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β-1,3-D-Glucan and Galactomannan as Biomarkers for the Detection of Invasive *Geotrichum* and *Magnusiomyces* Infections: a Retrospective Evaluation

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ABSTRACT *Magnusiomyces* and *Geotrichum* species are ascomycetous yeasts that can cause potentially life-threatening invasive fungal infections commonly referred to as geotrichosis. In this study, we aimed to estimate the incidence and mortality of these infections in a German tertiary care center. Furthermore, we evaluated the suitability of the fungal biomarkers galactomannan (GM) and β-1,3-D-glucan (BDG), which are both recommended as surrogate markers for *Magnusiomyces capitatus* infection by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the European Confederation of Medical Mycology (ECMM) joint clinical guidelines for the diagnosis and management of rare invasive yeast infections for detection of invasive geotrichosis. Cases meeting the inclusion criteria for invasive *Magnusiomyces/Geotrichum* infection were retrospectively identified. Serum samples and culture supernatants were analyzed with two commercially available fungal antigen tests (Platelia *Aspergillus* Ag EIA and Wako β-glucan test). For a control cohort, outpatient samples sent for lues testing were included. Thirty-eight cases of *Magnusiomyces/Geotrichum* infection were identified over an 11-year observation period. In the majority of cases, the fungus was isolated from intra-abdominal specimens of patients with a history of abdominal surgery/procedures (n = 32). All cases of fungemia occurred exclusively in haemato-oncologic patients (n = 14). Thirty-day survival was 42% in the fungemia and 43% in the intra-abdominal geotrichosis group. Serum samples were available for 23 patients (14 bloodstream and nine intra-abdominal infections). While BDG sensitivity was 65%, none of the sera was GM positive. This finding was supported by *in vitro* experiments analyzing fungal culture supernatants: *M. capitatus* secretes significant amounts of BDG but not GM. Specificity was 96% for BDG and 100% for GM. *Magnusiomyces* and *Geotrichum* infections are not limited to haemato-oncologic patients. Contrasting the current ESCMID/ECMM recommendation, our results indicate that GM is no suitable biomarker for the diagnosis of *Magnusiomyces* infection. Contrarily, BDG sensitivity is comparable to that of candidemia.

KEYWORDS *Magnusiomyces*, *Geotrichum*, *Saprochaete*, geotrichosis, invasive fungal infection, galactomannan (GM), beta-1,3-D-glucan (BDG), fungal antigen testing, serology

Over the last decades, a rising incidence of fungal infections has been observed (1). This increase was not solely caused by species of the four most prominent fungal genera, *Aspergillus*, *Candida*, *Cryptococcus*, and *Pneumocystis*, but also by a significant

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number of rarely reported opportunistic fungal pathogens (1). Among these emerging pathogens are arthroconidial yeastlike ascomycetes like *Geotrichum* and *Magnusiomyces* spp. Infections caused by these fungi are still summarized as geotrichosis in the clinical routine because the *Magnusiomyces* species with the utmost epidemiological significance, i.e., *Magnusiomyces capitatus*, was formerly referred to as *Geotrichum capitatum* (2, 3). *M. capitatus* (teleomorph)/*Saprochaete capitata* (anamorph) was the subject of extensive taxonomic reclassification and renaming (2): other previous names of this species were *Blastoschizomyces capitatus*, *Trichosporon capitatum*, *Dipodascus capitatum*, and *Blastoschizomyces pseudotrichosporon* (3).

Infections caused by *Geotrichum* and *Magnusiomyces* spp., which both belong to the family of Dipodascaceae, are mainly reported from Mediterranean Europe (75 to 87% of all cases) (2, 4, 5). Since members of the Dipodascaceae family were rarely detected in large surveillance studies from areas north of the Alps, some authors even consider the respective species endemic pathogens (6). However, in recent years, an increasing number of geotrichosis cases have been reported in Central Europe (6, 7). It was hypothesized that this increase is linked to climatic changes caused by global warming (6, 8).

