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NOTES

Detection of (1→3)-β-D-Glucan as an Adjunct to Diagnosis in a Mixed Population with Uncommon Proven Invasive Fungal Diseases or with an Unusual Clinical Presentation▽

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This single-center observational prospective study evaluated the performance of (1→3)-β-D-glucan as an adjunct diagnostic tool in 12 patients with proven invasive fungal disease with different risk factors. The infections were due to either uncommon fungal pathogens such as dematiaceous molds (*Scedosporium apiospermum*, *Alternaria infectoria*, and *Cladosporium macrocarpum*) and hyaline septate molds (*Fusarium solani* and *Blastoschizomyces capitatus*) or *Aspergillus* spp. with unusual clinical presentations.

In the setting of clinical diagnosis in a tertiary hospital, invasive fungal disease (IFD) appears in a wide range of patients with different risk factors and underlying conditions. The diagnosis of IFD is challenging, and up to 75% of patients are not identified before autopsy (1).

Conventional microbiological and radiological techniques that have been used for the diagnosis of IFD are relatively insensitive. Recently, noninvasive culture-independent diagnostic tools have been developed to improve diagnosis and clinical management.

(1→3)-β-D-Glucan (BG) is a cell wall component of fungi. Its presence in serum and normally sterile body fluids is a marker of IFD and is an indirect mycological criterion in the revised definitions of IFD (4). Different studies have established its diagnostic value in invasive candidiasis and invasive aspergillosis (9, 10). However, currently numerous non-*Candida* and non-*Aspergillus* fungi are important causes of IFD in the immunocompromised host, and in this setting, clinical and mycological experience with the use of BG as a tool for diagnosis is scarce (7–11). The aim of this report was to assess the usefulness of BG detection (Fungitell; Associates of Cape Cod, Falmouth, MA) as a diagnostic adjunct in proven IFD in a mixed population with uncommon fungal infections due to emerging dematiaceous and hyaline septate molds (14) or with an unusual clinical presentation.

In this single-center observational study, when there were patients with clinical suspicion of IFD, a prospective diagnostic workup was started. This included high-resolution computed tomography followed, when possible, by biopsies of deep tissues for bacterial, mycobacterial, fungal, and viral cultures. BG detection was performed according to the manufacturer's instructions. A BG level of ≥80 pg/ml was considered to be positive. Serum assays were performed in triplicate. The galactomannan (GM) assay was performed as recommended by the manufacturer (Platelia *Aspergillus* assay; Bio-Rad, Marnes La Coquette, France). An index of ≥0.5 was considered positive.

We herein report the performance of BG and GM reactivity assays (when appropriate) in sera from 12 patients with proven IFD (Table 1). There were seven non-*Aspergillus* infections, including three *Scedosporium apiospermum* (clade 4) infections (5), one *Alternaria infectoria* infection, one *Cladosporium macrocarpum* infection, one *Fusarium solani* infection, and one mixed infection by *Blastoschizomyces capitatus* and *Candida kefyr*. The samples involved were brain, subcutaneous tissue, peritoneal abscesses, and tissues with disseminated fungal infections (Table 1). *Aspergillus* species (three isolates of *Aspergillus fumigatus*, one of *Aspergillus versicolor*, and one of *Aspergillus flavus*) caused lung infection, brain abscesses, middle ear mastoiditis, and subungual proximal onychomycosis with subcutaneous involvement (2). Fungi were identified following the guidelines of Gilgado et al. (5) and de Hoog et al. (3).

All patients with dematiaceous fungi had BG reactivity in sera, and all of them were immunocompromised and had not been previously treated with antifungals. There were six patients with brain abscesses (patients 1, 3, 5, 10, 11, and 12) (Table 1), three of the patients with abscesses caused by dematiaceous fungi (*S. apiospermum* for patients 1 and 3 and *C. macrocarpum* for patient 5). It is well known that black fungi

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TABLE 1. Demographic and clinical findings for 12 patients with proven IFD[a]

