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Significance of lower respiratory tract cultures yielding *Aspergillus* spp. growth in a hospital without transplant patients

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ABSTRACT

Introduction: Isolation of *Aspergillus* spp. in non-neutropenic, non-transplant patients with chronic obstructive pulmonary disease (COPD) usually treated with corticosteroids is not easily interpretable. A retrospective review of clinical records corresponding to cultures (respiratory samples) yielding *Aspergillus* spp. in non- transplant patients was carried out.

Methods: Patients were assigned to four categories: colonization, possible, probable or definitive aspergillosis. A logistic regression model (step-wise procedure) was performed using as dependent variable mortality, and as independent variables those showing differences ($p \leq 0.1$) in the bivariant analysis.

Results: Sixty-nine patients were identified. Most were elderly (68.1% ≥65 years), male (73.9%), presented comorbidities (84.1% Charlson index ≥3), COPD (76.8%), were receiving high corticosteroid doses (66.7%), and had previously received antibiotics (94.2%). Forty-five cases were colonizations, 4 possible, 15 probable and 5 definitive aspergillosis. *A. fumigatus* was isolated in 75.4% patients: 66.7% colonized, 75% possible, 93.3% probable and 100% definitive aspergillosis. Colonized patients were older (71.9 ± 11.9 vs. 65.1 ± 9.2 years; p= 0.018) and presented higher (p=0.034) comorbidity index than patients with aspergillosis. Mortality was 31.1% in colonized vs. 62.5% in aspergillosis (p=0.012).

Conclusion: The isolation of *A. fumigatus* was associated with an increased probability of aspergillosis, with statistical association in the multivariate analysis between mortality and variables related to chemotherapy (no antifungal treatment), disease (diagnostic category) and immunity (leukocytosis).

Key words: Aspergillosis; Aspergillus fumigatus; Chronic obstructive pulmonary disease; Invasive disease; Colonization

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Significado del cultivo de *Aspergillus* en muestras del tracto respiratorio inferior en un hospital sin pacientes trasplantados

RESUMEN

Introducción: El aislamiento de *Aspergillus* spp. en pacientes no neutropénicos, no-trasplantados con enfermedad pulmonar obstructiva crónica (EPOC) habitualmente tratados con corticoesteroides no es fácilmente interpretable. Se realizó una revisión retrospectiva de las historias clínicas correspondientes a pacientes no trasplantados con cultivos de muestras respiratorias que presentaban crecimiento de *Aspergillus* spp.

Métodos: Se asignó a los pacientes a cuatro categorías: colonización, posible, probable o aspergillosis definitiva. Se realizó un modelo de regresión logística (procedimiento por pasos sucesivos) usando la mortalidad como variable dependiente y como variables independientes las variables que mostraron diferencias ($p \leq 0.1$) en el análisis bivariado.

Resultados: Se identificaron 69 pacientes. La mayoría era de edad avanzada (68,1% ≥65 años), varones (73,9%), presentaban comorbilidades (84,1% índice de Charlson ≥3), EPOC (76,8%), recibían dosis altas de corticosteroides (66,7%), o habían sido tratados con antibióticos previamente (94,2%). Cuarenta y cinco casos fueron clasificados como colonizaciones, 4 como posible, 15 como probable y 5 como aspergilosis definitiva. *A. fumigatus* fue aislado en el 75,4% de los pacientes: 66,7% colonizados, 75% posible, 93,3% probable y 100% con aspergilosis definitiva. Los pacientes colonizados presentaron mayor edad (71,9 ± 11,9 vs. 65,1 ± 9,2 años; p= 0,018) y mayor (p=0,034) índice de comorbilidad que los pacientes con aspergilosis. La mortalidad fue del 31,1% en pacientes colonizados vs. 62,5% en pacientes con aspergilosis (p=0,012).

