

REVIEW

Health care-acquired aspergillosis and air conditioning systems

M.L. CRISTINA, M. SARTINI, A.M. SPAGNOLO
Department of Health Sciences, University of Genoa, Italy

Key words

Aspergillosis • Nosocomial infections • HEPA filters

Introduction

Aspergillus species (Fig. 1) are conidia-forming fungi that are responsible for a wide spectrum of human illnesses.

About 19 species of *Aspergillus* have been documented as agents of human disease, but the majority of infections are caused by *Aspergillus fumigatus* (Fresenius, 1850), *Aspergillus flavus* (Link, 1809), *Aspergillus niger* (Van Tieghem, 1867), and *Aspergillus terreus* (Thom, 1918) [1, 2].

Clinical syndromes and diseases associated to *Aspergillus spp.* can be: “cutaneous” (primary skin and burn-wound infections, especially in neonates and children); “hypersensitivity” (allergic bronchopulmonary aspergillosis); “acute invasive” (pneumonia, ulcerative tracheo-bronchitis, osteomyelitis,...); “chronic invasive” (chronic pneumonitis) [3-5].

The primary route of acquiring *Aspergillus* infections is by the inhalation of conidia [6].

The respiratory tract is therefore the most common portal of entry of *Aspergillus spp.* conidia [7]. Conidia of the most commonly involved pathogenic *Aspergillus spp.* are relatively small, with sizes ranging from 2 to 5 µm [8, 9]. The small size of conidia permits them to deposit deep into the lung after inhalation and to reach the pulmonary alveolar spaces, where they may germinate

to form hyphae. Humans inhale approximately 200 conidia of filamentous fungi per day, 7 of which reach the alveoli. These conidia are phagocytosed by the alveolar macrophages and destroyed [10].

The outcome of infection depends more on host factors than on the virulence of the individual *Aspergillus species* [2, 4, 11]. Therefore, host immunity plays a major role in determining who may be at risk of developing invasive aspergillosis (IA). The principal host defences against invasive *Aspergillus* infection are alveolar macrophages, which target inhaled conidia, and neutrophils, which target the hyphal stage [12-14].

Immunocompromised patients are extremely susceptible to local invasion of respiratory tissues by deposited conidia, resulting in invasive aspergillosis [12, 15]. Most cases of health care-acquired invasive aspergillosis present with pneumonia [8]. However, while invasive aspergillosis occurs typically in severely immunocompromised patients, cases of surgical site infection have been reported in immunocompetent individuals [8, 16].

Risk factors for invasive aspergillosis

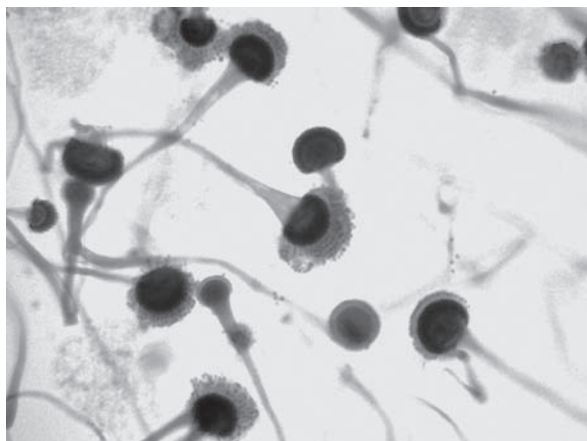
The major risk factor for invasive aspergillosis is prolonged and severe neutropenia [17], both disease- and therapy-induced.

Patients at highest risk have severe immunosuppression resulting from both granulocytopenia and compromised cell-mediated immunity.

Guidelines for Preventing Health-Care associated Pneumonias [18] highlights to maintain a high index of suspicion for health-care-associated pulmonary aspergillosis in severely immunocompromised patients (i.e., patients with severe, prolonged neutropenia [absolute neutrophil count < 500/ mm³ for 2 weeks or < 100/ mm³ for 1 week], most notably haematopoietic stem cell transplantation recipients and including recipients of solid organ transplants or patients with hematologic malignancies who are receiving chemotherapy, when they are severely neutropenic as defined previously) and persons receiving prolonged high-dose steroids.

