We report a reactive *Aspergillus* galactomannan enzymatic immunoassay against the serum of a patient with invasive *Prototheca zopfii* infection. Analysis of the supernatants of suspensions of *P. zopfii* and other *Prototheca* isolates revealed positive results as well. These data suggest cross-reactivity with the serum *Aspergillus* galactomannan assay in invasive protothecosis.

*Prototheca* species are classified as achorophylic algae and are opportunistic pathogens (7). *Prototheca* infections are generally divided into three groups: olecranon bursitis, localized cutaneous infections, and disseminated disease. The latter is more commonly reported in severely immunocompromised individuals, whereas localized infections can present in patients with normal host immunity (9). Therapeutic recommendations for protothecal infections are limited because susceptibility profiles are variable and do not correlate with successful treatment (8). Generally, treatment consists of medical and/or surgical therapy. Based on data from several case reports and in vitro studies, amphotericin B is presumably the most effective therapy (8, 9). Correct identification of *Prototheca* species is of therapeutic and prognostic value but may be difficult due to their yeast-like colony appearance on routine media (14). Here, we report on another possible misleading aspect of invasive protothecal infections.

We describe the case of a 63-year-old man diagnosed with follicular B-cell non-Hodgkin’s lymphoma (stage IV) in 1999. Following two relapses (2007 and 2010), autologous (2008) and allogeneic (2010) stem cell transplantation were performed. Subsequently, he developed chronic graft versus host disease (GVHD) in the summer (2010) stem cell transplantation were performed. Subsequently, he developed chronic graft versus host disease (GVHD) in the summer (2010). At hospital admittance, two out of three aerobic blood cultures (2007/2010), autologous (2008) and allogeneic (2010) stem cell transplantation were performed. Subsequently, he developed chronic graft versus host disease (GVHD) in the summer (2010). At hospital admittance, two out of three aerobic blood cultures were positive, and growth on blood and Sabouraud agar (BD Difco Laboratories, Le Pont De Claix, France) revealed yeast-like colonies. Pink colonies were observed on Candida CHROMagar (CHROMagar Company, Paris, France). Both unstained and calcofluor-stained wet mounts of the colonies showed characteristic asymmetrical morula-like structures. Protothecal infection was suspected, but the API 20C AUX (bioMérieux, Marcy l’Etoile, France) assimilation profile did not allow identification. Furthermore, identification could not be obtained with matrix-assisted laser desorption ionization—time of flight mass spectrometry (Microflex, database version 3.1.2.0; Bruker Daltonik GmbH, Bremen, Germany). Finally, identification of *Prototheca zopfii* by 18S rRNA sequencing was established by the Belgian Coordinated Collections of Micro-Organisms/Institute of Hygiene and Epidemiology, Mycology (BCCM/IHEM) (now the Scientific Institute of Public Health).

Two follow-up aerobic blood cultures taken 2 days after admission were still positive. Treatment with amphotericin B was continued with an initial good clinical response, and additional blood cultures were negative. Nevertheless, 2 weeks later, the patient’s condition deteriorated, and he died. No autopsy was performed. During these 2 weeks, serum galactomannan antigen was repeatedly positive (Fig. 1), and bronchoalveolar lavage (BAL) fluid showed a galactomannan index of 1.33. The normal value for BAL fluid samples is currently debated (4), but this result should be considered positive.

Despite the repeatedly positive galactomannan results, conventional diagnostic methods for *Aspergillus* infection gave negative results: *no Aspergillus* species could be cultured, and no radiographic patterns compatible with the European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) definitions (3) were seen. However, in GVHD patients, elevated galactomannan levels due to translocation of dietary galactomannan through the intestinal mucosa into the blood have been described (1, 6, 11). Other possible known causes of false-positive galactomannan results were excluded (12, 16, 19). Because coinfection with *P. zopfii* and *Aspergillus* spp. was rather unlikely, cross-reactivity in the Platelia *Aspergillus* assay caused by *Prototheca* was suspected.

To investigate the occurrence of reactivity in the serum galac-
Galactomannan assay caused by *Prototheca* species, we retrospectively analyzed a serum sample from a patient recently diagnosed in our laboratory with *Prototheca wickerhamii* infrapatellar bursitis (21). The serum sample from this patient did not show reactivity in the galactomannan assay (index, 0.11). The infection, however, was limited to the knee and was not disseminated.

Subsequently, we analyzed supernatants from these two species (*P. zopfii* and *P. wickerhamii*) and three additional strains, which were selected from the BCCM/IHEM collection (*P. zopfii* IHEM 20268, *Prototheca stagnora* IHEM 4201, and *Prototheca cutis* IHEM 23764). Distilled water, a *Candida albicans* clinical isolate (negative control), and an *Aspergillus fumigatus* clinical isolate (positive control) were included as controls. Using the conditions described by Dalle et al. (2), suspensions of three isolates—the *P. zopfii* clinical isolate, *P. wickerhamii* clinical isolate, and *P. stagnora* IHEM 4201—resulted in high galactomannan assay index values of 3.29, 1.92, and 1.77, respectively. For the two other strains—*P. cutis* IHEM 23764 and *P. zopfii* IHEM 20268—only weak reactivity (indexes between 0.53 and 1.06) was found when heavy suspensions were used. These results show that *Prototheca* spp. can cause reactivity in the galactomannan assay and suggest that the positive serum and BAL fluid galactomannan tests in the patient with invasive infection are caused by *Prototheca*, although a coinfection with *Aspergillus* could not be excluded.

The serum galactomannan enzymatic immunoassay is widely used for the diagnosis of aspergillosis in immunocompromised hosts. Since its introduction, some false-positive results caused by cross-reactivity with other fungi and yeasts, such as *Cryptococcus neoformans*, *Geotrichum capitatum*, *Penicillium* spp., *Alternaria* spp., *Histoplasma* spp., and *Paeilomyces* spp., have been demonstrated (2, 5, 18, 20, 22). To the best of our knowledge, no data are available on the detection of galactomannan antigen in serum from patients with invasive protothecosis. Galactomannan is not reported to be present in the cell wall of *Prototheca* species. *P. zopfii* β-glucan consists of a 1,4-linked (90%) and 1,3-linked (10%) glucose backbone (17), whereas *Aspergillus* galactomannan has a 1,4-linked mannose basic structure with galactose branching in position 6. However, comparison of the monosaccharide cell wall compositions of *Aspergillus niger* and *P. zopfii* illustrated the presence of glucose and mannose in both species but the absence of galactose in *P. zopfii* (10). It is unclear how cross-reactivity originated. The molecule responsible for cross-reactivity might be a structural component of the cell wall or a secreted glycoprotein.

The impact of this newly detected cross-reactivity is expected to be limited, as invasive protothecosis has a low prevalence (9). However, differentiation between *Aspergillus* and *Prototheca* is important for the choice of antifungal therapy; echinocandins can be used to treat invasive aspergillosis, whereas no data are available for their activity against *Prototheca* spp. (15). This report confirms once more that positive galactomannan levels should be interpreted with caution.

Both clinical *Prototheca* strains have been deposited in the BCCM/IHEM collection (P. wickerhamii, IHEM 25446; and *P. zopfii*, IHEM 25445).

**Nucleotide sequence accession number.** The 18S rRNA sequence for *P. zopfii* has been deposited in GenBank under accession no. JQ679396.

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**REFERENCES**