Conclusión: El aislamiento de *A. fumigatus* se asoció a un aumento de la probabilidad de aspergilosis, con una asociación estadística en el análisis multivariado entre mortalidad y variables relacionadas con la terapia (ausencia de tratamiento

antifúngico), la enfermedad (categoría diagnóstica) y la inmunidad (leucocitosis).

Palabras clave: Aspergilosis; *Aspergillus fumigatus*; EPOC; Enfermedad invasiva; Colonización

INTRODUCTION

Exposure to *Aspergillus* spp. is common since this fungus grows on a variety of organic material and the conidia are easily aerosolized. *Aspergillus fumigatus* (in 60 to 85% cases) followed by *Aspergillus niger* and *Aspergillus flavus* are the most common species isolated from lower respiratory tract samples[1,2]. But the value of isolating *Aspergillus* spp. from these samples is highly debatable because this finding is associated with colonization in a high percentage of cases[2], colonization may represent temporary passage, long-term benign carriage or a sign preceding invasive disease since the incubation period is unknown[3,4]. Sensitivity of isolation for diagnosis of invasive infection ranges from 5% to 75% depending on the population[2].

Patient populations at risk for invasive aspergillosis have been categorized into high-risk (such as allogenic bone marrow transplant, neutropenia, haematological cancer), intermediate-risk (such as autologous bone marrow transplant, solid organ transplant) and low-risk (such as cystic fibrosis, diabetes)[5,6]. For high-risk patients, standard definitions of opportunistic infections as proven, probable or possible have been proposed[1,7]. But there are an increasing number of reports of invasive infection in patients with less immunodeficiency such as chronic obstructive pulmonary disease (COPD) especially under steroids therapy[1,6,8-10].

Lack of sensitivity for diagnosis has therapeutic implications since while treatment is mandatory in severely immunocompromised patients (neutropenia and use of immunosupressants) with suggestive clinical manifestations or isolation of *Aspergillus* in respiratory samples, the therapeutic approach is not well defined in patients with *Aspergillus* isolation without neutropenia or transplantation.

The aim of this study was to investigate the significance of cultures yielding growth of *Aspergillus* spp. from respiratory samples in a general hospital without transplant patients.

MATERIAL AND METHODS

Clinical records of adult patients corresponding to cultures of respiratory samples yielding *Aspergillus* spp. in a two-year period (June 2004- May 2006) were retrospectively reviewed in 2007 at Hospital Severo Ochoa (Leganés, Madrid, Spain), a 406-beds hospital where patients candidate for transplantation are referred to other hospitals. Patients who had *Aspergillus* isolation on more than one occasion were counted once. Demographic data, underlying illnesses, clinical and radiological data, laboratory data, previous treatments (corticosteroids, antibiotics, antifungals…), antifungal treatment and hospitalization outcome were recorded. Neutropenia was defined[11] as a neutrophil count <1,000 cells/mm^3. The Charlson comorbidity index and its correction by age were calculated as previously described[12,13] as well as the functional classification according to the New York Heart Association (NYHA) (http://www.hcoa.org/hcoacme/chf-cme/chf00070.htm). The retrospective review of clinical records included the assignment of the patients to four diagnostic categories (colonization, possible aspergillosis, probable aspergillosis or definitive aspergillosis) according to previously defined criteria for immunocompromised patients[6,14].

Comparisons between proportions were performed by the χ^2 test and the Fisher's exact test, when necessary. For quantitative variables, since data did not showed normality in the Kolmogorov – Smirnoff test, the Kruskal-Wallis and Mann-Whitney tests, when necessary, were used. The bivariant analysis was performed comparing all variables between patients who died and those that did not. A logistic regression model (step–wise procedure) was performed using as dependent variable mortality, and as independent variables those showing differences ($p \leq 0.1$) in the bivariant analysis. Statistical analysis was performed using SPSS v 14 programme (SPSS Inc, Chicago IL). The model showing the highest R^2 was considered.

