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Impact of Zygomycosis on Microbiology Workload: a Survey Study in Spain▽

Marta Torres-Narbona, Jesús Guinea,* José Martínez-Alarcón, Patricia Muñoz, Ignacio Gadea, and Emilio Bouza as a representative of the MYCOMED Zygomycosis Study Group

Clinical Microbiology and Infectious Diseases Department, Hospital General Universitario Gregorio Marañón, Universidad Complutense, Madrid, Spain

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This multicenter, population-based study evaluated the laboratory workload produced by zygomycetes and the number of cases of zygomycosis in Spain during 2005. Less than 8% of the patients who harbored zygomycete isolates had zygomycosis. The incidence of zygomycosis (6 cases) was 0.43 cases/1,000,000 inhabitants and 0.62 cases/100,000 hospital admissions.

Zygomycosis, a life-threatening infection caused by molds of the class *Zygomycetes* (2, 17, 22, 24, 28), presents only sporadically (19). More recently, however, a remarkable increase in its incidence has been reported from single institutions in the United States and Europe (9, 10, 12, 14–16, 21, 23, 26, 27). The widespread use of voriconazole for the prophylaxis of invasive fungal infections in those institutions has been claimed to be responsible for this problem (10, 14, 23).

Furthermore, the frequency of isolation of these fungi in clinical microbiology laboratories and the proportion of patients who harbor such isolates and who really have zygomycosis are not reported.

We aimed to clarify the workload produced by zygomycetes in clinical microbiology laboratories to determine their clinical significance and to determine the incidence of zygomycosis in a population-based study in Spain.

(This study was partially presented at the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 2006 [M. Torres-Narbona, J. Guinea, J. Martínez-Alarcón, T. Peláez, E. Bouza, and the Zygomycosis Study Group, Abstr. 46th Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-907].)

Study period and participating hospitals. We invited 140 hospitals to participate in the study, and 50 (36%) participated. The 50 hospitals cared for 14,069,094 inhabitants and were uniformly distributed throughout Spain (which has 44 million inhabitants) (3). The institutions were classified as follows: more than 1,000 beds (9 hospitals), between 500 and 1,000 beds (18 hospitals), and less than 500 beds (23 hospitals). All institutions were general hospitals, and 48 were tertiary-care and/or teaching hospitals. Among the 50 participating hospitals, 28 had bone marrow transplant units. The size and geographical distributions of the nonparticipating institutions and the participating centers were similar.

Information requested. Every hospital completed a preestablished form that allowed us to assess the clinical and microbiological information for each hospital for the whole of 2005. The microbiological data included the number of samples processed during the study period, the number of samples processed for the isolation of fungi, the number of samples with fungal isolates, the number of samples from which zygomycetes were isolated, the number of patients with one or more samples positive for zygomycetes, and the number of samples from patients with zygomycosis. Information regarding the population that the hospital cared for, the number of beds, and the number of total admissions during 2005 was requested. All isolates were derived from clinical samples.

Zygomycetes were identified by conventional methods, based on morphological features.

We requested the completion of a preestablished protocol for every patient meeting the diagnostic criteria of proven or probable invasive fungal disease, according to the EORTC/MSG international standards developed for the diagnosis of invasive aspergillosis in hematological patients (1). In summary, a diagnosis of zygomycosis may be achieved by use of a combination of clinical evidence of the disease and detection of the fungus in tissues (either by histopathological findings or by isolation of zygomycetes from clinical samples).

Clinical and microbiological data were requested for each patient with zygomycosis.

Evaluation of incidence of zygomycosis and description of the clinical forms. During the study period, 78 patients harbored one or more isolates of zygomycetes, but only 6 patients (7.7%; 95% confidence interval [CI], 1.8 to 13.6) from four different institutions had zygomycosis. Five of the six patients had clinical evidence of the infection and the fungus was isolated from clinical samples microbiologically. The other two patients had clinical evidence of infection, and a histopathological diagnosis was made without microbiological isolation (Table 1). This enabled us to calculate an incidence of 0.43 cases per 1,000,000 inhabitants/year (95% CI, 0.00 to 1.72) and 0.62 cases/100,000 hospital admissions.