In the “National Guidelines for the Prevention of Nosocomial Invasive Aspergillosis during Construction/Renova-

Fig. 1. *Aspergillus* sp. (Orlando P. et al. - Department of Health Sciences, University of Genoa, Italy).



tion Activities” [19], at risk patients of invasive aspergillosis are classified in four groups: “Very high risk”, “High risk”, “Increased risk”, “No evidence of risk” (Tab. I).

Invasive aspergillosis incidence and mortality

Clinical reports show that the incidence of invasive aspergillosis differs greatly worldwide, at different treatment centers and even within the same institution, ranging from as low as 0% to 25% or more. Several factors are responsible for this variability. One crucial factor influencing the incidence of invasive aspergillosis is the degree of environmental exposure.

Invasive aspergillosis is associated with a high mortality rate ranging from 50% to 90% depending mostly on the recovery of the host immune response [20].

Mortality among leukaemia patients range from 13% to 80% and can reach 95% in bone marrow transplant recipients [21, 22]. Mortality rate can be as high as 100% if severe neutropenia persists.

Therefore, prevention of invasive aspergillosis is of major importance.

Hospital sources of *Aspergillus spp.*

Aspergillus spp. are ubiquitous fungi that commonly occur in soil, organically enriched debris, water, and decaying vegetation [23, 24]. They also survive in air

and dust [24]. Air plays a crucial role in the spread of *Aspergillus spp.* in the environment and in the transmission to patients [25].

Due to their small size *Aspergillus* conidia may remain airborne for prolonged periods. As conidia gradually settle out, anything in contact with air will become contaminated with conidia. When an environmental reservoir (soil, dust, ...) is disturbed, fungal conidia may be released into the air again. The process of settling out and becoming airborne again can repeat itself for prolonged periods of time [25].

The reduction in the concentration of airborne conidia is paralleled by a decrease in the frequency of invasive infections, but does not reduce it to zero. Although inhalation of airborne conidia is believed to be the primary route of acquiring *Aspergillus* infections, alternative modes of transmission may be present.

Recently, attention has been drawn to the role of hospital water as a possible source of filamentous fungi [26]. Warris et al. have revealed that 94% of water samples taken inside the examined hospital harboured filamentous fungi.

A. fumigatus was recovered from 49% of water samples taken from the taps in the paediatric bone marrow transplantation unit of this hospital [27].

The forming of bio-films in water distribution systems might be responsible for sudden increases in the contamination level of hospital water. So hospital water distribution systems may be a potential indoor reservoir of *Aspergillus* species and other molds, leading to

Tab. I. Classification of at-risk patients.

Risk level	At-risk patients
Very high risk	<ol style="list-style-type: none"> 1. Allogeneic and autologous bone marrow transplantation 3. Peripheral stem cell transplantation 4. Non-myeloablative transplantation 5. Children with severe combined immuno-deficiency syndrome 6. Prolonged neutropenia for greater than 14 days following chemotherapy or immunosuppressive therapy 7. Aplastic anaemia patients
High risk	<ol style="list-style-type: none"> 1. Neutropenia for less than 14 days following chemotherapy 2. Adult acute lymphoblastic leukaemia on high dose steroid therapy 3. Solid organ transplantation 4. Chronic Granulomatous Disease of Childhood 5. Neonates in intensive care units
Increased risk	<ol style="list-style-type: none"> 1. Patients on prolonged courses of high dose steroids particularly those hospitalised for prolonged periods 2. Severely immunosuppressed AIDS patients 3. Patients undergoing mechanical ventilation 4. Patients having chemotherapy who are not neutropenic 5. Dialysis patients
No evidence of risk	All patients not listed in previous Groups

From the “National Guidelines for the Prevention of Nosocomial Invasive Aspergillosis During Construction/Renovation Activities”. National Disease Surveillance Centre (2002).

aerosolization of fungal conidia and potential patient exposure.