Impaired host defense due to antineoplastic chemotherapy of hematologic malignancies was reported to be the most important risk factor (9). The by far most common manifestation of *Geotrichum*/*Magnusiomyces* infection is fungemia, which is sometimes associated with deep organ involvement as a sign of dissemination (2, 5, 9). The initial misdiagnosis of this yeast infection as *Candida* infection is considered to be a major reason for the very poor outcome of *Geotrichum*/*Magnusiomyces* infection with mortality rates of up to 65% (2, 5): medically important *Geotrichum* and *Magnusiomyces* spp. are characterized by intrinsic resistance to echinocandin antifungals, which are commonly used as an empirical therapy in suspected *Candida* infection (2, 9). Therefore, early and correct identification of the pathogen is crucial for the outcome.

To date, diagnosis still relies almost exclusively on cultivation, especially in blood culture (BC) (2, 9). BC is of outstanding importance for diagnosis of bacterial bloodstream infections. Compared to this, diagnosis of mycotic bloodstream infections is often more challenging due to decreased BC sensitivity and prolonged time to positivity (10).

Specific methods to prove *Geotrichum*/*Magnusiomyces* infection with rapid turnaround times such as PCR or serologic biomarkers have not been established. In a very small number of partially contradicting case reports, a potential use of fungal antigen tests, i.e., galactomannan (GM) and β-1,3-D-glucan (BDG), for detection of invasive *Geotrichum*/*Magnusiomyces* infections has been proposed (7, 11–16). While BDG is a pan-fungal marker that can be detected in invasive fungal infection (IFI) caused by many different pathogens, including *Aspergillus* spp., *Candida* spp., and *Pneumocystis jirovecii*, GM antigenemia can particularly observed in invasive aspergillosis (17). Due to a lack of evidence, here are no or only weak recommendations for antigen testing in mycoses caused by *Geotrichum* spp. or *M. capitatus* (9).

This study aims to evaluate the characteristics and outcomes of cases of *Geotrichum*/*Magnusiomyces* infection in a German tertiary care center over an 11-year period. In addition, this study provides the first systematic comparison of two serological biomarkers that have been recommended for the diagnosis of emerging fungal infections caused by *Geotrichum* and *Magnusiomyces* spp. to date.

MATERIALS AND METHODS

This retrospective analysis was performed at the Max von Pettenkofer Institute of Hygiene and Medical Microbiology that hosts the central microbiology laboratory for the University Hospital of Ludwig Maximilians University (LMU) Munich, a 2,000-bed university medical center in Munich, Germany. Antigen testing was performed at the Max von Pettenkofer Institute and the Institut für Hygiene und Mikrobiologie (Julius Maximilians University Würzburg, Würzburg, Germany).

In the period between 2008 and 2018, we identified all patients who were positive for growth of *Geotrichum* or *Magnusiomyces* spp. from primarily sterile specimens. If applicable, cases were categorized as proven invasive infection according to the European Organization for Research and Treatment of Cancer Mycoses study group (EORTC-MSG) consensus definitions (18). However, in some cases, data

on the time point of drain placement, which are required for the diagnosis of proven intra-abdominal infection, were missing, impeding the categorization. In contrast to most other relevant fungal pathogens, for *Geotrichum* or *Magnusiomyces* spp., no EORTC/MSG criteria have been defined for the categories of probable or possible infection. The respective cases, in which the missing information concerning the proven IFI subcriterion "a freshly placed (<24 h ago) drain" impeded categorization, are referred to as "invasive infection" following the clinical diagnosis.

In 23 of 38 included cases, a serum sample was available which had been obtained within ±10 days from the sampling date (day 0) of the specimen positive for the respective fungi (mean of −1 day and median of 1 day). For a negative cohort, we included time-matched serum samples (same date of sampling) of outpatients tested for syphilis.

Geotrichum/Magnusiomyces spp. from BC were cultured using the BD Bactec blood culture system (BD, Franklin Lakes, USA). Intra-abdominal specimens were incubated on Columbia blood agar and/or Sabouraud agar (BD). Thirty-five of 38 isolates were identified using the matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) system (Bruker Daltonics, Bremen, Germany) as previously described (19) and three isolates by analytical profile index testing (API ID 32C; bioMérieux, Marcy-l'Étoile, France).

