MAJOR ARTICLE

Outbreak of Invasive Aspergillosis After Major Heart Surgery Caused by Spores in the Air of the Intensive Care Unit

T. Peláez,[1,2,3] P. Muñoz,[1,2,3] J. Guinea,[1,2,3] M. Valerio,[1,2] M. Giannella,[1,2] C. H. W. Klaassen,[4] and E. Bouza[1,2,3]

[1]Department of Clinical Microbiology and Infectious Diseases, Hospital General Universitario Gregorio Marañón, [2]Department of Medicine, Faculty of Medicine, Universidad Complutense, Madrid, [3]Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES CD06/06/0058), Palma de Mallorca, Spain; and [4]Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands

Background. Outbreaks of invasive aspergillosis (IA) are believed to be caused by airborne *Aspergillus* conidia. Few studies have established a correlation between high levels of *Aspergillus fumigatus* conidia and the appearance of new cases of IA or have demonstrated matching genotypes between clinical isolates and those from the environment.

Methods. We detected an outbreak of IA (December 2006 through April 2008) in the major heart surgery intensive care unit (MHS-ICU) of our institution. Our local surveillance program consists of monthly environmental air sampling in operating rooms and ICUs for quantitative and qualitative identification of filamentous fungi. During the study period, we obtained 508 environmental samples from 3 different periods: 6 months before the outbreak, during it, and 6 months after it. Available environmental and clinical strains were genotyped according to the short tandem repeats assay.

Results. Seven patients developed proven or probable IA (5 with lung infection, 1 with mediastinitis, and 1 with lung infection and mediastinitis). *A. fumigatus* was involved in 6 cases. The underlying conditions of the patients were heart transplantation (n = 3), corticosteroid-dependent conditions (n = 2), and diabetes mellitus (n = 2). The mortality rate was 85.7%. Before and after the outbreak (±6 months), the median airborne *A. fumigatus* conidia levels were 0 colony-forming units (CFUs) per cubic meter, and no cases of IA occurred during these periods. However, during the outbreak period, the occurrence of the 6 cases of IA caused by *A. fumigatus* was linked to peaks of abnormally high *A. fumigatus* airborne conidia levels (175, 50, 25, 20, 160, and 400 CFUs/m^3) in the MHS-ICU, whereas counts in the air of both operating rooms remained negative. Matches between *A. fumigatus* genotypes collected from the air of the MHS-ICU and from representative clinical samples were found in 3 of the 6 patients. The outbreak abated when high-efficiency particulate air filters were installed in the affected areas.

Conclusions. Our study revealed that abnormally high levels of airborne *A. fumigatus* conidia correlated with new cases of IA, even in patients who were not severely immunocompromised. The demonstration of matches between air and clinical genotypes reinforces the role of environmental air in the acquisition of IA during the period following MHS. Environmental monitoring of *Aspergillus* spores in the air of postoperative units is mandatory, even when these units receive nonimmunocompromised patients undergoing major surgery.

Invasive aspergillosis (IA) mainly affects severely immunocompromised patients such as those with neutropenia due to hematological and oncological diseases and other entities associated with immunosuppression [1–4]. In this population, pulmonary invasion is the most common clinical presentation of IA.

IA may also occur in nonimmunocompromised patients, particularly those who have just undergone surgery or those with burns [5–9]. The disease has also been reported in patients who have undergone major heart surgery (MHS), mainly as case reports or reports on

Received 6 June 2011; accepted 15 September 2011.

Presented in part: 19th European Congress on Clinical Microbiology and Infectious Diseases, 16–19 May, 2009, Helsinki, Finland (abstract 0-244).

Correspondence: Patricia Muñoz, MD, PhD, Servicio de Microbiología Clínica y Enfermedades Infecciosas, CIBERES, Hospital General Universitario Gregorio Marañón, Dr. Esquerdo 46, 28007 Madrid, Spain (pmunoz@micro.hggm.es).

Clinical Infectious Diseases 2012;54(3):e24–e31

DOI: 10.1093/cid/cir771

small outbreaks of perioperatively acquired infective endocarditis, which is usually attributed to airborne *Aspergillus* conidia in operating rooms [8, 10–12]. However, due to the implementation of air quality regulations requiring high-efficiency particulate air (HEPA) filtration and positive pressure in the air of MHS operating rooms in many countries, these outbreaks have been minimized [13]. IA is rarely acquired during the period following MHS [9].

Some studies have demonstrated an epidemiological relationship between the presence of *Aspergillus* in the air and the occurrence of nosocomial IA [14–17]. However, the demonstration of genotypic identity between *Aspergillus fumigatus* strains from air and those found in clinical samples is difficult, because environmental samples are rarely taken routinely or only after clinical outbreaks are detected. In addition, molecular genotyping of *A. fumigatus* remains uncommon.

We describe an outbreak of IA acquired during the post-MHS period and its relationship with high counts of genotypically related *Aspergillus* in the environment.

