JCM
Journals.ASM.org

Cross-Reactivity of *Fusarium* spp. in the *Aspergillus* Galactomannan Enzyme-Linked Immunosorbent Assay

Anna Maria Tortorano,[a] Maria Carmela Esposto,[a] Anna Prigitano,[a] Anna Grancini,[b] Cristina Ossi,[c] Caterina Cavanna,[d] and Giuliana Lo Cascio[e]

Dipartimento di Sanità Pubblica-Microbiologia-Virologia, Università degli Studi di Milano, Italy[a]; Laboratorio di Microbiologia, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli, Regina Elena, Milano, Italy[b]; LabRaf, IRCCS Ospedale San Raffaele, Milano, Italy[c]; Laboratorio, IRCCS Ospedale San Matteo, Pavia, Italy[d]; and Servizio di Microbiologia, Azienda Ospedaliera di Verona, Italy[e]

Nine of 11 hematological patients with disseminated/deep-seated *Fusarium* infection tested at least twice for *Aspergillus* galactomannan (GM) had repeated positive results in the absence of *Aspergillus* isolation in culture. The centrifuged supernatants of 12 *Fusarium* isolates were tested by a GM enzyme-linked immunosorbent assay (EIA). All the isolates produced positive reactions when tested undiluted. These results show cross-reactivity of *Fusarium* spp. with *Aspergillus* GM that may constitute a drawback with respect to the specificity of the Platelia EIA.

Fusarium is a large genus of hyaline filamentous molds that have emerged as pathogen of immunocompetent and immunocompromised hosts. While *Fusarium* infections, such as onychomycosis, peritonitis in association with peritoneal dialysis, and keratitis as a consequence of corneal trauma or contact lens wear, in immunocompetent patients tend to be superficial or locally invasive, disseminated *Fusarium* infection remains an uncommon and yet severe opportunistic infection among highly immunocompromised patients (9). The European Confederation of Medical Mycology (ECMM) recently launched a survey on this infection in Europe.

The treatment of *Fusarium* infection in immunocompromised hosts is a frustrating task due to the limited susceptibility of *Fusarium* species to antifungal agents. Most of the species have shown *in vitro* resistance to azoles, polyenes, and echinocandins; numerous experimental studies in animals carried out to evaluate different antifungal treatments have had disappointing results, and a favorable outcome was found in less than 50% of the cases (5, 7, 9, 14, 15, 19).

The detection of *Aspergillus* galactomannan (GM) using a sandwich enzyme-linked immunosorbent assay (EIA) is now included among the microbiological criteria of the revised European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) classification of invasive aspergillosis (2). The clinical sensitivity of GM EIA is variable, ranging from 29% to 100% according to the host group and the underlying pathological processes (13). A major problem is the occurrence of false-positive results related in some cases to cross-reactivity with other fungi. The epitope detected by the EB-A2 monoclonal antibody employed in the EIA is not exclusively present in *Aspergillus* species. This epitope is contained also in antigens of other molds, including *Penicillium*, *Paecilomyces*, *Trichothecium*, *Geotrichum*, and *Myceliophthora*, of dimorphic fungi, such as *Blastomyces dermatitidis* and *Histoplasma capsulatum*, and also of yeasts (1, 3, 4, 8, 20, 21). Cross-reactivity of *Fusarium* spp. in the GM assay is controversial and seems to be related to the method used and the tested species (1, 6, 17). While cross-reactivity of *F. oxysporum* was found using Pastorex *Aspergillus* antigen latex agglutination but not using the Platelia test (1, 6, 16), *F. solani* was reported to lack cross-reactivity in both the assays (1, 6, 16).

The aims of the present study were to check GM index results in patients with disseminated *Fusarium* infection included in the ECMM survey conducted in Italy by the Federazione Italiana di Micopatologia Umana e Animale (FIMUA) and to test the cross-reactivity of different *Fusarium* spp. in the GM EIA.

Aspergillus GM index results of patients with *Fusarium* disseminated or deep-seated infection notified during the ECMM-FIMUA survey were checked in the microbiological database.

