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 Gilmour 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...
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 TABLE 1. Demographic and clinical findings for 12 patients with proven IFD

| Characteristic(s) | Data for patient: |
|-------------------|------------------|
| Gender/age (yr)   | 1 | 2 | 3 | 4 | 5 | 6 |
| Underlying disease(s) | F/63 | F/78 | M/62 | M/71 | M/45 | F/3 |
| Risk factor(s)     | RA, DM, COPD | DM | MM | RT | CP, AA, Ma, PH, L | HS, P |
| Clinical syndrome  | Nasal blockage, cephalic, sinusitis (sphenoidal, ethmoidal, and frontal) | Earache, ear discharge, mastoiditis, perichondritis, facial palsy | Cephalic, blindness, convulsive attacks | Pain due to CP, cephalic, facial palsy | Septic shock, cutaneous nodules |
| Risk factor(s)     | Steroids, azathioprine, infliximab | Topical steroids, cholesteatoma | Steroids, VBCMP/VBAD | Steroids, tacrolimus, mycophenolate mofetil, occupation as gardener | Steroids, cyclosporine |
| Risk factor(s)     | Sinuses (all) and brain | Mastoid bone | Brain | Subcutaneous tissue | Brain abscesses |
| Risk factor(s)     | Abscesses (two frontal brain, sphenoidal, and nasal) | Middle ear, mastoid | Occipital abscess | Subcutaneous tissue | Brain abscesses |
| Risk factor(s)     | Scedosporium apiospermum clade IV | Scedosporium apiospermum clade IV | Scedosporium apiospermum clade IV | Alternaria infectoria | Cladosporium macrocarpum |
| Risk factor(s)     | Fungus species | Fungus species | Fungus species | Fungus species | Fungus species |
| Site(s) of isolation of fungal species | Sinuses (all) and brain | Mastoid bone | Brain | Subcutaneous tissue | Brain |
| HRCT scan          | Abscesses (two frontal brain, sphenoidal, and nasal), erosion of cribiform plate | Mastoid bone erosion, demineralization of facial canal, middle ear occupation | Occipital abscess | Not done | Brain abscesses |
| 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 |

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 autologuous bone marrow transplant; IM, immunodeficiency (ongoing study); ARLT, acute rejection of liver transplant; AML, acute mylogenous leukemia; EUGCPN, endoscopic ultrasonography-guided celiac plexus neurolysis; VBCMP/VBAD, vincristine, bischlorehylnitrosourea, cyclophosphamide, melphalan, prednisone/vincristine, bischlorehylnitrosourea, cyclophosphamide, melphalan; HRCT, high-resolution computed tomography; NA, not applicable.
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.

### TABLE 1—Continued

| Data for patient: | 7 F/71 | 8 F/65 | 9 F/67 | 10 F/73 | 11 M/61 | 12 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, osteomyelitis, 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 bone abscess | Abscesses (frontal brain and sphenoidal) | Middle ear, mastoid, temporal bone 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 bone, middle ear occupation, temporal bone abscess | Not done | Mastoid bone erosion, demineralization of facial bone, middle ear occupation, temporal bone 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, 0.137/54; wk 22, 0.13/124 | Wk 4, 0.284/158 | Wk 1, 0.168/374; wk 2, 0.945/264; 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 |

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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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