Antigen testing was conducted using the Wako β-glucan test (FujiFilm Wako Chemicals Europe, Neuss, Germany) at a cutoff of 7 pg/ml and the Platelia *Aspergillus* Ag EIA (Bio-Rad Laboratories, Hercules, CA, USA) at a cutoff optical density index (ODI) of 0.50. Serum samples had been stored for up to 12 years at −20°C and thawed once for analysis. All measurements were performed in parallel. For analysis of *in vitro* antigen production, clinical isolates of *A. fumigatus* (D141), *Geotrichum candidum*, and *Magnusiomyces* spp. from the strain collections of the Max von Pettenkofer Institute and the German Reference Centre for invasive mycoses (NRZMyk) were cultivated in synthetic defined (SD) medium (yeast nitrogen base with amino acids [Merck, Darmstadt, Germany; catalog no. Y1250], 2% [wt/vol] glucose). *Geotrichum/Magnusiomyces* cells in the exponential growth phase were inoculated in 10 ml of medium at an optical density (600 nm) of 0.1 (Ultrospec 10 cell density meter; Amersham Biosciences, Uppsala, Sweden). For *A. fumigatus*, 5×10^5 conidia were added to the medium. After 22 h at 37°C, cultures were centrifuged at 2,500 × *g* for 15 min, and supernatants were sterile filtered and processed for antigen testing using the same protocol as for serum samples. For BDG analysis, all culture supernatants were diluted 1:100 in SD medium, and for GM analysis, only the *A. fumigatus* supernatant was diluted 1:1,000 in SD medium. GM measurement was performed from three biological replicates. All assays were performed according to the manufacturers' instructions. The supernatant results were multiplied by the respective dilution factor.

This retrospective study was reviewed and approved by the ethics committee of our university hospital (Ethikkommission der Medizinischen Fakultät der LMU Munich) and conducted in accordance with the Declaration of Helsinki. A waiver of informed consent was granted. Sample processing and data analysis were performed anonymously. Clinical information and reference standards results were not available to the performers and readers of the assay.

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, La Jolla, USA). Bonferroni's multiple-comparison test and Fisher's exact test were used for calculation of statistical significance of *in vitro* GM results and serum BDG results. Assessment of the significance of serum GM results had to be omitted upon a lack of positive results. Statistical significance was assumed based on an α-level of 0.05.

RESULTS

In an 11-year observation period in a university hospital, 31 and 7 cases of invasive *M. capitatus* and *Geotrichum* spp. infections could be identified, respectively (Tables 1 and 2). The vast majority of infections (82%) and all bloodstream infections were caused by *M. capitatus*. All isolates were cultured from blood cultures (37%) or intra-abdominal specimens (63%), e.g., peritoneal fluid, intraoperative swabs, or drains. The criteria for a proven invasive fungal infection according to the EORTC/MSG definitions (18) were met in 71% of cases (Table 1). In the other cases (29%), the date of abdominal drain insertion was not available, thereby impeding the EORTC/MSG categorization (criterion, "freshly placed drain"). Eight of 24 (33%) cases of intra-abdominal infections were characterized by specimens that only yielded growth of *M. capitatus* or *Geotrichum* spp., while the remaining patients suffered from polymicrobial infections, including bacterial pathogens, e.g., *Enterobacterales* or enterococci.

The vast majority of infections (82%) and all bloodstream infections were caused by *M. capitatus*. Infections occurred almost exclusively in individuals with underlying haemato-oncologic disease (37%) or a history of abdominal surgery/procedures (61%). Notably, in our cohort, cases of fungemia were exclusively found in haemato-oncologic patients. In contrast, intra-abdominal *M. capitatus/Geotrichum* infections were not observed in haemato-oncologic patients. The most common indication for abdominal procedures (including surgery) was related to preexisting infections, i.e., exploratory

TABLE 1 Demographic, clinical, and microbiologic characteristics of invasive *M. capitatus* and *Geotrichum* spp. cases[a]

Characteristic	No.	%
Demographic characteristics		
Female sex	16	42
Age (mean)	59	
Serum available	23	61
Clinical characteristics		
Underlying conditions		
Abdominal procedure	23	61
Type of procedure		
Surgery	17	74
ERCP	6	26
Indication for procedure		
Infection, source control	11	29
Infection, exploratory laparotomy	5	13
Other	7	18
History of SOT	3	8
History of nonhematologic malignancy	16	42
Hematologic malignancy	14	37
History of HSCT	5	13
IMS	1	3
Outcome		
Date of death available	19	50
Survival $<$ 30 days	16	42
Microbiological characteristics		
Focus of infection		
Intra-abdominal	24	63
Bloodstream	14	37
Fungal species		
M. capitatus	32	84
G. candidum	2	5
Geotrichum silvicola	1	3
Geotrichum species (not differentiated)	3	8
EORTC/MSG category		
Proven	27	71
Not applicable	11	29