RESULTS

In the study period 71 patients with at least one culture from respiratory samples (47 sputum samples and 34 bronchoaspirates) positive to *Aspergillus* spp. were identified. Two *Aspergillus* isolates (one *A. fumigatus* and one *A. terreus*) corresponded to two patients with allergic bronchopulmonary aspergillosis (≥65 years of age, with emphysema treated with systemic corticosteroids and comorbidity index ≥3). Of the 69 remaining patients 45 were classified as colonized patients, 4 as patients with possible, 15 as patients with probable and 5 as patients with definitive aspergillosis. Table 1 shows the distribution of isolates per category. Fifty-two of the 69 patients (75.4%) had positive culture to *A. fumigatus*, 7.2% to *A. terreus*, 5.8% to *A. flavus* and 11.6% to other species. Species other than *A. fumigatus* were isolated from colonized patients except in two cases. With respect to *A. fumigatus*, 30 out of 45 (66.7%) were isolated from colonized patients, 3 out of 4 (75%) from patients with possible, 14 out of 15 (93.3%) from patients with probable and 5 out of 5 (100%) from patients with definitive aspergillosis. When comparing species from colonized patients versus those from patients with aspergillosis (regardless the category), 30 out of 45 (66.7%) vs. 22 out of 24 (91.7%) (p=0.00003, OR= 16.50, 95%CI= 3.36 - 109.89) corresponded to *A. fumigatus*, respectively.

Table 2 shows demographic data and characteristics of patients distributed by categories. Colonized patients were significantly older (71.9 ± 11.9 vs. 65.1 ± 9.2 years; p= 0.018) and presented also higher (p=0.034) comorbidity index according to Charlson classification than patients with aspergillosis. Most frequent comorbid conditions were (colonized versus patients with aspergillosis): diabetes (24.4%

Table 1 **Species distribution per category (n (%))**

		Aspergillosis		
	Colonized	Possible	Probable	Definitive
	45	4	15	5
A. fumigatus	30 (66.7)	3 (75.0)	14 (93.3)	5 (100)
A. terreus	5 (11.1)			
A. flavus	3 (6.7)	1 (25.0)		
Aspergillus spp.	7 (15.6)		1 (6.7)	

vs. 16.7%; p=0.652), malignancies (17.8% vs. 16.7%; p=0.709) and cor pulmonale (15.6% vs. 12.5%; p=0.738). Most patients (53 out of 69; 76.8%) in this series presented COPD, with higher rate of emphysema in those patients presenting aspergillosis (13% in colonized versus 33% in patients with aspergillosis; p= 0.049). The number of patients with aspergillosis that had been previously admitted to ICU or that had received previous antifungal treatment was higher than among those colonized: 22.2% in colonized vs. 41.7% in patients with aspergillosis (p= 0.09) for ICU stay and 20.0% in colonized vs. 41.7% in patients with aspergillosis (p= 0.055) for previous antifungals, although differences did not reach statistical significance. No significant differences in colonized vs. patients with aspergillosis were found with respect to exacerbation of COPD (24.4% vs. 41.7%; p= 0.14), bronchospasms (26.7% vs. 20.8%; p= 0.59) and haemoptysis (6.7% vs. 12.5%; p= 0.41), although differences in fever almost reached statistical significance (26.7% vs. 50.0%; p= 0.053). Pleuritic pain was present in 8.9% colonized patients versus in 33.3% patients with aspergillosis (p= 0.018). Leukocytosis was present in 51.1% colonized patients and in 50.0% patients with aspergillosis, and leukopenia in 2.2% and 8.3%, respectively. A total of 22 (48.9%) colonized patients (p= 0.080) presented X-ray findings (17 of them with infiltrates) whereas this occurred in 17 (70.8%) patients with aspergillosis. Computerized tomography (CT) was performed in 24 (53.3%) colonized patients and in 18 (75.0%) patients with aspergillosis. Infiltrates and bronchiectasis were recorded in 33.4% and 29.2%, respectively, in CT of colonized patients and in 52.6% and 31.6%, respectively, in CT of patients with aspergillosis.