Characteristic(s)	Data for patient:					
	1	2	3	4	5	6
Gender/age (yr)	F/63	F/78	M/62	M/71	M/45	F/3
Underlying disease(s)	RA, DM, COPD	DM	MM	RT	CP, AA, Ma, PH, L	HS, P
Risk factor(s)	Steroids, azathioprine, infliximab	Topical steroids, cholesteatoma	Steroids, VBCMP/VBAD	Steroids, tacrolimus, mycophenolate mofetil, occupation as gardener	Steroids, EUGCPN	Steroids, cyclosporine
Clinical syndrome	Nasal blockage, cephalea, sinusitis (sphenoidal, ethmoidal, and frontal)	Earache, ear discharge, mastoiditis, perichondritis, facial palsy	Cephalea, blindness, convulsive attacks	5 mo of painful ankle nodule	Pain due to CP, cephalea, facial palsy	Septic shock, cutaneous nodules
Organ involvement of IFD	Abscesses (two frontal brain, sphenoidal, and nasal)	Middle ear, mastoid	Occipital abscess	Subcutaneous tissue	Brain abscesses	Blood, skin
Fungal species	*Scedosporium apiospermum* clade IV	*Scedosporium apiospermum* clade IV	*Scedosporium apiospermum* clade IV	*Alternaria infectoria*	*Cladosporium macrocarpum*	*Fusarium solani*
Site(s) of isolation of fungal species	Sinuses (all) and brain	Mastoid bone	Brain	Subcutaneous tissue	Brain	Blood, nodules
HRCT scan	Abscesses (two frontal brain, sphenoidal, and nasal), erosion of cribriform plate	Mastoid bone erosion, demineralization of facial canal, middle ear occupation	Occipital abscess	Not done	Brain abscesses	Not done
Baseline serum GM (index)/BG (pg/ml)	NA/109	NA/109	NA/166	NA/94	NA/71	NA/413
Monitoring serum GM (index)/BG (pg/ml)	Wk 2, NA/67	Wk 4, NA/84	Wk 2, NA/54; wk 4, NA/72	Wk 4, NA/33	Wk 2, NA/190; wk 4, NA/72	Wk 1, NA/912
Treatment	Voriconazole	Voriconazole, surgery	Voriconazole, terbinafine	Itraconazole	Voriconazole, surgery	Voriconazole
Clinical outcome	Death	Infection resolved	Continuing infection	Infection resolved	Death	Death

[a] Abbreviations: M, male; F, female; RA, rheumatoid arthritis; DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease; MM, multiple myeloma; RT, renal transplant; CP, chronic pancreatitis; AA, alcohol addiction; Ma, malnutrition; PH, portal hypertension; L, lymphopenia; HS, hemophagocytic syndrome; P, pancytopenia; RF, end-stage diabetes renal failure; HD, hemodialysis; CA, colon adenocarcinoma; LA, lung adenocarcinoma, unknown origin; ADOC, adriamycin, cisplatin, oncovin, cyclophosphamide; NHL, non-Hodgkin's lymphoma; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; PBSCA, peripheral blood stem cell autologous bone marrow transplant; IM, immunodeficiency (ongoing study); ARLT, acute rejection of liver transplant; AML, acute myelogenous leukemia; EUGCPN, endoscopic ultrasonography-guided celiac plexus neurolysis; VBCMP/VBAD, vincristine, bischloroethylnitrosourea, cyclophosphamide, melphalan, prednisone/vincristine, bischloroethylnitrosourea, adriamycin, dexamethasone; HRCT, high-resolution computed tomography; NA, not applicable.

are neurotropic (12), and while *S. apiospermum* is a known pathogen, *C. macrocarpum* is a ubiquitous saprophyte. In patient 5, three brain abscesses appeared following an invasive procedure (endoscopic ultrasonography-guided celiac plexus neurolysis); this procedure was done for the treatment of severe pain due to chronic pancreatitis (6). Patients 11 and 12 were severely immunocompromised and had a very rapid progression with a severe complication (brain abscesses) of the baseline fungal disease (sinusitis and middle ear infections). In both cases there was serum BG reactivity, which appeared 2 weeks before GM serum positivity in patient 12, as has been reported for other patients elsewhere (10). In the case of patient 10, who had a mild immunodeficiency (ongoing study) and a long progression with mastoid bone erosion, both fungal markers were negative and only when a brain abscess appeared was there BG reactivity in serum. Interestingly, when the baseline fungal disease is in the middle ear, the clinical outcome and fungal marker performance seem to depend on the immune situation of the patient. In the case of patient 2, there was a long uncomplicated course of the disease and final resolution of the infection.