[a]ERCP, endoscopic retrograde cholangiopancreatography; SOT, solid organ transplantation; IMS, immunosuppressive therapy in the nonhematologic and nontransplant setting; HSCT, hematopoietic stem cell transplantation.

laparotomy and source control. The available data do not allow to differentiate whether the yeasts were the primary cause of disease or represented a secondary infection following abdominal surgery/procedures. Seventy percent of patients with intra-abdominal infection were prediagnosed with solid organ malignancy.

Forty-two percent of the patients died within 30 days after first cultural proof of the fungus. The mortality rate might be underestimated since some patients were discharged

TABLE 2 Frequency of isolation of *Geotrichum* spp./*M. capitatus* in the case group[a]

			No. of cases with the following no. of					
			Positive samples/case			Samples from the site of infection/other site		
Cases	Mean	Median	1	2–5	>5	1/0	>1/0	≥1/≥1
All cases	6	4	11	12	15	12	10	16
Cases with sera	9	9	0	9	14	0	8	15
Cases without sera	2	1	11	3	1	12	2	1

[a]Positive samples are defined by growth of fungi. Site of infection refers to the body site from which the specimen was obtained that allowed inclusion in this study. Other sites comprise all nonprimary sterile body sites that were tested positive for *Geotrichum* spp./*M. capitatus*.

TABLE 3 Measurement results of antigen testing[a]

Characteristic or result	GM	BDG
Sensitivity (%)	0	65
Focus of infection (%)		
Bloodstream	0	64
Intra-abdominal	0	67
Fungal species (%)		
M. capitatus (*n* = 22)	0	64
G. candidum (*n* = 1)	0	100
Sex (%)		
Female	0	88
Male	0	53
Distance to day 0 (%)		
≤1 day (*n* = 15)	0	80
≤3 days (*n* = 19)	0	79
>3 days (*n* = 4)	0	0
Specificity (%)	100	96
GM index/BDG level (pg/ml)		
Mean		
All samples	0.2	78.4
Positive samples		126.1
Median		
All samples	0.1	21.6
Positive samples		47.2
Maximum	0.4	1,034.8

[a]GM results in optical density indices. Day 0, day of sampling of the specimen positive for *M. capitatus/G. candidum* growth.

from our hospital into the palliative care setting. Notably, 30 days survival did not significantly differ between the fungemia group and the group with intra-abdominal infection. Most patients with intra-abdominal *M. capitatus/Geotrichum* infection that died within 30 days after proven diagnosis of fungal disease suffered from solid organ malignancy.

Sera were available for 23 of 38 cases (Table 1). All of the 23 cases included in the serologic analysis were characterized by at least two culture-positive samples, e.g., swabs or drains, excluding contamination (Table 2). Mean and median serum sampling dates were −1 day and day 0, respectively, with 65% of sera dating from the period of ±1 day from day 0, which was defined by the sampling date of the specimen that yielded fungal growth (Table 3). None of the included sera of the 23 *M. capitatus/Geotrichum* patients yielded positive GM results (Table 3). In contrast, BDG antigenemia was detected in 15 of 23 cases of *M. capitatus/Geotrichum* infections: 14 of 22 cases of *M. capitatus* infection and the sole case of *G. candidum* infection were positive for BDG. Interestingly, the latter case (*G. candidum* peritonitis) was characterized by the far-highest concentration of BDG (>1 μg/ml). However, close to the sampling date of the respective serum, *Candida glabrata* was recovered from bile fluid of this patient, which might be an alternative cause of BDG antigenemia. For all other BDG-positive individuals, there was no evidence for invasive infection with fungal pathogens other than *M. capitatus* in a period of ±3 weeks from day 0. Sensitivity significantly increased with closer proximity of the sampling dates of serum and BC/intra-abdominal specimen (87% versus 25% for sera sampled in the period of ± 1 days from day 0 versus more distant sera; $P < 0.01$). Sensitivities for bloodstream and intra-abdominal infections were 64% and 67%.

