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Clinical implications of environmental sources for *Aspergillus*

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Invasive aspergillosis presents a formidable problem for both diagnosis and therapy. Therefore, prevention is a very important strategy in controlling this disease. Preventing invasive aspergillosis demands a clear understanding of the environmental sources of *Aspergillus* spp. and how this mould is transmitted to patients. Insight into the sources of exposure, mechanisms of transmission, and host susceptibility to infection are vital to appropriately direct preventive strategies to those settings where the risk of infection is the highest and consequently the impact of prevention the greatest.

Keywords aspergillosis, environment, prevention

Introduction

Invasive filamentous fungal infections are a serious threat to immunocompromised patients, causing increased morbidity and mortality [1–3]. *Aspergillus fumigatus* is the most frequent cause of invasive filamentous fungal infection in bone marrow transplant (BMT) patients, followed by *Aspergillus flavus*, *A. terreus* and *A. niger* [4,5]. Incidence rates of invasive aspergillosis depend principally on host factors. In transplant patients the incidence of invasive aspergillosis ranges from 0.7% to 8.4% depending on the organ transplanted. Lung and BMT recipients possess the highest incidence, whereas pancreas and kidney transplant patients have lower risk of developing invasive aspergillosis [6,7]. The incidence of invasive aspergillosis in patients with acute leukemia, chronic granulomatous disease, and AIDS are estimated at 5%–24%, 25%–40% and 0%–12%, respectively [2].

Aspergillus infections in immuncompromised hosts range from primary cutaneous aspergillosis (especially in neonates and children), rhinosinusitis, tracheobronchitis, pulmonary aspergillosis (the most common site of infection), cerebral aspergillosis to disseminated aspergillosis [2]. Clinical symptoms are non-specific and related to the localization of the infection. Furthermore, the clinical presentation varies among the different patient groups. The most immunocompromised patients are those least likely to have overt symptoms, and progression of the disease is usually fast.

Despite the incorporation of new diagnostic and therapeutic measures in management strategies, the number of patients with a favorable response remains low and mortality rates range from 50% to 90% depending mostly on the recovery of the host immune responses [2,8,9]. Therefore, prevention of invasive aspergillosis is of major importance.

To develop preventive control measures, we need to have a clear understanding of how and where most infections are acquired. We have to take into account that the incidence of invasive aspergillosis differs among transplantation centers and may require different preventive measures in different institutions. Furthermore, the most appropriate control measures for the healthcare setting may differ from those advised for patients at home.

Given the ubiquitous presence of *Aspergillus* spp. in our environment, the question arises if it is feasible to protect immunocompromised patients from this opportunistic mould. We may succeed to prevent exposure in the well-controlled hospital environment during admission, but patients are increasingly being treated in the setting of the out-patient clinic, thereby significantly reducing the ability to control exposure to moulds. Preventing disease by antifungal prophylaxis

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or immunomodulating agents seems to be a logical alternative approach in the setting of out-patient management. However, no regimen has been reported to be clearly effective or superior in preventing disease.

Amphotericin B, in various dosages and formulations, has been evaluated for preventing invasive aspergillosis but safety and efficacy data are lacking in high-risk patient groups. Itraconazole prophylaxis is not recommended due to the poor and variable absorption of the capsules and the potential of drug interactions. Although two studies suggested that itraconazole oral suspension can offer protection against deep fungal infections [10,11], two meta-analyses found no beneficial effect on survival with either the use of mould-active azoles or amphotericin B for chemoprophylaxis against invasive aspergillosis in hematology patients with severe neutropenia [12,13]. In some patients on prophylaxis, infection due to primary or secondary resistant moulds was described [14].

Even less data exist on the benefits of the use of immunomodulating agents and we are still far from any recommendations for clinical practice. Therefore, efforts should be made to develop rational control measures to prevent exposure of the immunocompromised host to *Aspergillus* spp. In order to implement effective prevention strategies we need to understand the routes of transmission by identifying environmental sources of *Aspergillus* spp. and activities that lead to high exposure of patients and, furthermore, to gain insight into the pathogenesis of invasive *Aspergillus* infections once patients have been exposed.

Epidemiology

Aspergillus spp. are ubiquitous saprophytic fungi found in every region of the world. *Aspergillus* spp. are commonly found in soil and decaying vegetation. They can be found in household dust, building materials, ornamental plants, flower arrangements, tobacco, food, and water. Conditions enhancing dispersal of moulds are activities like construction, demolition, and excavation; inadequate or defective air handling; disturbance of dust accumulations during routine cleaning; water leaks and moisture accumulation.