Overall, five of the six patients had malignant hematological conditions (83%), including leukemia and lymphoma. Five of the patients were under immunosuppressive treatment, three had neutropenia, and only one had diabetes and had received corticosteroid treatment. Only one of the six patients had received voriconazole as prophylaxis during the 15 days before

* Corresponding author. Mailing address: Servicio de Microbiología Clínica y Enfermedades Infecciosas-HIV, Hospital General Universitario Gregorio Marañón, c/ Dr. Esquerdo, 46, 28007 Madrid, Spain. Phone: 0034-914265104. Fax: 0034-913721721. E-mail: jguineaortega@yahoo.es.

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TABLE 1. Clinical and microbiological data for the six patients with true invasive zygomycosis

Characteristic	Patient no.					
	1	2	3	4	5	6
Clinical form	Rhinosinusal	Rhinosinusal	Pulmonary and cutaneous	Pulmonary	Cutaneous-facial	Cutaneous-abdominal
Underlying condition						
Neutropenia	Yes	No	Yes	Yes	No	No
Hematological malignancies	Yes (acute myeloid leukemia)	Yes (chronic lymphatic leukemia and BMT[a])	Yes (acute myeloid leukemia)	Yes (lymphatic leukemia)	Yes (non-Hodgkin's lymphoma)	No
Transplant	No	Yes	No	No	No	Yes (liver)
Diabetes	No	No	No	No	Yes	No
Imaging data (X ray, CT scan, NMR[b])	Yes	Yes	Yes	Yes	No	No
Previous immunosuppressive treatment	Yes	Yes	Yes	Yes	No	Yes
Previous antifungal treatment (30 days previously)	No	Yes (voriconazole)	No	No	No	No
Previous antibiotic treatment (30 days previously)	No	Yes	No	Yes	No	Yes
Previous corticosteroid treatment	No	No	No	No	Yes	No
Samples	Rhinosinusal sample	None	Wound	Sputum ($n = 3$)	None	Skin biopsy
Histopathology	Not done	Presence of hyphae	Not done	Not done	Presence of hyphae	Presence of hyphae
Culture result	*Mucor* sp.	Not done	*Rhizomucor* sp.	*Cunninghamella bertholletiae*	Not done	*Mucor* sp.
Diagnostic method	Clinical and culture	Clinical and histopathology	Clinical and culture	Clinical and culture	Clinical and histopathology	Clinical, culture and histopathology
Outcome	Cured	Death	Death	Death	Death	Cured

[a] BMT, bone marrow transplant.
[b] CT, computed tomography; NMR, nuclear magnetic resonance.

the diagnosis. Of the six cases, four died and two were cured by surgery plus antifungal treatment.

Workload evaluation. The participating hospitals cared for a population of 14,069,094 inhabitants, and their microbiology laboratories processed 2,815,873 samples. Of these, 187,031 (6.6%) were processed for the isolation of fungi. Overall, 37,192 (19.9%) of them were positive for yeasts and/or molds. Of the 50 hospitals, only 19 (38%) reported the clinical isolation of zygomycetes from 171 samples (0.45%). The laboratories isolated 12.2 zygomycetes per 1,000,000 inhabitants (95% CI, 5.36 to 19.06), 6 per 100,000 total samples processed in the microbiology laboratory (95% CI, 2 to 10), 0.62 per 100,000 hospital admissions (95% CI, 0.00 to 1.3), and 5 per 1,000 samples submitted for fungal isolation (95% CI, 1 to 9). Of the 171 positive samples, only 6 were from infected patients and the probability of having zygomycosis when a culture was positive for zygomycetes was 3.5%; only 7.7% of the patients from whom zygomycetes were isolated had zygomycosis.