A total of 12 *Fusarium* isolates—2 *F. oxysporum*, 3 *F. proliferatum*, 3 *F. verticillioides*, and 4 *F. solani* species complex (FSSC), namely, 1 *F. falciforme* and 3 *F.* sp. *cucurbitae* MPV—identified by sequencing the elongation factor 1α gene (10, 11, 12) were studied. Seven isolates were from the patients included in the study. The other five, namely, four isolates from blood and one from a skin biopsy specimen, were from patients not included in this study, and the GM test was not performed for these patients. One *Aspergillus fumigatus* isolate was used as a control.

The isolates were grown on Potato dextrose agar for 72 h at room temperature. A loop of biomass was transferred in 50 ml of Sabouraud broth and incubated in the dark at room temperature on a rotary shaker (20 rpm). After 72 h at room temperature, 15 ml of the supernatant was centrifuged at 2,000 rpm for 5 min. The centrifuged supernatants (undiluted and diluted 1:100) were tested twice by Platelia *Aspergillus* EIA (Bio-Rad, Marne La Coquette, France).

Eleven patients were tested at least twice for GM (Table 1). Eight with hematological malignancy had proven disseminated *Fusarium* infection, documented by isolation of the fungus from blood or by the presence of hyphae at histology of a skin

Received 29 September 2011 **Returned for modification** 9 October 2011
Accepted 4 December 2011
Published ahead of print 28 December 2011
Address correspondence to Anna Maria Tortorano, annamaria.tortorano@unimi.it.

doi:10.1128/JCM.05946-11

TABLE 1 Serum GM index results for patients with disseminated/deep-seated *Fusarium* infection[a]

Patient	Sex	Age (yr)	Predisposing factor(s)	*Fusarium* infecting isolate	Positive biological sample(s)	Serum GM index range
1	M	29	Allo-HSCT	*F. oxysporum*	Pleural fluid	0.69–0.90
2	M	63	Leukemia	*F. oxysporum*	Bronchial secretions	1.40–2.30
3	M	55	AML	*F. proliferatum*	Blood	0.89–0.86
4	F	61	Allo-HSCT	*F. proliferatum*	Blood	1.37–2.33
5	F	56	AML, allo-HSCT	*F. proliferatum*	Blood	0.7–2.15
6	M	8	Non-Hodgkin's lymphoma	*F. proliferatum*	Blood	0.53–7.7
7	M	19	ALL, auto-HSCT	*F. proliferatum*	Blood	0.54–1.45
8	F	57	Postchemotherapy aplasia, RAEB	FSSC	Purulent nasal discharge	0.50–0.60
9	M	41	Non-Hodgkin's lymphoma, allo-HSCT	*F. verticillioides*	Skin biopsy	Negative
10	M	5	ALL	*F. verticillioides*	Blood	Negative
11	F	9	ALL, auto-HSCT	*F. verticillioides*	Blood	0.70–4.16

[a] M, male; F, female; allo-HSCT, allogeneic hematopoietic stem cell transplantation; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; RAEB, refractory anemia with excess blasts; auto-HSCT, autologous hematopoietic stem cell transplantation; FSSC, *Fusarium solani* species complex.

biopsy specimen combined with growth of *Fusarium* in culture. Another three neutropenic patients had probable infections according to the EORTC/MSG criteria, as hyphae were seen by direct microscopy of respiratory tract samples or pleural fluid and *Fusarium* spp. were isolated in culture. Nine of these 11 patients had repeated positive GM test results, with an index ranging from 0.50 to 7.7 in the absence of isolation of *Aspergillus* spp. in culture of bronchial secretions or of other respiratory tract specimens (Table 1).

The means of the indices of reactivity in the Platelia assay of fungal cultures are reported in Table 2. All the *Fusarium* isolates produced positive reactions (index ≥ 0.5) when tested undiluted. The highest values (approximately 4) were detected in two FSSC isolates, one of which (*F. falciforme*) tested positive (0.55) also at the 1:100 dilution. The *A. fumigatus* isolate, tested as a control, showed a positive GM index when tested undiluted and diluted 1:100.