Only three patients (4.3%) presented neutropenia. They were three males of 60-69 years old receiving chemotherapy for lymphoma (2 cases, one classified as colonization and one as probable aspergillosis) or rheumatoid arthritis (1 case classified as definitive aspergillosis).

Antifungal treatment was initiated in 21 out of 45 (46.7%) colonized patients (9 with voriconazole, 5 with voriconazole in combination with caspofungin, itraconazole or amphotericin and 7 with itraconazole) vs. in 20 out of 24 (83.3%) patients with aspergillosis (10 with voriconazole in combination with caspofungin, itraconazole or amphotericin, 6 with voriconazole, 2 with amphotericin and 2 with itraconazole), the difference being statistically significant (p= 0.03). All patients with definitive aspergillosis had been treated. Mortality was 31.1% in colonized patients compared to 62.5% in patients with aspergillosis (p=0.012). By categories, mortality was 31.1% in colonized patients, 25% in possible, 80% in probable and 40% in definitive aspergillosis (p= 0.009). The three patients with neutropenia died.

In the multivariate analysis the logistic regression was statistically significant (p= 0.03, R^2 Cox= 0.304), with mortality being associated with absence of antifungal treatment (p= 0.068; OR= 3.46, 95%CI= 0.91-13.08), with categories of aspergillosis (p= 0.030) due to the association with definitive aspergillosis (p= 0.055, OR= 11.91, 95%CI= 0.10-149.17) when using colonization as reference, and with leukocytosis (p= 0.003, OR= 7.41, 95%CI= 1.96-28.57) when using leukopenia as reference.

Focusing on the 24 patients with aspergillosis, and splitting them by clinical diagnosis, 7 patients had simple tracheobronchitis (1 possible, 3 probable and 3 definitive) and 17 had invasive disease (3 possible, 12 probable and 2 definitive): 14 aspergillus pneumonia, 2 chronic necrotizing pulmonary aspergillosis and 1 invasive tracheobronchitis. Mortality in tracheobronchitis (2 out of 7; 28.6%) was lower (p= 0.061) than in invasive aspergillosis (13 out of 17; 76.5%). This difference became statistically significant (p= 0.037) when considering only probable plus definitive aspergillosis: 2 out of 6 (33.3%) for tracheobronchitis and 12 out of 14 (85.7%) for invasive aspergillosis.

DISCUSSION

The value of recovering *Aspergillus* spp. from culture of lower respiratory tract secretions depends on patient population. While the significance of *Aspergillus* isolation has been extensively studied in immunocompromized hosts who develop invasive disease[6], the significance of *Aspergillus* isolation in immunocompetent or mild immunocompromized

Table 2 Characteristics of patients distributed by categories

			Aspergillosis			
		Colonized (n=45)	Possible (n=4)	Probable (n=15)	Definitive (n=5)	All aspergillosis categories (n=24)
Age (mean ± SD)		71.9 ± 11.9	64.5 ± 7.6	64.3 ± 9.6	68.2 ± 10.3	65.1 ± 9.2[a]
Males (%)		71.1	100	73.3	80.0	79.2
Charlson (median (IQ))		5.0 (5.0 - 6.0)	4.0 (3.0 - 5.7)	4.0 (2.0 - 5.0)	4.0 (2.0 - 5.0)	4.0 (2.2 - 5.0)[b]
% patients with	0	4.4	0.0	0.0	0.0	0.0
	1-2	6.6	0.0	33.3	20.0	25.0
	≥3	89.9	100	66.7	80.0	75.0
NYHA (% patients)	1	24.4	0.0	26.7	40.0	25.0
	2	42.2	75.0	33.3	40.0	41.7
	3	28.9	25.0	40.0	20.0	33.3
	4	4.4	0.0	0.0	0.0	0.0
COPD (% patients)		75.6	100	80.0	60.0	79.2
Previous stay in ICU (%)		22.2	25.0	53.8	20.0	41.7
Previous mechanical ventilation (%)		20.0	25.0	40.0	20.0	33.3
Previous antibiotic treatment (%)		93.3	100	93.3	100	95.8
Previous antifungal treatment (%)		20.0	25.0	53.0	20.0	41.7
Treatment with corticosteroids[c] (%)		62.2	75.0	81.0	60.0	75.0