MATERIALS AND METHODS

Setting and Description of the MHS Intensive Care Unit

Our institution is a large teaching hospital attending a population of ~715 000 inhabitants. The heart surgery division performs ~600 MHS interventions per year. The 2 MHS operating rooms have positive pressure and HEPA filters.

The MHS intensive care unit (MHS-ICU) was divided into 3 areas: 1 area in which air is HEPA-filtered with positive pressure and 2 adjacent areas for immediate postoperative care in which the air is regularly filtered (non-HEPA) without positive pressure.

Patients in the MHS-ICU are followed up by a team of specialized anesthetists and cardiovascular surgeons. Infectious diseases specialists perform daily rounds to collaborate in the diagnosis and treatment of infectious complications and in the implementation of preventive measures.

Patients

The outbreak lasted from December 2006 through April 2008. Each patient included was classified according to the probability of having IA following the guidelines of the European Organization for Research and Treatment of Cancer/Mycoses Study Group [18]. According to these criteria, patients with a positive *Aspergillus* culture were classified as proven IA, probable IA, or colonization. The *Aspergillus* score of Bouza et al [19] was also applied to these patients. Demographic, clinical, microbiological, and histopathological data were recorded.

Environmental Surveillance

Volumetric air samples from the 3 MHS-ICUs and from the 2 operating rooms are obtained at least once a month for quantitative and qualitative identification of filamentous fungi. Additional samples were also obtained when a suspicious case of *Aspergillus* infection was detected.

During the study period, we obtained 508 environmental samples from 3 different periods: June–November 2006 (pre-outbreak period), December 2006 through April 2008 (outbreak period), and May–October 2008 (post-outbreak period).

Air was sampled using a volumetric sampler (Merck Air Sampler MAS 100; http://www.merck-chemicals.com/pharmaceutical-ingredients/mas-100-microbial-air-monitoring-systems/spanish/c_FMCb.s1OQgYAAAEoFldh9ENU) [11, 20]. Sealed Sabouraud-dextrose irradiated plates were incubated at 30°C for 5 days. The plates were examined daily to check for fungal growth. Colonies of *A. fumigatus* growing on the plates were isolated and identified by morphological procedures and stored.

Clinical Samples and Other Diagnostic Procedures

Clinical samples were requested by physicians. All specimens were cultured in fungal media (Sabouraud-dextrose agar with chloramphenicol, brain-heart infusion agar with antibacterial agents, and potato-dextrose agar) and incubated at 30°C for 3 weeks. Molds were identified based on macroscopic and microscopic characteristics according to conventional procedures [21]. Antifungal susceptibility testing was performed on all the isolated clinical strains following the criteria of the Clinical Laboratory Standards Institute. *Aspergillus* serum galactomannan was determined using the Platelia *Aspergillus* system (Bio-Rad, Marnes-la-Coquette, France), with a positivity cutoff of ≥0.5 ng/mL.

Molecular Typing

Available environmental and clinical strains were genotyped according to the short tandem repeats (STR*Af*) assay developed by de Valk et al [22]. This approach is based on analysis of 9 short tandem repeat markers that are amplified by means of 3 multiplex polymerase chain reactions.

The number of repeats in each marker was assigned using Fragment Profiler software (version 1.2; GE Healthcare). Typing data were imported into Bionumerics software (version 6.0.1; Applied Maths, Sint-Martens-Latem, Belgium). In line with previous definitions, environmental and clinical isolates were considered matched when genotypes were identical (same allele composition for all 9 markers) or clonally related (genotypes with a total of up to 2 repeats' difference in a single locus) [23]. Due to the highly discriminatory and reproducible results of this method, quality control isolates are not necessary. Further details on the criteria for STR*Af* are presented by Guinea et al [24].

Statistical Analysis

Spore counts of *A. fumigatus* during the 3 periods (pre-outbreak, outbreak, and post-outbreak) were compared using the Mann–Whitney *U* test.

RESULTS

Incidence of IA and Clinical Characteristics of the Patients

From December 2006 through April 2008, *Aspergillus* species was recovered from the clinical samples of 10 patients admitted to the MHS-ICU. Isolation was considered colonization in 3 cases and proven or probable IA in 7 cases. The incidence of IA in the MHS-ICU during the outbreak period was 5.2 cases per 1000 admissions compared with no cases during the pre- and post-outbreak periods (Figure 1).

The clinical characteristics of the 7 patients with IA are shown in Table 1. Five patients had pulmonary IA, 1 patient had postsurgical mediastinitis, and 1 patient had both mediastinitis and pulmonary IA. The main underlying conditions included heart transplantation (n = 3), diabetes mellitus (n = 2), corticosteroid-dependent asthma (n = 1), and corticosteroid-dependent chronic obstructive pulmonary disease with multiple myeloma (n = 1). All these patients had a very complicated postsurgical course, which required prolonged mechanical ventilation. Four patients required continuous arteriovenous hemofiltration before the diagnosis of IA, and 3 patients had undergone further surgery, mainly due to hemorrhage. IA was diagnosed 3–72 days after the initial surgical procedure. Six of the 7 patients with IA died (85.7%). Three patients died before receiving optimal therapy: 2 received caspofungin as empirical therapy for invasive candidiasis, and 1 died before antifungal therapy could be started. The 4 remaining patients received combination therapy with voriconazole and caspofungin; only 1 of them survived.