This population-based study shows that the workload generated by zygomycetes in Spanish clinical microbiology laboratories is very low, and only a small proportion of patients from whom a zygomycete is isolated from one or more clinical samples presented with zygomycosis. The study also shows the low incidence of zygomycosis.

Data that can be used to compare the workload produced by zygomycetes in microbiology laboratories are scarce. Zygomycetes can be isolated from the environment. Previous studies showed that they can be isolated in 20% to 39% of outdoor air samples (4), 1.6% to 9.5% of hospital air samples (18), and occasionally as colonizers in human samples (11). The isolation of zygomycetes in clinical laboratories should be interpreted with caution.

Recently, an increased incidence of zygomycetes has been reported in individual institutions or even in individual units within those institutions (6–10, 12–16, 20, 23, 27, 29). The incidence of zygomycosis in the United States was estimated to be about 1.7 cases per 1,000,000 inhabitants between 1992 and 1993, or about 500 patients per year (19). When the disease is diagnosed postmortem, it is 10- to 50-fold less frequent than candidiasis or aspergillosis and is supposed to be present in one to five cases/10,000 autopsies (5, 25, 30).

Institutions reporting on the increased incidence of zygomycosis usually relate it to the widespread use of voriconazole as antifungal prophylaxis (12, 15, 23, 29). The rate of consumption of voriconazole/1,000,000 inhabitants in 2005 was 3,227 daily defined doses in Spain (G. Armenteros, Pfizer Spain, personal communication), while in the United States it was 5,506 daily defined doses. This difference may explain the low incidence of zygomycosis in Spain, but without population-based studies evaluating its incidence in the United States or studies specially designed to clarify this issue, the reason for this difference remains a suggestion.

Our data suggest that the reemergence of zygomycosis cannot be assumed from reports from single units or single institutions. In Spain, voriconazole is not widely used for antifungal prophylaxis. A word of caution regarding the widespread use of voriconazole for prophylaxis seems pertinent.

The staff of the microbiology services of all participating hospitals are members of the MYCOMED Zygomycosis Study Group and are as follows: A. Sánchez Porto, Hospital del S.A.S. La Línea de La Concepción, Cádiz; R. M. Gordillo Urbano, Hospital Universitario Reina Sofia (S.A.S.), Córdoba; M. de la Rosa Fraile and C. Miranda Casas, Hospital General Virgen de las Nieves, Granada; I. Cuesta Lendínez and L. Parras Jiménez, Hospital Universitario Ciudad de Jaén, Jaén; V. Pérez Valero and P. Blanc Iribarren, Hospital Regional Carlos Haya, Málaga; A. Pascual Hernández and A. I. Suárez Barrenechea, Hospital Universitario Virgen Macarena, Sevilla; E. Martín Mazuelos and C. Martín Martín de la Escalera, Hospital Universitario Nuestra Señora De Valme, Dos Hermanas, Seville; M. J. Revillo Pinilla and A. Rezusta López, Hospital