Other sources of *Aspergillus spp.* in hospital environment are various foodstuffs including biscuits, tea, herbs and spices, shellfish, and fruit [25].

However, airborne transmission is the principal route of transmission of *Aspergillus spp.* within the hospital environment. To date, the minimal airborne concentration of *Aspergillus* conidia necessary to cause infection in patients with significant immunodeficiency remains unknown. Even concentrations of airborne *Aspergillus* conidia below 1 colony-forming unit (cfu)/m³ have been shown to be sufficient to cause outbreaks in immunocompromised patients. Rhame [28] reported a higher risk of invasive aspergillosis when the average density of *A. fumigatus* was 0,9 cfu/m³.

The concentration of airborne fungi in patient care areas during outbreak investigations ranged from 0 to more than 100 conidia/m³. In rooms with high-efficiency particulate air (HEPA) filtration counts below 0.1 CFU/m³ have been reported.

Due to the prevalence of *Aspergillus spp.* in the hospital environment, many clinicians believe that invasive aspergillosis is hospital-acquired but this is not often proved definitively.

Patterson et al. [29] propose that an invasive aspergillosis episode should be considered nosocomially-acquired if the clinical syndrome begins after the first week of hospital admission or within 14 days of hospital discharge with no evidence of pre-existing or incubating infection on admission. Invasive aspergillosis is considered community-acquired when the clinical syndrome was present on admission to hospital, occurred within the first week of hospital admission, or occurred more than 14 days after hospital discharge.

Proof of the health care-acquired origin of an infection can only be established definitively by demonstrating the identity of strains isolated from the environment and from infected patients.

It might be supposed that tracing the sources of *Aspergillus* infections has become less difficult with the advent of reliable molecular typing methods, which are designed to determine the relatedness of particular subspecific strains. Often, however, it is not possible to demonstrate a correlation between patient and environmental isolates and, on the rare occasions that some patient and environmental isolates have been shown to be identical, not all of the patient isolates could be matched with those from the environment. In most of these studies, isolates of *Aspergillus* from some of the patients with invasive aspergillosis were found to be similar to environmental hospital isolates, but these strains were not compared with isolates recovered from the patients' home environments [30].

The demonstration of a relationship between invasive aspergillosis and airborne strains of *Aspergillus* is difficult because of the limited practice of environmental sampling for *Aspergillus* and for the difficulties in establishing the molecular identity of different strains. On the other hand, environmental investigation starts some

time after an increased incidence of health care-acquired aspergillosis is recognized, but the concentration of environmental airborne fungal conidia varies constantly, so a hospital-acquired *Aspergillus* infection cannot be excluded even if the infecting strain is not found in the hospital environment.

Causes of invasive aspergillosis outbreaks

Many Authors have described outbreaks of health care-acquired invasive aspergillosis reported in intensive care units, transplantation units, renal unit, haematology and oncology units and medical wards where immunosuppressed patients were housed [19].

The presence of *Aspergillus spp.* in the health-care facility environment is a substantial extrinsic risk factor for opportunistic invasive aspergillosis outbreaks [5].

Some of these outbreaks have become a complication of construction, demolition or renovation works in or near hospital wards. Any patients exposed to health care facility construction activities or soil excavation may be at increased risk of acquiring a construction-related nosocomial infection. A study found that hospitalization during construction was an independent risk factor for development of invasive nosocomial fungal infection ($p = 0.09$). Similarly, some Authors reported an increased incidence of aspergillosis over 20 years that coincided with the increasing number of internal hospital renovation projects and, to a lesser extent, external construction projects [20, 24, 25].

Site renovation and construction can disturb *Aspergillus*-contaminated dust and produce bursts of airborne fungal conidia [19, 31].