To the best of our knowledge, patient 4 represents the first reported case of cutaneous alternariasis with BG reactivity. The treatment with oral itraconazole led to clinical cure after 4 weeks, rendering the BG detection negative. Treatment was maintained for a further 3 months, and 1 year after the end of

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TABLE 1—*Continued*

Data for patient:					
7	8	9	10	11	12
F/71	F/65	F/67	F/73	M/61	M/65
DM, RF, HD, CA	DM, LA	NHL	IM, L	ARLT	AML
Colonic surgery, postsurgical anastomotic leakages	ADOC, steroids	R-CHOP, steroids, PBSCA, neutropenia, traumatic removal of hand nail cuticle	Topical steroids, cholesteatoma	Steroids, cyclosporine	Cytarabine, daunorubicin, neutropenia
Peritonitis	Fever, coughing	Fever, finger pain, cellulitis, proximal onychomycosis	5 mo of earache, ear discharge, mastoiditis, perichondritis, facial palsy, cephalea	Cephalea, sinusitis (sphenoidal and ethmoidal)	Cephalea, earache, ear discharge, mastoiditis, perichondritis, facial palsy
Peritoneal abscesses	Lung	Subcutaneous tissue	Middle ear, mastoid, temporal brain abscess	Abscesses (frontal brain and sphenoidal)	Middle ear, mastoid, temporal brain abscess
Blastoschizomyces capitatus, *Candida kefyr*	*Aspergillus fumigatus*	*Aspergillus fumigatus*	*Aspergillus fumigatus*	*Aspergillus versicolor*	*Aspergillus flavus*
Peritoneal abscess	Cavitated lung nodule	Subcutaneous tissue and nail	Brain, mastoid bone	Sphenoidal and ethmoidal sinuses	Mastoid bone
Abdominal abscesses	Cavitated lung nodule in medium right lobule	Not done	Mastoid bone erosion, demineralization of facial canal, middle ear occupation, temporal brain abscess	Not done	Mastoid bone erosion, demineralization of facial canal, middle ear occupation, temporal brain abscess
NA/1,313	0.297/462	0.927/247	0.140/44	0.144/95	0.144/347
Wk 1, NA/2,465	Wk 1, 0.199/1,277; wk 2, 0.263/1,203	Wk 1, 0.540/542; wk 2, 0.383/214; wk 3, 0.460/508; wk 6, 0.231/59	Wk 1, 0.90/36; wk 2, 0.166/23; wk 3, 0.235/81; wk 4, 0.185/18; wk 5, 0.373/54; wk 22, 0.13/124	Wk 4, 0.284/158	Wk 1, 0.168/374; wk 2, 0.945/284; wk 3, 0.589/165
Liposomal amphotericin B	Voriconazole, caspofungin	Caspofungin	Voriconazole, caspofungin, surgery	Voriconazole, caspofungin	Voriconazole, caspofungin
Death	Death	Infection resolved	Continuing infection	Death	Death

treatment the patient was cured even though immunosuppressed.

Patient 9 had an unusual portal of entry of IFD due to *A. fumigatus*, as has been reported elsewhere (2). Both markers were positive, and the patient achieved a total cure, possibly due to the introduction of an early treatment that rendered both markers negative.

Patient 6 had a very high fungal load since *F. solani* was cultured in blood and several nodules in skin. BG was positive, which is in agreement with previously reported studies (7, 9).

Case 8 suggests that since both fungal markers (GM and BG) have limitations for the diagnosis of invasive aspergillosis, the two markers should be combined as diagnostic tools (10).

Patient 7 had fungal peritonitis caused by two different etiologic agents. There is no real consensus on the diagnostic criteria for fungal peritonitis, but in gastrointestinal surgery patients with anastomotic leakage, the isolation of fungi in tissue obtained in a surgical procedure establishes a sound diagnosis, as is the case for this patient (13).

Although the number of patients with invasive aspergillosis was small, BG detection tended to be superior to GM detection in establishing the diagnosis. However, since both BG and GM testing have limitations, detection of both markers should be used in combination to improve the diagnostic workup of the disease (10).

Non-*Aspergillus* emerging mycelial invasive disease lacks an early indirect diagnosis. However, the majority of these isolates produce high levels of BG in vitro with apparent species-specific BG levels (8).

In nine patients BG positivity appeared a mean of 12 days (range, 5 to 29 days) before the fungal culture was grown. In patient 3, both markers appeared at the same time, and in two patients (patients 9 and 11) BG positivity appeared 2 and 30 days later than the fungal culture, respectively.

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As described previously (10), these preliminary results suggest that monitoring BG antigenemia would also be a valuable tool in predicting therapeutic outcome in patients with IFD, since rising levels of BG tended to correspond with treatment failure.

In conclusion, data presented in this report suggest that BG is a useful noninvasive tool for the diagnosis of IFD in patients with uncommon fungal infections or with unusual clinical presentations. All new diagnostic techniques such as BG should be validated against postmortem findings or biopsies of deep tissues because only proven cases of IFD offer the most valuable and sound information.

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