Community-acquired or nosocomial

The many nosocomial outbreaks of invasive aspergillosis described have contributed to the idea that the hospital is the place where the patients become infected. A major problem in determining where the patients actually become infected is that the incubation period is unknown. A number of studies in recent years have supported the idea that invasive aspergillosis is mainly community-acquired instead of originating in the hospital [15,16].

The airborne route

Air plays a crucial role in the spread of *Aspergillus* spp. in the environment and in the transmission to patients. *Aspergillus* spp. efficiently release large amounts of conidia in the air from conidiophores that protrude from the mycelial mass. As conidia gradually settle out anything in contact with air will become contaminated with conidia. The process of settling out and becoming airborne again can repeat itself for prolonged periods of time, since *Aspergillus* conidia remain viable for months. The conidia of the *Aspergillus* spp. most commonly involved in human disease (*A. fumigatus*, *A. flavus* and *A. terreus*) are relatively small, with sizes ranging from 2 to 5 microns [17]. Their small size allows them to become deposited deep into the lung after inhalation. In most individuals, inhaled conidia will be cleared by the alveolar macrophages, without affecting the individuals' health. Immunocompromised patients are extremely susceptible to local invasion of respiratory tissue by deposited conidia, resulting in invasive growth of hyphae. Most cases of invasive aspergillosis (80%–90%) present with pneumonia [2]. Therefore, it has been hypothesized that the inhalation of airborne *Aspergillus* conidia, either directly or through intermediate nasopharyngeal colonization [18–20], is a direct cause of pulmonary infection in immunocompromised patients.

Numerous outbreaks of invasive aspergillosis have been described, linking activities like construction and renovation, which increase the conidial air counts, with the reported cases. However, there are several drawbacks preventing us from drawing firm conclusions based on those reports. Most studies are retrospective in nature, creating the possibility of unrecognized environmental risk factors involved in the outbreak. Additionally, before the ability to compare isolates at a molecular level it was not possible to relate environmental strains to clinical isolates due to the ubiquitous presence of *A. fumigatus* in the environment

Recognition of the importance of the airborne route in the transmission of invasive aspergillosis has lead to the recommendation that high-efficiency particulate air (HEPA) filtration (alone or in combination with laminar air flow [LAF] systems) be installed in hematopoietic stem cell transplant (HSCT) and hematology units. These measures have been shown to decrease the number of conidia in the air [21–24].

Despite this, there are conflicting results concerning the relationship between reduction in the concentration of airborne conidia and a decrease in the frequency of invasive fungal infections [24–27]. Molecular tools have been applied to detect genetic relatedness between environmental and clinical isolates and various studies suggest a nosocomial origin of *A. fumigatus* infection [28–30]. However, other researchers have failed to detect genetic relatedness between environmental, especially airborne strains, and clinical isolates [21,31–34]. The use of HEPA-filtration has reduced the incidence of invasive aspergillosis but infections were not eliminated, indicating that patients might be exposed to conidia from alternative sources.

The waterborne route

The presence of filamentous fungi in hospital water and their supply systems suggests that water plays a role in the epidemiology of invasive aspergillosis [35]. Several reports show the presence of *Aspergillus* spp. and other moulds in water used in hospitals, hemodialysis units and in potable water sources [36–44]. Biofilm formation in water distribution systems or in pipe-work supplying automatic cleaners for medical equipment might be responsible for sudden increases in the fungal contamination of hospital water [45,46]. There are remarkable differences in the recovery of the various moulds and the levels of contamination between countries as well within countries.

We previously showed that surface water is heavily contaminated with *Aspergillus* spp., while no moulds could be recovered from ground water [47]. The main difference between ground water and surface water is that the latter has contact with ambient air, leading to fungal contamination from the air into the water. Likewise, the natural reservoir may be responsible for the disparity noted in the recovery of *A. fumigatus* between the various studies reported. Different methods of storage and handling of water may also lead to variations in the observed fungal contamination.