[a]p=0.018 vs. colonized

[b]p=0.034 vs. colonized

[c]daily dose >30 mg prednisone (equivalent)

patients has been scarcely investigated. It is clear that neutropenia is the main risk factor for aspergillosis in immunocompromized patients because polymorphonuclear cells and macrophages are the first line of defence against *Aspergillus*. But altered cellular response with macrophage deactivation[15] and corticosteroids use that suppresses neutrophil action against *Aspergillus* hyphae[16] may be risk factors in non-neutropenic or mild immunocompromized patients. Elderly patients (a growing segment of the hospitalized population) may present these latter immunological risk factors, as they are concurrent in patients with previous prolonged hospitalizations and chronic lung diseases with subsequent corticosteroid treatment[2]. In this type of patients without currently recognized risk factors, diagnosis of aspergillosis is more difficult since: a) as the fungus is ubiquitous, caution should be taken in ascribing it a pathogenic role when isolated from non sterile respiratory tract samples, b) gold standard for definitive diagnosis of invasive disease remains histopathological tissue examination, and c) invasive methods are usually non possible in critically patients or in those requiring ventilation[6,17]. Since no invasive diagnostic test is sensitive or specific enough to establish a definitive diagnosis, in this study we tried to assess the clinical significance of cultures from respiratory samples positive to *Aspergillus* spp. by using diagnostic categories initially used in clinical trials for immunocompromised patients (with transplants or cancer) by retrospectively reviewing clinical records in a hospital without patients receiving transplants.

Excluding the two cases of allergic aspergillosis in this series, the 69 positive cultures identified corresponded to a well defined population: elderly (68.1% ≥65 years), mainly males (73.9%), with high comorbidity (84.1% with Charlson index ≥3), presenting COPD (76.8%), receiving high dose of corticosteroids (66.7%), and with previous antibiotic treatment (94.2%). It should be noted that only three patients (4.3%) had neutropenia as corresponds to a population different from that of patients receiving transplants. Most patients (65.2%) were categorized as colonized without differences between them and patients with aspergillosis in the characteristics described except age that was significantly higher in colonized patients. As in other series most patients with *Aspergillus* colonization had chronic lung disease[11], and although the present retrospective study is descriptive and had not been designed to identify variables that differentiate colonization from infection, significantly higher number of infected patients had previous admission in ICU or received previous antifungals.

As previously described *A. fumi*gatus was the most prevalent isolate[1,2], but in the present study isolation of this

species was significantly linked to the diagnostic category ranging from 66.7% in colonized patients to 100% in patients with definitive aspergillosis. That means that the isolation of *A. fumigatus* was associated with an increased probability of aspergillosis (66.7% in colonized vs. 91.7% aspergillosis).

In non-immunocompromized patients the success of antifungal treatment depends on early diagnosis although delayed diagnosis is the rule[1]. Logically, antifungal treatment was initiated in a higher (p=0.03) number of infected patients (83%) than colonized, but surprisingly treatment was initiated in 46.7% patients classified in this retrospectively revision as colonized patients, a substantial difference with previous studies[1,2]. This may be due to the retrospective study design where assignment to diagnostic categories (colonization, possible, probable or definitive aspergillosis) was performed by study investigators, not by treating physicians. Probably, physicians that had prescribed the antifungals recorded in clinical records did not use these criteria (designed for transplant and oncology clinical trials), and in the face of the lack of confidence and timely diagnosis of aspergillosis in critically patients, administered the antifungals because early administration may be life-saving. In patients with aspergillosis the main antifungal used was voriconazole (alone or in combination) that in previous studies has shown improved response and survival versus amphotericin B in invasive aspergillosis[18], and effective treatment of chronic pulmonary aspergillosis.