Monitoring of GM serum levels is currently used for early diagnosis of invasive aspergillosis due to its standardization and simple application in routine practice. The reported values of sensitivity and specificity of the Platelia *Aspergillus* assay differ widely in the literature. A 93% specificity for proven and probable cases was reported in a meta-analysis (13), whereas a low level of specificity is reported in some experiences (18).

Among the different causes of false-positive results, including the use of antibiotics such as piperacillin and amoxicillin, infections caused by mold containing cross-reactive GM have been reported. Cross-reactivity with antigens released by a large number of fungi has been reported in the literature (1, 3, 4, 8, 20, 21). Test specificity with exoantigens of *Fusarium* cultures is controversial (1, 6, 16).

Positive GM test results for patients with *Fusarium* infection have been anecdotally reported in the literature (18). In our survey, 9 out of 11 patients with disseminated or deep-seated *Fusarium* infection had repeated positive GM test results in the absence of isolation of *Aspergillus* spp.

To verify the hypothesis that a cross-reaction with exoantigens released by *Fusarium* might be the cause of *Aspergillus* GM positivity, we tested 12 *Fusarium* isolates, representing the species that most frequently cause infection, for their reactivity in the Platelia *Aspergillus* assay. All the *Fusarium* tested isolates showed positive indices of reactivity, even if lower than that shown by the *A. fumigatus* isolate used as control. The reactivity indices of *Fusarium* cultures ranged from 1.15 (*F. verticillioides*) to 4.14 (*F. falciforme*).

Our cases as well as *in vitro* studies show that *Fusarium* exoantigens may be cross-reactive in the Platelia *Aspergillus* assay. Therefore, a positive GM test in an immunocompromised host may represent invasive aspergillosis or another fungal infection,

TABLE 2 Reactivities of exoantigens from 12 clinical isolates of *Fusarium* species and from one *A. fumigatus* isolate in the Platelia *Aspergillus* assay

IUM isolate[a]	Patient (source)	*Fusarium* sp.	Index of reactivity in Platelia assay	
			Undiluted reaction mixture	Reaction mixture diluted 10^{-2}
07-0123	1 (pleural fluid)	*F. oxysporum*	2.35	0.25
09-1022	2 (bronchial secretions)	*F. oxysporum*	1.68	0.30
10-0049	4 (blood)	*F. proliferatum*	2.48	0.24
09-0505	3 (blood)	*F. proliferatum*	1.49	0.35
09-0782	7 (blood)	*F. proliferatum*	2.04	0.37
10-0035	(blood)	*F.* sp. *cucurbitae* MPV	2.05	0.18
10-0036	(blood)	*F.* sp. *cucurbitae* MPV	2.50	0.42
07-0259	(blood)	*F.* sp. *cucurbitae* MPV	3.97	0.36
08-0221	(skin biopsy)	*F. falciforme*	4.14	0.55
09-0620	(blood)	*F. verticillioides*	1.84	0.38
09-0780	10 (blood)	*F. verticillioides*	1.15	0.22
09-0781	11 (blood)	*F. verticillioides*	1.82	0.22
11-0088		*A. fumigatus*	6.05	1.72

[a] IUM, Igiene Università Milano culture collection.

including *Fusarium* etiology. On the other hand, this unexpected reactivity of the test could be considered a useful diagnostic and prognostic tool in the management of *Fusarium* infections, as no antigen system for detection of this fungal infection exits.

In conclusion, these results show cross-reactivity of *Fusarium* spp. with *Aspergillus* GM that may constitute a drawback with respect to the specificity of the EIA. Therefore, a positive GM result, as with the presence of septate acute-angle branching hyphae in tissue and a positive β-D-glucan test, cannot help medical practitioners reach a specific diagnosis. This may represent a crucial point for the choice of antifungal therapy and for the evaluation of treatment outcome of the use of antifungals, such as echinocandins, that have been shown to be inactive *in vitro* against all *Fusarium* spp.