The hypothesis that aerosolization of water contaminated with *Aspergillus* may lead to an increase in the conidial air count was tested by obtaining air samples before and after allowing a hot shower run for about 15 min [39]. Although no differences were found in the frequency and concentration of *Aspergillus* conidia; when all air samples were analyzed, conidial air counts of *Aspergillus* spp. were significantly higher in wet areas (bathrooms) compared to dry areas (rooms, hallways). Furthermore, the results of this study showed strikingly similar rank-order distributions of *Aspergillus* spp. in both water and air. *A. niger* was the most frequently recovered species, followed by *A. fumigatus*, *A. terreus*, and *A. flavus*. These data strongly suggest that in hospitals with adequate air filtration systems, *Aspergillus* can be secondarily airborne.

The increase in data supporting hospital water as a potential source of *A. fumigatus* gives rise to the question if a so-called 'wet route of transmission' exists. This wet route of transmission may be either directly by ingestion, aspiration, inoculation of vulnerable skin and wounds, or indirectly by aerosolization leading to inhalation of secondarily airborne *Aspergillus* conidia. Earlier suggestions for a wet route of transmission can be found in anecdotal reports linking invasive aspergillosis with aspiration of contaminated surface water in near-drowning patients [48,49]. Furthermore, ingestion of contaminated water may lead to the development of gastric or intestinal aspergillosis [50,51]. Cases of primary cutaneous aspergillosis are mainly described in neonates and in trauma patients with burns or penetrating wounds [52].

In a prospective study we compared environmental (air and water) and clinical isolates of *A. fumigatus* collected during an 18-month period by molecular typing studies [53]. Amplified fragment length polymorphism (AFLP) fingerprinting was used to determine the epidemiologic relatedness between the collected isolates. The 'gold standard' for fingerprinting of *A. fumigatus* is restriction fragment length polymorphism (RFLP) analysis [54]. Although yielding high-quality fingerprints with excellent discriminatory power, RFLP requires large amounts of high quality DNA and is very laborious to perform. For obvious practical reasons, PCR-based fingerprinting methods have gained more interest. AFLP fingerprinting has proven to have superior reproducibility and a high degree of discriminatory power compared to other random amplification methods [55,56]. The results showed that *A. fumigatus* isolates recovered from water showed genetic relatedness with isolates recovered from patients. Clinical isolates from two different patients, a patient with leukemia suffering from invasive pulmonary aspergillosis and a patient with chronic granulomatous disease colonized with *A. fumigatus* in his lungs, were genotypically indistinguishable from respectively closely related to strains recovered from water sources. Furthermore, we showed that the intake reservoir was indeed the source of *A. fumigatus* strains found in tap water inside the hospital. A retrospective study performing molecular characterization of *A. fumigatus* strains showed that a clinical *A. fumigatus* isolate was genotypically indistinguishable from an isolate recovered from the shower wall.

However, this retrospective study did not determine if the *A. fumigatus* isolate from the shower wall originated from water or air [39].

By analyzing the environmental *A. fumigatus* isolates we showed that they could be divided in two main clusters. One cluster consisted almost exclusively of *A. fumigatus* isolates recovered from air, while the other cluster consisted of mainly *A. fumigatus* isolates recovered from water [53]. This might imply that some *A. fumigatus* strains are better adapted to growth in a wet environment than a dry environment. It remains to be seen if the differences between the clusters are based on subtle genetic differences. Although highly speculative at this point, if we were able to distinguish the *A. fumigatus* strains originated from water as opposed to the air, then we might have a very useful epidemiologic tool for outbreak management.

It remains unclear how patients become infected with waterborne *A. fumigatus* strains. Aerosolization appears to be the most logical route of infection since all our patients infected with a waterborne strain had a pulmonary infection. Waterborne *Aspergillus* species can aerosolize and their concentration seem to be higher in area's with water activities like bathrooms [39]. This aerosolization can occur directly from running water sources, and/or splashing of contaminated environmental surfaces with water. Anaissie and colleagues showed that cleaning water-related surfaces immediately before showering is associated with a significant reduction in the concentration of airborne filamentous fungi, including *Aspergillus* spp. [57]. These findings support the idea that aerosolization of filamentous fungi results in part from the back splashing of contaminated surfaces with water.

Clinical implications

Within the relatively controlled hospital environment, preventive control measures should be directed against contaminated air, water, objects known for being a habitat for *Aspergillus* spp., and activities increasing conidial air counts.