As can be expected, mortality was significantly higher in patients with aspergillosis, and among infected patients, higher for patients with invasive disease than with simple tracheobronchitis.

The results of this study show that isolation of *Aspergillus* spp. in non-neutropenic, non-transplant COPD patients (usually treated with corticosteroids) is not easily interpretable and this may be important since in the multivariate analysis there was an association between mortality and the absence of antifungal treatment, the diagnostic category and leukocytosis.

ACKNOWLEDGEMENTS

Pfizer S.A., Madrid, Spain supported part of the study with an educational grant.

N.M. is an employee of Pfizer S.A. (Madrid, Spain).

Part of this study was presented at the XIV Congreso de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC), 19-22 May 2010, Barcelona, Spain.

REFERENCES

1. Garnacho-Montero J, Amaya-Villar R, Ortiz-Leyba C, León C, Alvarez-Lerma F, Nolla-Salas J, et al. Isolation of *Aspergillus* spp. from the respiratory tract in critically ill patients: risk factors, clinical presentation and outcome. Crit Care 2005;9:R191-9.
2. Soubani AO, Khanchandani G, Ahmed HP. Clinical significance of lower respiratory tract *Aspergillus* culture in elderly hospitalized patients. Eur J Clin Microbiol Infect Dis 2004;23:491-4.
3. Bulpa P, Dive A, Sibille Y. Invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease. Eur Respir J 2007;30:782-800.
4. Hope WW, Walsh TJ, Denning DW. The invasive and saprophytic syndromes due to *Aspergillus* spp. Med Mycol 2005;43 Suppl 1:S207-38.
5. Perfect JR, Cox GM, Lee JY, Kauffman CA, de Repentigny L, Chapman SW et al. The impact of culture isolation of Aspergillus species: a hospital-based survey of aspergillosis. Clin Infect Dis 2001;33:1824-3.
6. Vandewoude KH, Blot SI, Depuydt P, Benoit D, Temmerman W, Colardyn F et al. Clinical relevance of *Aspergillus* isolation from respiratory tract samples in critically ill patients. Crit Care 2006;10:R31.
7. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T et al. 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 2008;46:1813-21.
8. Bulpa PA, Dive AM, Garrino MG, Delos MA, Gonzalez MR, Evrard PA et al. Chronic obstructive pulmonary disease patients with invasive pulmonary aspergillosis: benefits of intensive care?. Intensive Care Med 2001;27:59-67.
9. Conesa D, Rello J, Vallés J, Mariscal D, Ferreres JC. Invasive aspergillosis: a life-threatening complication of short-term steroid treatment. Ann Pharmacother 1995;29:1235-7.
10. Rello J, Esandi ME, Mariscal D, Gallego M, Domingo C, Valles J. Invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease: report of eight cases and review. Clin Infect Dis 1998;26:1473-5.
11. Nalesnik MA, Myerowitz RL, Jenkins R, Lenkey J, Herbert D. Significance of *Aspergillus* species isolated from respiratory secretions in the diagnosis of invasive pulmonary aspergillosis. J Clin Microbiol 1980;11:370-6.
12. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40:373-83.
13. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. J Clin Epidemiol 1994;47:1245-51.
14. Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis 2002;34:7-14.
15. Lederer JA, Rodrick ML, Mannick JA. The effects of injury on the adaptive immune response. Shock 1999;11:153-9.
16. Roilides E, Uhlig K, Venzon D, Pizzo PA, Walsh TJ. Prevention of corticosteroid-induced suppression of human polymorphonuclear leukocyte-induced damage of Aspergillus fumigatus hyphae by granulocyte colony-stimulating factor and gamma interferon. Infect Immun 1993;61:4870-7.
17. Denning DW. *Aspergillus* in "nonimmunocompromised" critically ill patients. Am J Respir Crit Care Med 2004;170:580-1.
18. Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE,

Oestmann JW et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med 2002;347:408-15.