Universitario Miguel Servet, Zaragoza; M. J. Santos and J. Clavena, Hospital Universitario Central de Asturias, Oviedo; F. Vázquez Valdés, Hospital Monte Naranco, Oviedo; D. Miguel Martinez, Hospital de Cabueñes, Gijón; E. Hidalgo Pérez, Hospital de Jove, Gijón; P. Prendes Peláez and H. Villar Pérez, Hospital San Agustin, Avilés; J. L. Pérez Sáez and N. Borrell Solé, Hospital Son Dureta—Complejo Hospitalario, Palma de Mallorca; N. Batista Díaz and J. M. Ode Febles, Hospital Nuestra Señora de La Candelaria, Sta. Cruz Tenerife; A. Sierra López and M. A. Miguel Gómez, Hospital Universitario de Canarias, La Laguna, Sta. Cruz de Tenerife; D. Crespo Sánchez and J. J. Blas Señalada, Complejo Hospitalario Universitario de Albacete, Albacete; I. Barba Ferreras, Hospital Nuestra Señora del Carmen—Hospital General de Ciudad Real, Ciudad Real; R. Ibáñez Pérez and R. Sánchez Arroyo, Hospital Ntra. Sra. de Sonsoles (Complejo Hospitalario de Ávila), Ávila; I. Pozas Pozas and M. García Bravo, Hospital General Yagüe, Burgos; J. A. García Rodríguez and N. Gutierrez Zufiaurre, Hospital Universitario de Salamanca, Salamanca; S. García Carbajosa and P. Carrero González, Complejo Hospitalario de Segovia (Sacyl), Segovia; A. Campos Bueno and F. Merino Fernández, Complejo Hospitalario de Soria (Santa Bárbara/Virgen del Mirón), Soria; P. Pérez Pascual and A. Gómez Nieto, Hospital del Río Hortega, Valladolid; M. F. Brezmes Valdivieso and L. López-Urrutia Lorente, Hospital Virgen de La Concha, Zamora; J. Lite Lite and E. Cuchi Burgos, Hospital Mutua de Terrassa, Barcelona; D. Fontanals and D. Mariscal, Consorcio Hospitalario del Parc Tauli, Sabadell; J. M. Santamaría Puig, F. Gómez Bertomeu, and J. Tapiol Oliva, Hospital de Tarragona Joan XXIII, Tarragona; J. Blanco Palenciana and E. Garduño Eseverri, Hospital Universitario Infanta Cristina, Badajoz; P. Teno Sánchez and J. Viñuelas Bayón, Complejo Hospitalario de Cáceres-Hospital San Pedro De Alcántara, Cáceres; D. Velasco Fernández, Complejo Hospitalario Universitario Juan Canalejo, A Coruña; P. Alonso García and J. Pita Carretero, Complejo Hospitalario Xeral-Calde, Lugo; G. Esteban Meruéndano, Complejo Hospitalario de Ourense-Hospital Santa María Nai, Ourense; R. Cortés Bohórquez, Hospital Central de La Cruz Roja Española—San José y Santa Adela, Madrid; M. López Brea, B. Buendía, and A. Pérez de Ayala, Hospital Universitario de La Princesa, Madrid; F. Baquero Mochales and A. Sánchez Sousa, Hospital Ramón y Cajal, Madrid; I. Gadea Gironés and A. Ortiz Pérez, Fundación Jiménez Díaz-Clínica Nuestra Señora de La Concepción, Madrid; A. de Urmeneta Rada, Hospital de Cantoblanco, Madrid; M. Sánchez Concheiro and C. García Esteban, Hospital Universitario de Getafe, Madrid; I. Wilhelmi de Cal and M. Cuétara García, Hospital Severo Ochoa de Leganés, Madrid; A. Delgado-Iribarren and J. F. Valverde Cánovas, Fundacion Hospital Alcorcón, Madrid; I. Galán Navarro, Hospital Comarcal de Melilla, Melilla; R. Blázquez Garrido, Hospital Morales Meseguer, Murcia; J. Leiva León and M. Rubio Vallejo, Clinica Universitaria de Navarra, Pamplona; I. Dorronsoro Ibero, Hospital de Navarra, Pamplona; J. A. Jiménez Alfaro and A. Jáuregui López, Policlínica Guipúzcoa, San Sebastián; E. Pérez Trallero and J. M. García-Arenzana, Hospital de Donostia de San Sebastián, Donostia; N. Corral and L. Eloduy, Hospital San Eloy, Baracaldo; A. Yagüe Muñoz, Hospital La Plana, Villarreal, Castellón; and A. Lloret Caballería and A. Farga Marti, Hospital Arnau de Vilanova, Valencia.