Microbiological Findings and Other Diagnostic Procedures

Diagnostic findings are summarized in Table 2. Six of the 7 patients with IA had *A. fumigatus* isolated. Three patients (1, 3, and 7) had a polyfungal infection. Three non–*A. fumigatus* species were isolated from patient 3 (*A. flavus, A. niger,* and *A. nidulans*).

All the strains were susceptible to amphotericin B, voriconazole, itraconazole, posaconazole, and caspofungin, with the exception of the *A. flavus* strain (patient 1), which was resistant to caspofungin (median inhibitory concentration, >32 μg/mL).

Calcofluor white staining revealed septate hyphae in all the clinical samples. In the 2 patients with mediastinitis, invasion by *Aspergillus* was also demonstrated in the tissue biopsies. Postmortem examination was not permitted in any of the patients.

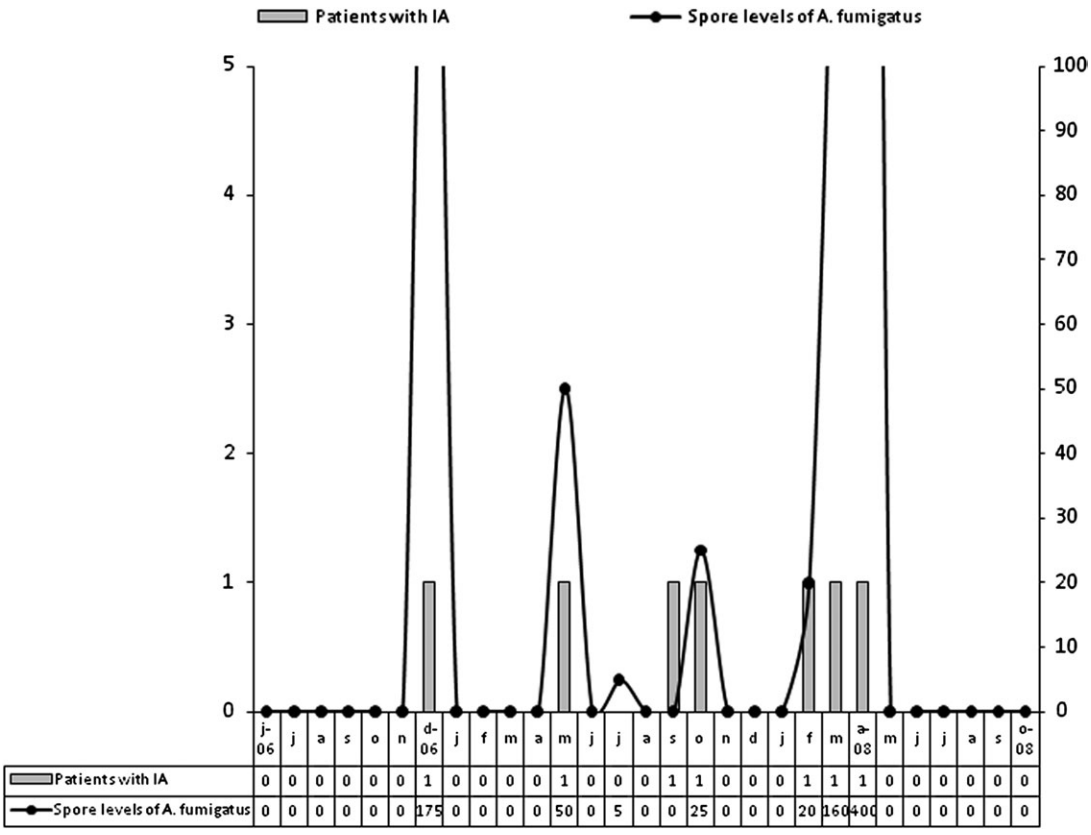

Figure 1. Spore levels of *Aspergillus fumigatus* in the major heart surgery intensive care unit and their relationship with the occurrence of invasive aspergillosis (IA).

