Madurella mycetomatis is a causative organism of eumycetoma, a disease characterized by tumorous masses, and formation of grains and purulent material (Ahmed et al., 2004). At present treatment of mycetoma is based on surgery and long-time antifungal therapy to prevent relapses. Ideally, continuous diagnostic monitoring of patients to determine the success or failure of antifungal therapy should be implemented. For the diagnosis of invasive aspergillosis, for instance, a sandwich ELISA is widely used (Mennink-Kersten et al., 2004). Circulating galactomannan or galactomannan-containing proteins are detected by the assay against the galactofuran epitopes present on these molecules (Mennink-Kersten et al., 2004). Immunological cross-reactivity has been described for Paecilomyces variotii, Penicillium spp., Geotrichum capitatum and Cryptococcus neoformans (Dalle et al., 2005; Swanink et al., 1997). In the present study such galactomannan cross-reactivity was investigated for M. mycetomatis.

Culture supernatant from 32 M. mycetomatis isolates was obtained by culturing a 70% transmission inoculum in RPMI medium for 7 days at 37°C (van de Sande et al., 2005). Serum was obtained from patients and healthy controls, and frozen at −20°C until needed. The Platelia Aspergillus assay (Bio-Rad) was performed according to the manufacturer’s instructions. The amount of galactomannan was determined from a concentration curve of pure galactomannan. Strain-dependent amounts of M. mycetomatis galactomannan-like compounds were secreted into the culture medium (Fig. 1). According to the cut-off value of 1 ng ml⁻¹, six isolates secreted no galactomannan-like compounds. The other 24 strains secreted these compounds in concentrations of up to 12.9 ng ml⁻¹.

In only 2 of the 16 patient sera galactomannan levels above the cut-off value of 1 ng ml⁻¹ were found (Fig. 1). There was no correlation with either the extent or the duration of the disease. In none of the healthy volunteer sera were galactomannan levels above 1 ng ml⁻¹ found.

Significant amounts of galactomannan-like compounds were found to be secreted into the culture medium. Cross-reactivity in the galactomannan assay has already been described (Dalle et al., 2005). The fact that galactomannan is secreted in the culture medium but not recovered from patient circulation could be due to a variety of reasons. It could be that the structure of the sclerotic in patients (cement material, melanin, tight package) prohibits diffusion of the galactomannan. For aspergillosis it has already been demonstrated that in some cases galactomannan can be detected in the bronchoalveolar lavage but not in the serum (Becker et al., 2003; Verweij et al., 2000). Furthermore, it could be that antifungal treatment of mycetoma hampers the performance of this test. All patients were treated with either high doses of itraconazole or ketoconazole. Marr et al. (2005) demonstrated that administration of antifungal agents on the day of test sampling decreased the sensitivity of the assay significantly. It could also be that the antigen is not secreted because of a lack of nutrients. It has already been shown for Aspergillus niger and Penicillium fellutanum that secreted antigens can be reused as a carbon source when the organisms are deprived of nutrients (Mennink-Kersten et al., 2004; Park et al., 1997). In nutrient-rich conditions, Aspergillus spp. release high amounts of galactomannan during growth, but these amounts can be reduced in a nutrient-poor environment such as serum. In conclusion, M. mycetomatis secreted compounds that are immunologically cross-reactive to

**Fig. 1.** Galactomannan concentration in *M. mycetomatis*. Galactomannan concentrations were determined in the culture supernatant after culturing for 7 days in RPMI culture medium (■). Galactomannan concentrations were also determined in patient serum (△) and serum from healthy Sudanese controls (▽).
Aspergillus galactomannan in vitro but not in vivo.

Wendy W. J. van de Sande,1 Ahmed H. Fahal,2 Henri Verbrugh1 and Alex van Belkum1

1Erasmus MC, University Medical Center Rotterdam, Department of Medical Microbiology & Infectious Diseases, Dr Molewaterplein 40, 3015 GD Rotterdam, The Netherlands
2Mycetoma Research Group, University of Khartoum, Khartoum, Sudan

Correspondence
Wendy W. J. van de Sande
w.vandesande@erasmusmc.nl