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REFERENCES

1. **Ascioglu, S., J. H. Rex, B. de Pauw, J. E. Bennett, J. Bille, F. Crokaert, D. W. Denning, J. P. Donnelly, J. E. Edwards, Z. Erjavec, D. Fiere, O. Lortholary, J. Maertens, J. F. Meis, T. F. Patterson, J. Ritter, D. Selleslag, P. M. Shah, D. A. Stevens, and T. J. Walsh.** 2002. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin. Infect. Dis. **34:**7–14.
2. **Bigby, T. D., M. L. Serota, L. M. Tierney, Jr., and M. A. Matthay.** 1986. Clinical spectrum of pulmonary mucormycosis. Chest **89:**435–439.
3. **Boletín Oficial del Estado.** 2005. Real decreto 1358/2005, p. 39422–39423. Ministry of Health, Madrid, Spain.
4. **Guinea, J., T. Peláez, P. Muñoz, and E. Bouza.** 2005. How frequent are agents responsible for mucormycosis in outdoor air? Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-962. American Society for Microbiology, Washington, DC.
5. **Hotchi, M., M. Okada, and T. Nasu.** 1980. Present state of fungal infections in autopsy cases in Japan. Am. J. Clin. Pathol. **74:**410–416.
6. **Imhof, A., S. A. Balajee, D. N. Fredricks, J. A. Englund, and K. A. Marr.** 2004. Breakthrough fungal infections in stem cell transplant recipients receiving voriconazole. Clin. Infect. Dis. **39:**743–746.
7. **Jacobs, P., L. Wood, A. Du Toit, and K. Esterhuizen.** 2003. Eradication of invasive mucormycosis–effectiveness of the echinocandin FK463. Hematology **8:**119–123.
8. **Kauffman, C. A.** 2004. Zygomycosis: reemergence of an old pathogen. Clin. Infect. Dis. **39:**588–590.
9. **Kobayashi, K., M. Kami, N. Murashige, Y. Kishi, G. Fujisaki, and T. Mitamura.** 2004. Breakthrough zygomycosis during voriconazole treatment for invasive aspergillosis. Haematologica **89:**ECR42.
10. **Kontoyiannis, D. P., M. S. Lionakis, R. E. Lewis, G. Chamilos, M. Healy, C. Perego, A. Safdar, H. Kantarjian, R. Champlin, T. J. Walsh, and I. I. Raad.** 2005. Zygomycosis in a tertiary-care cancer center in the era of *Aspergillus*-active antifungal therapy: a case-control observational study of 27 recent cases. J. Infect. Dis. **191:**1350–1359.
11. **Lionakis, M. S., and D. P. Kontoyiannis.** 2004. The significance of isolation of saprophytic molds from the lower respiratory tract in patients with cancer. Cancer **100:**165–172.
12. **Lionakis, M. S., and D. P. Kontoyiannis.** 2005. Sinus zygomycosis in a patient receiving voriconazole prophylaxis. Br. J. Haematol. **129:**2.
13. **Marr, K. A., R. A. Carter, F. Crippa, A. Wald, and L. Corey.** 2002. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin. Infect. Dis. **34:**909–917.
14. **Marty, F. M., L. A. Cosimi, and L. R. Baden.** 2004. Breakthrough zygomycosis after voriconazole treatment in recipients of hematopoietic stem-cell transplants. N. Engl. J. Med. **350:**950–952.
15. **Oren, I.** 2005. Breakthrough zygomycosis during empirical voriconazole therapy in febrile patients with neutropenia. Clin. Infect. Dis. **40:**770–771.
16. **Pagano, L., M. Caira, and L. Fianchi.** 2005. Pulmonary fungal infection with yeasts and pneumocystis in patients with hematological malignancy. Ann. Med. **37:**259–269.