Other outbreaks of health care-acquired invasive aspergillosis have been associated with malfunctioning or contamination of hospital ventilation or air filtration systems.

Heating, ventilation, and air conditioning (HVAC) systems in health-care facilities allow to:

- maintain the indoor air temperature and humidity at comfortable levels for patients, staff, and visitors;
- remove contaminated air to protect susceptible patients from airborne health-care-associated pathogens.

Malfunction of healthcare facility HVAC systems, improper installation, filter inefficiencies, and poor maintenance can contribute to the spread of health-care-associated airborne infections [5].

Several studies have identified the type of air filter, air changes per hour in room, direction of airflow and air pressure, humidity, and ventilation-system cleaning and maintenance as factors related to air quality and infection rates. Microorganisms proliferate in environments wherever air, dust, and water are present, and air-handling systems can be ideal environments for microbial growth. Accumulation of dust and moisture within the HVAC systems increase the risk for the spread of environmental fungi and bacteria [32].

Lutz et al. [8] describe an outbreak of *Aspergillus* infection at a tertiary care hospital among surgical patients associated with a contaminated air-handling system. This outbreak was due to insulation in variable airflow volume units that had deteriorated after becoming wet. These units were located downstream of final filters, and, therefore, conidia released from mould growing on insulation were not filtered out before entering the operating theatre. No additional invasive *Aspergillus* wound infections were identified after the operating theatre air-handling systems were remediated, suggesting that this outbreak was due to the deterioration of insulating material in variable airflow volume units.

Furthermore, a failure or malfunction of the HVAC system may expose patients to airborne contaminants. Clusters of infections caused by *Aspergillus spp.* have been linked to poorly maintained and/or malfunctioning air conditioning systems [5]. HVAC systems require routine maintenance and monitoring to provide acceptable indoor air quality efficiently and to minimize the risk of transmission of airborne pathogens.

Infection control measures

Prevention of invasive aspergillosis is particularly important because of the high mortality rate for at risk patients as patients with prolonged and severe neutropenia. There is no real consensus worldwide as to how this should be done; nevertheless, high-efficiency particulate air (HEPA) filtration of incoming air, directed room airflow with high frequency air changes ($\geq 12/h$), and a well-sealed room together with positive air pressure in the patient's room in relation to the corridor are uniformly accepted control measures to lower the conidia air count [20].

Outbreaks of invasive aspergillosis reinforce the importance of maintaining an environment as free as possible of *Aspergillus spp.* conidia for patients who have severe granulocytopenia. To achieve this goal, specialized services in many large hospitals (particularly bone-marrow transplant services) have installed "protected environments" for the care of their high-risk, severely granulocytopenic patients and have increased their vigilance during hospital construction and routine maintenance of hospital air-filtration and ventilation systems to prevent exposing high-risk patients to bursts of fungal conidia [21].

Providing clean filtered air and effectively controlling indoor air pollution through ventilation are two key aspects of maintaining good air quality in hospital environments [32]. The use of air filtration systems and other preventive measures limits drastically the access of outdoor fungi, reducing its concentrations.

Guidelines for Environmental Infection Control in Health-Care Facilities [5] show that filtration system with HEPA filters is adequate for most patient-care areas in ambulatory-care facilities and hospitals, including the operating room environment and areas providing central

services. HEPA filters are at least 99.97% efficient for removing particles $> 0.3 \mu\text{m}$ in diameter [33].

Several studies that have employed high efficiency particulate air (HEPA) filters in the rooms of at risk patients have noted significant declines in both airborne levels of *Aspergillus* and in aspergillosis infection rates [34].