Table 1. Clinical Characteristic of the Seven Patients With Invasive Aspergillosis

Patient	Age, Years (Sex)	Surgery Date	IA Diagnosis Date (Days After Surgery)	Underlying Condition	Procedure	Clinical Manifestation	Treatment	Clinical Outcome
1	57 (male)	3 December 2006	9 December 2006 (6)	Diabetes mellitus, postsurgical ventricular dysfunction	Cardiac bypass surgery, additional surgery due to hemorrhage	Mediastinitis	CAS and surgical drainage	Death
2	52 (male)	7 May 2007	23 May 2007 (16)	FK and MMF, corticosteroids, hypogammaglobulinemia, prolonged MV, CAVHF	Heart transplantation	IPA	CAS and VOR	Death
3	56 (female)	11 September 2007	15 September 2007 (4)	FK and MMF, corticosteroids, breast cancer, multiorgan failure	Emergency heart transplantation after CABG and ventricular assistance	IPA	CAS	Death
4	82 (female)	10 October 2007	13 October 2007 (3)	COPD, chronic renal failure, myeloma, corticosteroids, CAVHF	Valve replacement and bypass	IPA	CAS and VOR	Death
5	82 (female)	11 January 2008	18 February 2008 (38)	Asthma, corticosteroids, CAVHF	Valve replacement	IPA	No treatment	Death
6	84 (female)	11 January 2008	24 March 2008 (72)	Diabetes mellitus, prolonged MV, additional surgery, hypogammaglobulinemia	Valve replacement	IPA	CAS and VOR	Death
7	57 (female)	25 February 2008	15 April 2008 (49)	FK and MMF, corticosteroids, COPD, hypogammaglobulinemia, CMV disease, CAVHF	Heart transplantation, additional surgery	Mediastinitis and IPA	CAS and VOR	Survival

Abbreviations: CABG: coronary artery bypass graft; CAS, caspofungin; CAVHF, continuous arteriovenous hemofiltration; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; FK, tacrolimus; IA, invasive aspergillosis; IPA, invasive pulmonary aspergillosis; MMF, mycophenolate mofetil; MV, mechanical ventilation; VOR, voriconazole.

All but patients 1 and 2 had a positive *Aspergillus* galactomannan test result in serum (≥0.5 ng/mL). The mean value of the *Aspergillus* score in the 7 patients with IA was 3.4 (range, 2–4).

Thoracic computed tomography scans were performed in 4 patients and revealed pulmonary infiltrates (n = 1), pleural effusion (n = 2), and nodular lesions (n = 2).

Table 2. Patients With Invasive Aspergillosis and Results Obtained Using Different Diagnostic Methods

Patient	Diagnosis	Histology Result	Calcofluor White Stain Result	Culture Result	*Aspergillus* Species	GM Level, ng/mL	*Aspergillus* Score	Image
1	Mediastinitis	Positive	Positive	Positive	*A. fumigatus, A. flavus*	0.298	2	ND
2	IPA	ND	Positive	Positive	*A. fumigatus*	0.465	4	Chest x-ray: bilateral infiltrates and pleural effusion
3	IPA	ND	Positive	Positive	*A. flavus, A. niger, A. nidulans*	0.719	4	Chest x-ray: mild pleural effusion
4	IPA	ND	Positive	Positive	*A. fumigatus*	1.056	4	CT: bilateral patchy infiltrates and mild bilateral pleural effusion
5	IPA	ND	Positive	Positive	*A. fumigatus*	0.708	4	CT: no pulmonary infiltrates, bilateral pleural effusion and atelectasis
6	IPA	ND	Positive	Positive	*A. fumigatus*	1.373	2	CT: multiple bilateral nodular infiltrates and a predominant nodule in upper left lung
7	Mediastinitis and IPA	Positive	Positive	Positive	*A. fumigatus, A. terreus*	2.557	4	CT: bilateral cavitated nodules predominantly in right lung

Abbreviations: CT, computed tomography; GM, galactomannan; IPA, invasive pulmonary aspergillosis; ND, not done.