17. **Pagano, L., M. Offidani, L. Fianchi, A. Nosari, A. Candoni, M. Piccardi, L. Corvatta, D. D'Antonio, C. Girmenia, P. Martino, and A. Del Favero.** 2004. Mucormycosis in hematologic patients. Haematologica **89:**207–214.
18. **Peláez, T., J. Guinea, J. Martínez- Alarcón, P. Muñoz, C. Bové, and E. Bouza.** 2005. Are agents responsible for mucormycosis commonly found in hospital air? Analysis of air samples in a general hospital over a six-year period. Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-963. American Society for Microbiology, Washington, DC.
19. **Rees, J. R., R. W. Pinner, R. A. Hajjeh, M. E. Brandt, and A. L. Reingold.** 1998. The epidemiological features of invasive mycotic infections in the San Francisco Bay area, 1992–1993: results of population-based laboratory active surveillance. Clin. Infect. Dis. **27:**1138–1147.
20. **Rickerts, V., A. Bohme, and G. Just-Nubling.** 2002. Risk factor for invasive zygomycosis in patients with hematologic malignancies. Mycoses **45:**27–30.
21. **Ritz, N., R. A. Ammann, C. C. Aebischer, M. Gugger, K. Jaton, R. A. Schmid, and C. Aebi.** 2005. Failure of voriconazole to cure disseminated zygomycosis in an immunocompromised child. Eur. J. Pediatr. **164:**231–235.
22. **Singh, S. K., P. Wadhwa, and V. Sakhuja.** 2004. Isolated bilateral renal mucormycosis. Urology **63:**979–980.
23. **Siwek, G. T., K. J. Dodgson, M. de Magalhaes-Silverman, L. A. Bartelt, S. B. Kilborn, P. L. Hoth, D. J. Diekema, and M. A. Pfaller.** 2004. Invasive zygomycosis in hematopoietic stem cell transplant recipients receiving voriconazole prophylaxis. Clin. Infect. Dis. **39:**584–587.
24. **Takabayashi, M., R. Sakai, H. Sakamoto, M. Kakinuma, Y. Iemoto, H. Kanamori, and Y. Ishigatsubo.** 2004. Cutaneous mucormycosis during induction chemotherapy for acute lymphocytic leukemia. Leuk. Lymphoma **45:**199–200.
25. **Tietz, H. J., D. Brehmer, W. Janisch, and H. Martin.** 1998. Incidence of endomycoses in the autopsy material of the Berlin Charite Hospital. Mycoses **41:**81–85.
26. **Verma, A., S. Williams, S. Trifilio, T. Zembower, and J. Mehta.** 2004. Successful treatment of concomitant pulmonary zygomycosis and aspergillosis with a combination of amphotericin B lipid complex, caspofungin, and voriconazole in a patient on immunosuppression for chronic graft-versus-host disease. Bone Marrow Transplant. **33:**1065–1066.
27. **Vigouroux, S., O. Morin, P. Moreau, F. Mechinaud, N. Morineau, B. Mahe, P. Chevallier, T. Guillaume, V. Dubruille, J. L. Harousseau, and N. Milpied.** 2005. Zygomycosis after prolonged use of voriconazole in immunocompromised patients with hematologic disease: attention required. Clin. Infect. Dis. **40:**e35–e37. [Epub ahead of print.]
28. **Walsh, T. J., A. Groll, J. Hiemenz, R. Fleming, E. Roilides, and E. Anaissie.** 2004. Infections due to emerging and uncommon medically important fungal pathogens. Clin. Microbiol. Infect. **10:**48–66.
29. **Wingard, J. R.** 2005. The changing face of invasive fungal infections in hematopoietic cell transplant recipients. Curr. Opin. Oncol. **17:**89–92.
30. **Yamazaki, T., H. Kume, S. Murase, E. Yamashita, and M. Arisawa.** 1999. Epidemiology of visceral mycoses: analysis of data in annual of the pathological autopsy cases in Japan. J. Clin. Microbiol. **37:**1732–1738.