Madurella mycetomatis compounds cross-reactive with galactomannan are detectable in culture supernatant but not in serum

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 through an antibody raised 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 sclerotia 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

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