases we have presented and in vitro tests showed a previously unknown cross-reactivity of *G. capitatum* with *Aspergillus* galactomannan. Although this cross-reactivity may constitute a drawback for the specificity of the test, the knowledge of this phenomenon may be useful in the diagnosis and management of invasive *G. capitatum* infections, which are rare but cause severe complications in patients with hematological malignancies.

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