Perdelli et al. [35] show that the use of air-conditioning systems equipped with HEPA filters markedly reduces the concentration of *aspergilli* in the environment. In this study, air samples were taken in 3 hospital wards with different air-conditioning features: no conditioning system (ward A), a conditioning system equipped with minimum efficiency reporting value (MERV) filters (ward B), and a conditioning system thoroughly maintained and equipped with high-efficiency particulate air (HEPA) filters (absolute) (ward C). Active sampling showed positive samples for fungi in wards A and B only, with average values of 0.50 colony-forming units (CFU)/m³ in A and 0.16 CFU/m³ in B. Passive sampling (sedimenting mycotic load) was positive only in ward A (mean, 0.14 CFU/cm²/h).

Hahn et al. [36] demonstrate that in the setting of an outbreak of aspergillosis, HEPA filters are protective for highly immunocompromised patients with haematologic malignancies and are effective at controlling outbreaks due to air contamination with *Aspergillus* conidia. Particularly, an outbreak of 10 cases of health care-acquired invasive infection with *Aspergillus flavus* occurred among haematologic oncology patients with prolonged granulocytopenia housed in an environment with neither HEPA filters nor laminar air flow units. After high-efficiency particulate air (HEPA) filters were installed as an infection control measure, there were only two additional cases of health care-acquired aspergillosis in the 2 years following the outbreak.

Outbreaks of health care-acquired invasive aspergillosis during the last two decades have been reported during hospital construction and indoor building renovation and were in correlation with increased air counts of *Aspergillus* organisms.

Oren et al. [37] show a health care-acquired outbreak of invasive pulmonary aspergillosis during extensive hospital construction and indoor renovation (period 1) in acute leukaemia patients treated in a regular ward that has only natural ventilation. The observed infection rate was 50%. Chemoprophylaxis with intravenous continuous low-dose amphotericin B was then instituted as a preventive measure. During the next 18 months (period 2) invasive pulmonary aspergillosis developed in 43% of acute leukaemia patients. After that period a new haematology ward was opened with an air filtration system through HEPA filters and none of the acute leukaemia or bone marrow transplantation patients who were hospitalized in the haematology ward developed invasive pulmonary aspergillosis during the following three years (period 3). Estimation of the number of *Aspergillus* was made by air sampling in different areas in different periods. During period 1 and 2 they found an average of 15 conidia/m³, while during period 3, in the new haematological ward with the HEPA ventilation,

there was an average count of 0.18 CFU/m³ in the air, while counts in regular wards remained as before.

Kruger et al. [38] show that fungal air contamination in HEPA-conditioned patient rooms was constantly low, independent from construction activity.

Nevertheless, other authors have suggested that HEPA filtration supplies alone are not sufficient to prevent health care-acquired aspergillosis, because outbreaks of invasive aspergillosis have been described in units with HEPA filtration.

A study of Cornet et al. [22] has shown that although HEPA filtration significantly reduced the air counts for *Aspergillus* during periods when no construction was taking place, only HEPA filtration plus laminar airflow (LAF) had a significant impact during periods of construction.

When LAF is provided, the air is usually swept across the room parallel to the floor driving contaminants out through the ducts. Nevertheless, laminar airflow

usually involves 400 air changes per hour or more and is expensive, uncomfortable due to draughts and noise [39].

In conclusion, it is practically impossible to eliminate the risk of aspergillosis entirely, but it can be reduced to a minimum. Indeed, the use of an appropriate air-conditioning system (equipped with HEPA absolute filters), proper maintenance of the system itself, the application of protocols for sanitization and staff behaviour recommended for critical wards, such as compliance with procedural norms (eg, avoid frequent opening of doors between the patient care areas and the outer environment) etc., contribute to reducing the risk of aspergillosis significantly.

Moreover, monitoring and evaluation of fungal conidia counts in healthcare settings is recommended to assess the efficiency of the air filtration process and a consensus on the tolerable breakpoint concentration of airborne fungi is needed [40].

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■ Correspondence: Prof. Maria Luisa Cristina, Department of Health Sciences, University of Genoa, via Pastore 1, 16132 Genoa, Italy - Tel. +39 010 353 8883 - Fax +39 010 353 8216 - E-mail: cristinaml@unige.it