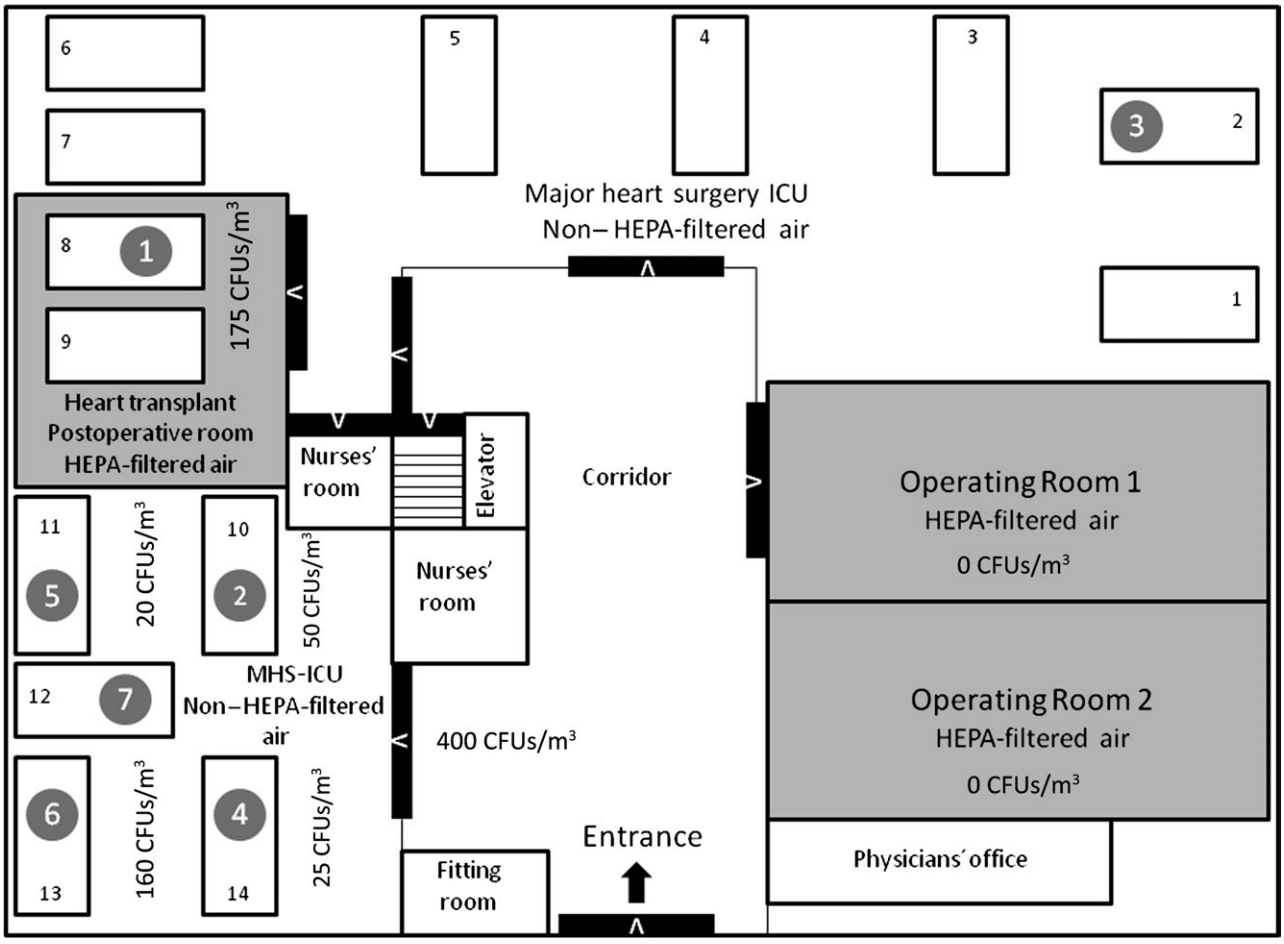

Figure 2. Map of the major heart surgery intensive care unit (MHS-ICU) showing the bed of the seven patients with invasive aspergillosis (*circles*) and *Aspergillus* conidia load. CFU, colony-forming unit; HEPA, high-efficiency particulate air.

Environmental Surveillance

The chronological distribution of the IA cases and the mean level of *A. fumigatus* conidia obtained in the MHS-ICU and in the operating rooms are summarized in Figures 1 and 2 and Table 3.

The first infected patient was recorded in December 2006 and the last in April 2008. During this period, we detected peaks (abnormally high levels) of *A. fumigatus* conidia in the air (20–400 colony-forming units [CFUs] per cubic meter)

Table 3. Relationship Between Spore Levels of *Aspergillus Fumigatus* and Patients With Invasive Aspergillosis

Patient	Date	Diagnosis	Spore Level of *A. fumigatus* in Operating Room	Spore Level of *A. fumigatus* in MHS-ICU, CFUs/m^3	Matches Between Air and Patient *A. fumigatus* Isolates
1	9 December 2006	Mediastinitis	0	175	Yes
2	23 May 2007	IPA	0	50	No
3	15 September 2007	IPA[a]	0	0	Not genotyped
4	13 October 2007	IPA	0	25	Yes
5	18 February 2008	IPA	0	20	No
6	24 March 2008	IPA	0	160	Yes
7	15 April 2008	Mediastinitis and IPA	0	400	No

Abbreviations: CFU, colony-forming unit; IPA, invasive pulmonary aspergillosis; MHS-ICU, major heart surgery intensive care unit.

[a] IPA caused by *A. flavus, A. niger,* and *A. nidulans*.

(Figure 2). Detection of cases with IA caused by *A. fumigatus* coincided with abnormally high levels of *A. fumigatus* conidia in the air of the MHS-ICU. During this period, extensive building work was being performed in the hospital. The building work involved the refurbishment of a day-hospital in the cardiology department, which is located on the same floor, just in front of the main entrance to the MHS-ICU. All recommended control measures were strictly followed. The air conditioning system was shared by both areas.

The first peak of high-level conidia in the air occurred in December 2006 (175 CFUs/m^3; patient 1), the second in May 2007 (50 CFUs/m^3; patient 2), the third in October 2007 (25 CFUs/m^3; patient 4), the fourth in February 2008 (20 CFUs/m^3; patient 5), the fifth in March 2008 (160 CFUs/m^3; patient 6), and the sixth in April 2008 (400 CFUs/m^3; patient 7) (Figure 2). After each case of IA, careful environmental cleaning was performed and air filters were changed in the MHS-ICU. During the study period, median *Aspergillus* conidia counts in operating rooms were 0 CFUs/m^3.