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 conidial air counts. The HEPA-filtration of incoming air is obvious as air serves as a transporter for the conidia, while the recommended high air changes in the room is thought to be effective in taking away sudden increases in conidial air counts by activities that disturb accumulated dust. Proper dusting methods are designed to prevent the formation of clouds of dust and subsequent increase in conidial air counts. Horizontal surfaces should be cleaned daily with a moistened cloth with either a commercial detergent or/and a hospital disinfectant. Vacuum cleaners need to be kept in good repair and preferably equipped with HEPA filters for use in the areas with high-risk patients. The use of carpeting is not allowed in hospital wards with severely immunocompromised patients. Fresh or dried flowers, potted plants, and fruits should not be allowed in the patient's room because these objects can serve as sources for *Aspergillus* spp.

Although the preventive control measures against contaminated air, surfaces, and objects by *Aspergillus* spp. can be found in the 'Prevention and Control of Health-Care-Associated Pulmonary Aspergillosis' guidelines recommended by the Centers for Disease Control and Prevention, no specific guidelines exist for preventing exposure to *Aspergillus* spp. found in hospital water. General recommendations include minimizing the exposure of high-risk patients to potential sources of *Aspergillus* spp. as well as activities that may aerosolize *Aspergillus* spp. Furthermore, the source of *Aspergillus* spp. should be eliminated [58]. These recommendations imply that water precautions need to be introduced in those hospitals where water is contaminated with *Aspergillus* spp.

Elimination of filamentous fungi either by general water purification or at the point of individual water use appear to be logical strategies designed to reduce exposure of high-risk patients. The efficacy of water disinfection methods against moulds is largely unknown, but water distillation will kill filamentous fungi. Chlorination has no effect, and other disinfection methods have not been evaluated for their effect on water contaminated with *Aspergillus* spp. or with other moulds. Distillation of small quantities of water used for drinking and personal hygiene seems to be a simple measure, although the efforts required to provide sterile water to a large number of hospitalized immunocompromised patients can be enormous. Commercially available bottled water is an appropriate alternative for personal drinking water due to its low cost. Caution should be taken what kind of bottled water is chosen. Bottled water has to meet different quality control standards compared with bottled mineral water and both are not necessarily sterile. Bottled (mineralized) water must meet the same microbiological criteria as natural water, while natural mineral water originates from an underground deposit or spring and is subjected to strict rules and recognition by local authorities, including freedom from parasites and pathogenic

bacteria. Studies examining fungal contamination of bottled water have shown that both bottled water, and to a lesser extent bottled mineral water, can be contaminated by moulds [59–61].

An inexpensive but potentially effective measure might be to minimize patient exposure to hospital water and activities leading to aerosolization of hospital water [62]. Possible recommendations such as avoiding showering and using sponges and bed baths instead might be useful and are easy to implement. In addition, avoiding exposure to faucet water and flushing toilets might be required to prevent transmission of *Aspergillus* spp. If using the bathroom, cleaning of the walls and floors properly results in lower conidial air counts and thereby decreases the exposure of immunocompromised patients to *Aspergillus* spp. [57].

Elimination of moulds through filters for taps and showers might be considered as a control measure to prevent exposure to waterborne *Aspergillus* spp. Point-of-use filters for taps and showers have been reported to effectively manage the potential of exposure to *Pseudomonas aeruginosa* and *Legionella* species in hematology-oncology units, a BMT unit, and a pediatric nephrology unit [63–65]. By using these kind of filters in the bathrooms of hospital wards for immunocompromised patients, it might be possible to prevent exposure to various waterborne micro-organisms and thereby decrease the risk of waterborne nosocomial infections.

Conclusions

A clear understanding of the epidemiology and routes of transmission is required to develop rational and effective control measures to prevent exposure of immunocompromised patients to *Aspergillus* spp. Inhalation of air contaminated with *Aspergillus* spp. seems to be the most important route of transmission irrespective of the environmental source for *Aspergillus* spp. The various environmental sources for *Aspergillus* spp. require specific preventive control measures and might differ between countries and even between hospitals. Furthermore, the risk of developing an infection in relation to the susceptibility of the patient should also play an important role in the implementation of protective measures. It is of utmost importance to characterize the relative importance of each environmental source and route of transmission to allow implementation and evaluation of preventive measures. Infection control measures to prevent nosocomial aspergillosis are not necessarily applicable in the prevention of community acquired aspergillosis. Future studies should have a focus on community-acquired aspergillosis to develop appropriate recommendations for the immunocompromised patients in their home environments. Disinfection methods and control measures to effectively eliminate *Aspergillus* spp. from water sources need to be urgently studied.

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