For each serum of the case group, a time-matched sample of an individual nonsuspicious for fungal infection was included in a control cohort. While there was one low-positive

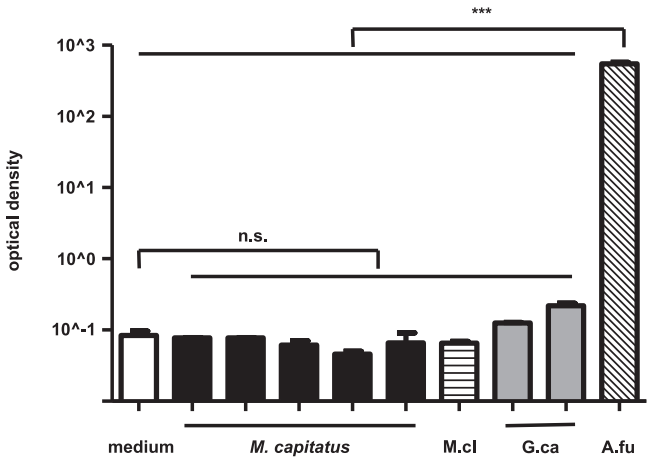

FIG 1 *In vitro* galactomannan (GM) production. Culture supernatants of clinical isolates of five *M. capitatus* strains, one *M. clavatus* (M.cl) isolate, two strains of *G. candidum* (G.ca), and one *A. fumigatus* (A.fu) patient isolate were tested for GM. Testing was performed in biological triplicates (*A. fumigatus* supernatant diluted 1:1,000 in medium). ***, $P < 0.0001$; n.s., not significant.

BDG result (7.5 pg/ml), GM antigenemia was not detected in this group ($n = 23$; BDG and GM specificity, 96% and 100%, respectively).

Culture supernatants of two *M. capitatus* strains isolated from blood samples of patients included in this study were tested for GM using the Platelia *Aspergillus* antigen enzyme-linked immunosorbent assay (ELISA) (Fig. 1). Additionally, further clinical isolates from the collection of the National Reference Center for Invasive Fungal Infections (NRZMyk; two *G. candidum* strains from BCs, three *M. capitatus* strains from intra-abdominal specimens, and one *Magnusiomyces clavatus* strain from a respiratory tract sample) were included. Surprisingly, none of the *Magnusiomyces/Geotrichum* culture supernatants contained significant amounts of GM compared to the medium control. In marked contrast, supernatants of a clinical isolate of *A. fumigatus* (D141), which was included as a control and cultivated under similar conditions, exceeded the optical density of the medium control by a factor of >6,500 ($P < 0.0001$). It cannot be ruled out that the culture conditions put the *Geotrichum* strains at a disadvantage compared to *A. fumigatus* in terms of antigen synthesis, e.g., by lower fungal biomass or impairment of secretion. Importantly, the *A. fumigatus* and the *M. capitatus* supernatants were also subject to testing for another fungal cell wall component, i.e., BDG. Culture supernatants of all *Magnusiomyces* and *G. candidum* strains contained high BDG concentrations comparable to the *A. fumigatus* sample, indicating relative comparability under the applied culture conditions (Fig. 2).

DISCUSSION

Geotrichosis has been considered an uncommon condition outside the Mediterranean region with its mild climate (4, 5, 9). However, a careful review of the literature indicates that the incidence of *Geotrichum* and *Magnusiomyces* infections in areas north of the Alps is not as low as generally assumed and may even be increasing over the recent years (2, 6, 7, 20). It was proposed that the possibly increasing incidence of geotrichosis and other endemic fungal infections could be linked to climate change and global warming (6, 8, 21). However, currently, there is insufficient evidence that clearly establishes this link. In any case, cited reports and our data indicate that the epidemiology of invasive *Magnusiomyces* and *Geotrichum* infections is probably underestimated in the presumable non-/low-risk areas.