When the mean spore counts in the air of the MHS-ICU were compared for the 3 periods analyzed (pre-outbreak, outbreak, and post-outbreak), statistically significant differences were observed between the outbreak period (17.4 CFUs/m^3) and both the pre-outbreak (0 CFUs/m^3) and post-outbreak (0 CFUs/m^3) periods ($P = .045$). The spore count was significantly higher during the outbreak period than during the pre- and post-outbreak periods. Our data showed that the presence of $>$17.4 CFUs/m^3 of *A. fumigatus* spores can increase the risk of IA even in MHS patients who are not severely immunocompromised.

Throughout the study period (6 months before the outbreak, during the outbreak, and 6 months after it), we obtained 508 environmental samples from the MHS-ICU. During the outbreak period, we genotyped 59 environmental isolates (from 30 environmental samples) and 109 clinical isolates (from 35 clinical samples) using STR*Af.* We found matches between clinical and environmental *A. fumigatus* genotypes in 3 of the 6 IA cases (patient 1, with mediastinitis, and patients 4 and 6, with invasive pulmonary aspergillosis).

After the last peak of abnormally high airborne conidia levels, the air conditioning system was replaced. HEPA filters were installed in all the rooms of the MHS-ICU. No further cases of IA have been recorded to date.

DISCUSSION

Our study revealed that abnormally high levels of airborne *A. fumigatus* conidia correlated with new cases of IA, even in patients who were not severely immunocompromised. The demonstration of matches between air and clinical genotypes reinforces the role of environmental air in the acquisition of IA during the period following MHS.

The incidence of IA is increasing in many institutions, and recent publications suggest important shifts in the underlying conditions of patients and in the species of *Aspergillus* causing the disease [2, 25–27]. The disease occurs mainly in profoundly immunocompromised neutropenic or nonneutropenic patients [1, 3, 4, 28–30]. However, IA is increasingly frequent in specific groups of nonimmunocompromised patients, such as patients undergoing major surgery and burn patients with tissue exposed to the environment [1, 7, 8, 31–34].

Invasive aspergillosis in patients undergoing MHS has mainly been reported as an intra-operative accident due to deficiencies in the air conditioning of operating rooms leading to isolated cases or outbreaks of infective endocarditis [9, 12]. Postoperative acquisition of IA after MHS occurs mainly in patients undergoing heart transplantation or with severe immunodeficiency, but also—albeit rarely—occurs in noninmunocompromised patients [35–38]. The most common clinical presentation is invasive pulmonary or wound aspergillosis [38, 39]. However, to the best of our knowledge, neither of the two has been related to high environmental levels of *Aspergillus* conidia.

The epidemiological link between outbreaks of IA and environmental contamination is relatively strong [11, 17]. Other than this epidemiological evidence, few reports have correlated the increasing concentrations of spores in air with an increasing incidence of IA [40, 41]. However, the weaknesses of those studies include the lack of a reference concentration of *Aspergillus* in air, making it impossible to distinguish normal from abnormal or ''dangerous'' air. Furthermore, the cutoff point for ''dangerous'' air depends on the degree of immunosuppression of the exposed population and the exposure of deep tissues to the environment.

In our opinion, the reason for this information gap is clear. Systematic monitoring of concentrations of *Aspergillus* in the air of nonprotected hospital spaces is very uncommon, and when performed, it is usually undertaken late, once an ongoing epidemic outbreak has been demonstrated. This was not the case in our institution, where we routinely monitor levels and identification of filamentous fungi in the air of the MHS-ICU.

Newer techniques for real-time particle counting could prove faster than culture-based procedures when monitoring air quality. The use of particle counters could anticipate abnormally high peaks and thus make it possible to prevent new cases of IA. However, these procedures cannot identify fungi and should be carried out in parallel with culture-based procedures, at least when the peak has been detected [16].

Although no conidia concentration above which the risk of increased incidence of IA has been established, our observations showed that air with a mean *A. fumigatus* load of $\geq$16.7 CFUs/m^3 should be considered ''dangerous'' when susceptible hosts are exposed.

Recent guidelines from the Spanish Society for Infectious Diseases [16] suggest that nonfiltered air should not contain >5 conidia per cubic meter; other authors accept between 10 and 25 CFUs/m^3 [42, 43]. Concentrations of >25 CFUs/m^3 should be regarded as "dangerous" in the hospital setting [13]. In our institution, cases of IA disappeared briefly after air control measures were undertaken, although they subsequently recurred. The outbreak finished only when the MHS-ICU was closed for 1 month to install HEPA filters in all areas of the ICU. Since then, air surveillance monitoring has shown excellent air quality with an *Aspergillus* conidia load of <5 CFUs/m^3. In our opinion, HEPA filtration should be implemented in MHS-ICUs and in ICUs that attend immunosuppressed patients. Environmental control measures must be reinforced when building work is in progress, and closure of the unit should be considered when an outbreak is declared.