REFERENCES

1. **Cummings JR, et al.** 2007. Cross-reactivity of non-fungal species in the *Aspergillus* galactomannan enzyme immunoassay. Diagn. Microbiol. Infect. Dis. **59**:113–115.
2. **De Pauw B, et al.** 2008. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin. Infect. Dis. **46**:1813–1821.
3. **Fekkar A, et al.** 2009. Serum cross-reactivity with *Aspergillus* galactomannan and cryptococcal antigen during fatal disseminated *Trichosporon dermatis* infection. Clin. Infect. Dis. **49**:1457–1458.
4. **Giacchino M, et al.** 2006. *Aspergillus* galactomannan enzyme-linked immunosorbent assay cross-reactivity caused by invasive *Geotrichum capitatum.* J. Clin. Microbiol. **44**:3432–3434.
5. **Guarro J.** 2011. Lessons from animal studies for the treatment of invasive human infections due to uncommon fungi. J. Antimicrob. Chemother. **66**:1447–1466.
6. **Kappe R, Schulzeberge A.** 1993. New cause for false-positive results with the Pastorex *Aspergillus* antigen latex agglutination-test. J. Clin. Microbiol. **31**:2489–2490.
7. **Lortholary O, et al.** 2010. International retrospective analysis of 73 cases of invasive fusariosis treated with voriconazole. Antimicrob. Agents Chemother. **54**:4446–4450.
8. **Morio F, et al.** 2011. Invasive *Myceliophthora thermophila* infection mimicking invasive aspergillosis in a neutropenic patient: a new cause of cross-reactivity with the *Aspergillus* galactomannan serum antigen assay. Med. Mycol. **49**:883–886.
9. **Nucci M, Anaissie E.** 2007. Fusarium infections in immunocompromised patients. Clin. Microbiol. Rev. **20**:695–704.
10. **O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC.** 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Nat. Acad. Sci. U. S. A. **95**:2044–2049.
11. **O'Donnell K, et al.** 2008. Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the *Fusarium solani* species complex. J. Clin. Microbiol. **46**:2477–2490.
12. **O'Donnell K, et al.** 2010. Internet-accessible DNA sequence database for identifying Fusaria from human and animal infections. J. Clin. Microbiol. **48**:3708–3718.
13. **Pfeiffer CD, Fine JP, Safdar N.** 2006. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. Clin. Infect. Dis. **42**:1417–1427.
14. **Raad II, et al.** 2006. Posaconazole as salvage treatment for invasive fusariosis in patients with underlying hematologic malignancy and other conditions. Clin. Infect. Dis. **42**:1398–1403.
15. **Spellberg B, et al.** 2006. Comparison of antifungal treatments for murine fusariosis. J. Antimicrob. Chemother. **58**:973–979.
16. **Stynen D, et al.** 1992. Rat monoclonal antibodies against *Aspergillus* galactomannan. Infect. Immun. **60**:2237–2245.
17. **Swanink CMA, Meis J, Rijs A, Donnelly JP, Verweij PE.** 1997. Specificity of a sandwich enzyme-linked immunosorbent assay for detecting *Aspergillus* galactomannan. J. Clin. Microbiol. **35**:257–260.
18. **Tanriover MD, Ascioglu S, Altun B, Uzun O.** 2010. Galactomannan on the stage: prospective evaluation of the applicability in routine practice and surveillance. Mycoses **53**:16–25.
19. **Tortorano AM, et al.** 2008. Species distribution and in vitro antifungal susceptibility patterns of 75 clinical isolates of *Fusarium* spp. from northern Italy. Antimicrob. Agents Chemother. **52**:2683–2685.
20. **Wheat LJ, et al.** 2007. Histoplasmosis-associated cross-reactivity in the BioRad Platelia *Aspergillus* enzyme immunoassay. Clin. Vaccine Immunol. **14**:638–640.
21. **Xavier MO, Pasqualotto AC, Cardoso ICE, Severo LC.** 2009. Cross-reactivity of *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, and *Cryptococcus* species in the commercial Platelia *Aspergillus* Enzyme Immunoassay. Clin. Vaccine Immunol. **16**:132–133.