The common perception that these fungi are pathogens almost exclusively found in haemato-oncologic patients might have resulted in underdiagnosis of such infections. In fact, of all 38 cases with *Geotrichum* and *Magnusiomyces* spp. identified from primarily sterile specimens in an 11-year period, 24 patients had a recent history of

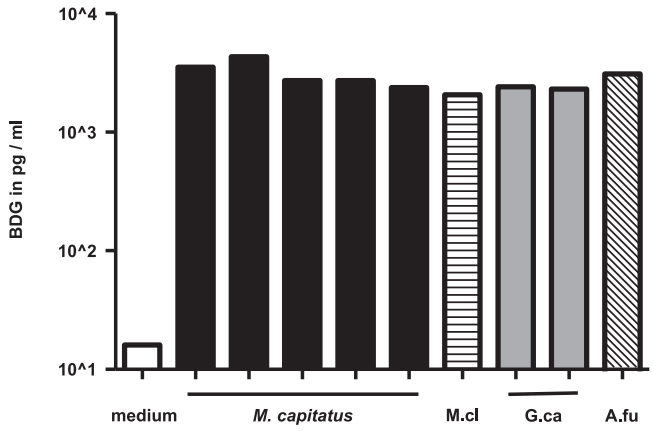

FIG 2 *In vitro* β-1,3-D-glucan (BDG) production. Culture supernatants of the respective isolates (see Fig. 1) were tested for BDG. All samples were diluted 1:100 in medium.

abdominal surgery/procedures. This indicates that these fungal infections might be more prevalent in nonhaemato-oncologic patients than commonly assumed. However, the clinical relevance of the intra-abdominal finding of *Magnusiomyces/Geotrichum* spp. remains unclear: discrimination between yeast contamination, colonization, and invasive infection in patients with suspected tertiary peritonitis is often not possible with certainty, resulting in the misinterpretation as a negligible finding (22). Awareness of this disease must be raised and the definition of patients at risk for *Magnusiomyces/Geotrichum* infection should be extended.

Interestingly, the focus of infection was strictly dependent on the underlying conditions. Bloodstream infections occurred only in haemato-oncologic patients and intra-abdominal infections only in nonhaemato-oncologic patients. This association indicates different etiologies: in haemato-oncologic patients, fungal cells presumably migrate from their typical habitat in the host, i.e., the gut, to the bloodstream by taking advantage of the impaired immunity caused by immunosuppressive antitumor therapy (9). In contrast, direct contamination during procedures penetrating the mucosal barrier is supposed to be the cause of intra-abdominal infections. In these patients, systemic infection is prevented by host immunity, which is typically not or, to a much lesser extent, compromised than in the first group. Consequently, one would expect the immunocompetent patients with yet-localized intra-abdominal fungal disease to have a better outcome than the haemato-oncologic patients with fungemia. In our cohort, 6 of 14 (43%) fungemia patients and 10 of 24 (42%) intra-abdominal mycosis patients died within 30 days after diagnosis. Importantly, the 30-day survival does not reflect the cause of death and, consequently, cannot be used to specifically attribute the fatal outcome to this infection. Particularly in cases of polymicrobial intra-abdominal infections, the significance of the detected fungi for the patients' conditions must be critically questioned: whether the recovery of *Magnusiomyces/Geotrichum* spp. evidences infection or only temporal contamination of the peritoneal cavity following intestinal trauma cannot be concluded with certainty from laboratory and clinical data. More detailed analyses should be performed in the future to identify factors that determine the outcome and to investigate the clinical relevance of intra-abdominal *Magnusiomyces/Geotrichum* infection. Furthermore, despite being based on the largest cohort of patients infected with these fungal pathogens, the present analysis could be biased due to the small sample size. For example, previous studies investigating *Geotrichum/Magnusiomyces* fungemia generally reported notably higher mortality rates (52 to 65%) than we found in our study (43%) (2, 4, 5).

Compared to bacteremia, detection of fungal bloodstream infection is hampered by lower BC sensitivity and prolonged time to positivity (10). Consequently, alternative techniques like antigen testing have a prominent role in the diagnosis of mycoses (17, 22). Analysis of the *Aspergillus*-specific cell wall polysaccharide GM in serum and BAL