One of the strengths of our study is that the isolates were collected prospectively, thus enabling us to genotype environmental and clinical isolates. Using a high-discriminatory molecular typing tool (STR*Af*), we found matches between environmental and clinical genotypes in 3 of the 6 patients with IA caused by *A. fumigatus*. Our study proved the epidemiological relationship between high concentrations of *A. fumigatus* spores in air and the appearance of cases of IA, as well as the presence of matches between patients and some of the environmental isolates.

Our results support environmental monitoring of *Aspergillus* spores in the air of postoperative units, even when these units receive nonimmunocompromised patients undergoing major surgery.

Notes

Acknowledgments. We thank Thomas O'Boyle for editing and proofreading the article.

Financial support. This work was supported by the Fondo de Investigación Sanitaria (grant PI070198 to T.P. and J.G. and contract CP09/00055 to J.G.); and the Instituto de Salud Carlos III.

Potential conflicts of interest. T.P. has participated as speaker for Astellas, and has received travel grants from Astellas. P.M. has participated as speaker for Astellas, Novartis, and Pfizer and has received payment for educational presentations from Astellas. J.G. has participated as speaker for Astellas, Gilead, Pfizer, and Merck Sharp & Dohme, and has received travel grants from Astellas and European Society of Clinical Microbiology and Infectious Diseases. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Cornillet A, Camus C, Nimubona S, et al. Comparison of epidemiological, clinical, and biological features of invasive aspergillosis in neutropenic and nonneutropenic patients: a 6-year survey. Clin Infect Dis **2006**; 43:577–84.
2. Denning DW. Invasive aspergillosis. Clin Infect Dis **1998**; 26:781–805.
3. Patterson JE, Peters J, Calhoon JH, et al. Investigation and control of aspergillosis and other filamentous fungal infections in solid organ transplant recipients. Transpl Infect Dis **2000**; 2:22–8.
4. Upton A, Kirby KA, Carpenter P, Boeckh M, Marr KA. Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality. Clin Infect Dis **2007**; 44: 531–40.
5. Karim M, Alam M, Shah AA, Ahmed R, Sheikh H. Chronic invasive aspergillosis in apparently immunocompetent hosts. Clin Infect Dis **1997**; 24:723–33.
6. Cook DJ, Achong MR, King DE. Disseminated aspergillosis in an apparently healthy patient. Am J Med **1990**; 88:74–6.
7. Denning DW. Aspergillosis in "nonimmunocompromised" critically ill patients. Am J Respir Crit Care Med **2004**; 170:580–1.
8. Pasqualotto AC, Denning DW. Post-operative aspergillosis. Clin Microbiol Infect **2006**; 12:1060–76.
9. Jensen J, Guinea J, Torres-Narbona M, Muñoz P, Pelaez T, Bouza E. Post-surgical invasive aspergillosis: an uncommon and under-appreciated entity. J Infect **2010**; 60:162–7.
10. El-Hamamsy I, Durrleman N, Stevens LM, Perrault LP, Carrier M. *Aspergillus* endocarditis after cardiac surgery. Ann Thorac Surg **2005**; 80:359–64.
11. Vonberg RP, Gastmeier P. Nosocomial aspergillosis in outbreak settings. J Hosp Infect **2006**; 63:246–54.
12. Badiee P, Alborzi A, Shakiba E, Ziyaeyan M, Pourabbas B. Molecular diagnosis of *Aspergillus* endocarditis after cardiac surgery. J Med Microbiol **2009**; 58:192–5.
13. Ruiz-Camps I, Aguado JM, Almirante B, et al. [Recommendations of the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) on the prevention of invasive fungal infection due to filamentous fungi]. Enferm Infecc Microbiol Clin **2010**; 28: 172.e1–21.
14. Richardson MD, Rennie S, Marshall I, et al. Fungal surveillance of an open haematology ward. J Hosp Infect **2000**; 45:288–92.
15. Arnow PM, Sadigh M, Costas C, Weil D, Chudy R. Endemic and epidemic aspergillosis associated with in-hospital replication of *Aspergillus* organisms. J Infect Dis **1991**; 164:998–1002.
16. Alberti C, Bouakline A, Ribaud P, et al. Relationship between environmental fungal contamination and the incidence of invasive aspergillosis in haematology patients. J Hosp Infect **2001**; 48:198–206.
17. Benet T, Nicolle MC, Thiebaut A, et al. Reduction of invasive aspergillosis incidence among immunocompromised patients after control of environmental exposure. Clin Infect Dis **2007**; 45:682–6.
18. De Pauw B, Walsh TJ, Donnelly JP, 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.
19. Bouza E, Guinea J, Pelaez T, Perez-Molina J, Alcala L, Muñoz P. Workload due to *Aspergillus fumigatus* and significance of the organism in the microbiology laboratory of a general hospital. J Clin Microbiol **2005**; 43:2075–9.
20. Guinea J, Pelaez T, Alcala L, Bouza E. Outdoor environmental levels of *Aspergillus* spp. conidia over a wide geographical area. Med Mycol **2006**; 44:349–56.
21. Murray P, Baron E, Pfaller M, Tenover F, Yolken R. Manual of clinical microbiology. Washington, DC: American Society for Microbiology, **1999**.
22. de Valk HA, Meis JF, Curfs IM, Muehlethaler K, Mouton JW, Klaassen CH. Use of a novel panel of nine short tandem repeats for exact and high-resolution fingerprinting of *Aspergillus fumigatus* isolates. J Clin Microbiol **2005**; 43:4112–20.
23. Balajee SA, de Valk HA, Lasker BA, Meis JF, Klaassen CH. Utility of a microsatellite assay for identifying clonally related outbreak isolates of *Aspergillus fumigatus*. J Microbiol Methods **2008**; 73:252–6.
24. Guinea J, García de Viedma D, Peláez T, et al. Molecular epidemiology of *Aspergillus fumigatus*: an in-depth genotypic analysis of isolates in-