fluids is a major tool in the diagnosis of invasive aspergillosis. Cross-reactivity with other pathogenic molds like *Fusarium* spp. or *Talaromyces marneffei* is a commonly observed feature of the GM ELISA (17). To date, no systematic studies that evaluate the performance of GM testing in clinical specimens in the setting of geotrichosis/*Magnusiomyces* infection have been published. There is a small number of anecdotal reports that suggest GM in serum of geotrichosis patients to become positive (11–13). Therefore, the 2014 clinical guidelines for the diagnosis of rare invasive yeast infections published by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the European Confederation of Medical Mycology (ECMM) recommend the use of GM as surrogate marker for *M. capitatus* infection (CIII) (9). However, none of the 23 patients for which sera were available in this study were positive for GM. In agreement with our data, there are a number of case reports and small case series of *Magnusiomyces* fungemia, in which all included patients (total number, 11) remained GM negative throughout the course of infection (14–16). Notably, the majority of tested sera were positive for BDG (14–16). The ESCMID/ECMM guideline, which was published before the respective reports, recommends BDG testing for the diagnosis of *M. capitatus* (CIII) (9). However, it is stated that this recommendation is based on *in vitro* findings only. In this study, BDG sensitivity in clinical samples was 65%, which is comparable to the sensitivity of 67% that we recently reported for the diagnosis of candidemia applying the same assay used in this study (Wako BDG assay) (23). Notably, the BDG cutoff was recently decreased from 11 pg/ml to 7 pg/ml by the manufacturer. Applying the outdated cutoff, one case of fungemia would be missed, resulting in a sensitivity of 61%. The sensitivity of the BDG assay in patients suffering from intra-abdominal *Geotrichum*/*Magnusiomyces* infection of about 67% was surprisingly high. Some of these cases might have become positive because of the pan-fungal nature of the antigen: it cannot be excluded that, besides *Geotrichum* or *Magnusiomyces*, also *Candida* spp. were transmitted from the gut to the peritoneum, resulting in BDG antigenemia due to invasive candidiasis. Furthermore, the possibility of BDG false positivity has to be considered: surgical materials that are typically used in the setting of intra-abdominal procedures, e.g., sponges and gauze, are a well-known cause of medical treatment-related BDG contamination (24).

To further investigate the unexpected finding of GM negativity of clinical samples, we aimed to analyze *in vitro* antigen production. It was demonstrated previously that also yeasts, i.e., *M. capitatus* and *Geotrichum* spp., are capable of producing different galactomannans *in vitro* (11, 25). To our surprise, we did not detect GM in the culture supernatant of several clinical isolates. It cannot be excluded that this result is an incidental finding due to the low number of isolates analyzed ($n = 8$). On the other hand, this discrepancy might be attributable to different experimental settings: in this study, we analyzed the culture supernatant of fungi grown in liquid culture with fully synthetic medium. Contrarily, Giacchino and colleagues grew fungal colonies on solid Sabouraud agar for 5 days, transferred colonies to double-distilled water (ddH_2O), and agitated this suspension prior to analysis (11). We cannot exclude that this processing may have resulted in the release of normally tightly bound cell wall constituents. Interestingly, the cell wall of pathogenic fungi such as Mucorales or *Cryptococcus neoformans* also contains BDG (26, 27), which is not (or at least in nondetectable quantities) released during infection so that BDG cannot be used for diagnosis of these mycoses (28).

Our data indicate that GM is not a suitable biomarker for the diagnosis of geotrichosis. This questions the recommendation of the ESCMID/ECMM guideline in which GM is suggested as surrogate marker for *M. capitatus* infection (9). In contrast to GM, significant amounts of BDG were detected in the culture supernatants of the analyzed *M. capitatus* strains (BC isolates of two cases included in this study). This is in good agreement with experimental data of Odabasi et al., who demonstrated comparable results analyzing *M. capitatus* and *Candida* spp. culture supernatants (29).

At the first glance, the finding of a BDG specificity of 96% is in good agreement with recent studies which evaluated the performance of the Wako BDG assay in

another yeast infection, i.e., candidemia (specificities of 93 to 96% in a comparable control cohort of bacteremia patients) (23, 30). Applying the outdated cutoff (11 pg/ml instead of 7 pg/ml), which was used in these previous studies, the specificity even increases to 100%. Besides statistical effects due to low case number, this finding is probably based primarily on selection bias: the control cohort consists of outpatients tested for lues who typically do not have any of the risk factors for BDG false positivity (24). This limitation must be considered upon assessment of the specificity analysis.

Conclusion. Our data indicate that invasive *Geotrichum* and *Magnusiomyces* infection is neither restricted to haemato-oncologic patients nor bloodstream infections. Awareness should be raised of the role of these mycoses as a complication of abdominal interventions. Based on our results, we suggest that BDG-based antigen testing can be a helpful tool for the detection of invasive *Magnusiomyces* infection. In contrast, GM-based antigen testing appears to be not suitable for detection of this mycosis.

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