volved in an outbreak of invasive aspergillosis. J Clin Microbiol **2011**; 49:3498–503.
25. Weber DJ, Peppercorn A, Miller MB, Sickbert-Benett E, Rutala WA. Preventing healthcare-associated *Aspergillus* infections: review of recent CDC/HICPAC recommendations. Med Mycol **2009**; 47(Suppl 1): S199–209.
26. Patterson TF, Kirkpatrick WR, White M, et al. Invasive aspergillosis: disease spectrum, treatment practices, and outcomes. I3 *Aspergillus* Study Group. Medicine (Baltimore) **2000**; 79:250–60.
27. Marr KA, Patterson T, Denning D. Aspergillosis: pathogenesis, clinical manifestations, and therapy. Infect Dis Clin North Am **2002**; 16: 875–94, vi.
28. Horn DL, Fishman JA, Steinbach WJ, et al. Presentation of the PATH Alliance registry for prospective data collection and analysis of the epidemiology, therapy, and outcomes of invasive fungal infections. Diagn Microbiol Infect Dis **2007**; 59:407–14.
29. Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. Blood **2002**; 100:4358–66.
30. Wald A, Leisenring W, van Burik JA, Bowden RA. Epidemiology of *Aspergillus* infections in a large cohort of patients undergoing bone marrow transplantation. J Infect Dis **1997**; 175:1459–66.
31. De Maubeuge J, Song M, Juvent M, Ley R, Achten G. *Aspergillus* infection of severe burns. Dermatologica **1983**; 167:78–85.
32. McGinnis MR. Infection of a burn wound by *Aspergillus niger*. Am J Clin Pathol **1980**; 74:118.
33. Panke TW, McManus AT, Spebar MJ. Infection of a burn wound by *Aspergillus niger*: gross appearance simulating ecthyma gangrenosa. Am J Clin Pathol **1979**; 72:230–2.
34. Mousa HA, Al-Bader SM, Hassan DA. Correlation between fungi isolated from burn wounds and burn care units. Burns **1999**; 25: 145–7.
35. Montoya JG, Chaparro SV, Celis D, et al. Invasive aspergillosis in the setting of cardiac transplantation. Clin Infect Dis **2003**; 37(Suppl 3): S281–92.
36. Paterson DL, Singh N. Invasive aspergillosis in transplant recipients. Medicine (Baltimore) **1999**; 78:123–38.
37. Vandecasteele SJ, Boelaert JR, Verrelst P, Graulus E, Gordts BZ. Diagnosis and treatment of *Aspergillus flavus* sternal wound infections after cardiac surgery. Clin Infect Dis **2002**; 35:887–90.
38. Levin T, Suh B, Beltramo D, Samuel R. *Aspergillus* mediastinitis following orthotopic heart transplantation: case report and review of the literature. Transpl Infect Dis **2004**; 6:129–31.
39. Forestier E, Remy V, Lesens O, et al. A case of *Aspergillus* mediastinitis after heart transplantation successfully treated with liposomal amphotericin B, caspofungin and voriconazole. Eur J Clin Microbiol Infect Dis **2005**; 24:347–9.
40. Muñoz P, Guinea J, Pelaez T, Duran C, Blanco JL, Bouza E. Nosocomial invasive aspergillosis in a heart transplant patient acquired during a break in the HEPA air filtration system. Transpl Infect Dis **2004**; 6:50–4.
41. Mellado E, Diaz-Guerra TM, Cuenca-Estrella M, et al. Characterization of a possible nosocomial aspergillosis outbreak. Clin Microbiol Infect **2000**; 6:543–8.
42. Bouza E, Peláez T, Pérez-Molina J, et al. Demolition of a hospital building by controlled explosion: the impact on filamentous fungal load in internal and external air. J Hosp Infect **2002**; 52:234–42.
43. Morris G, Kokki MH, Anderson K, Richardson MD. Sampling of *Aspergillus* spores in air. J Hosp Infect **2000**; 44:81–92.