Original article

# Invasive pulmonary aspergillosis in neutropenic patients and the influence of hospital renovation

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## Summary

To evaluate the effects of airborne *Aspergillus* contamination during and after the renovation work of a Florentine haematology unit, we conducted (November 2003–January 2005) a strict programme of environmental fungal surveillance. Air samples were taken from patients' rooms, along the corridors inside the wards, along the corridor between wards and outside the building. The concentration of *Aspergillus fumigatus* was high along the corridor between the two haematology wards (2.98 CFU $m^{-3}$), lower in the non-neutropenic patients' rooms and outside the hospital building (1.53 and 1.42 CFU $m^{-3}$, respectively), very low in the neutropenic patients' rooms (0.09 CFU $m^{-3}$). During this period, three proven cases (*A. fumigatus*), two probable ones and two possible cases of invasive pulmonary aspergillosis were documented in 97 patients with acute leukaemia (7%). The three cases of proven aspergillosis coincided with the period of renovation work and with the period in which we have found the maximum concentration of *A. fumigatus* along the corridor. These data suggest a possible relationship between environmental fungal contamination and the incidence of invasive aspergillosis, and underline the importance of environmental surveillance.

**Key words:** *Aspergillus fumigatus*, hospital renovation, neutropenic patients, invasive aspergillosis.

## Introduction

Invasive pulmonary aspergillosis (IPA) is a serious infection arising prevalently in immunocompromised patients where there is often a dramatic evolution due to diagnostic and therapeutic difficulties.

The incidence of this disease has been increasing dramatically in the last 30 years due to the increasingly frequent use of immunosuppressive therapy, and is the most common pulmonary mycosis in patients with haematological malignancies.[1–3]

This infection comes about prevalently aerogenically and *Aspergillus fumigatus* is the main aetiological agent;[4] nevertheless the mycosis can also be caused by other species such as *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus* and so on.

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These are environmental fungi isolated mainly in the soil from decomposing vegetation, but also from air and domestic dust. Therefore, it is possible that immunocompromised patients contract the infection in the hospital setting as well.

Fungal contamination inside the hospital is the result of a combination of various factors that are difficult to investigate; however, different authors have established that hospital infections caused by *Aspergillus* spp. occur with greater frequency when construction work in hospital wards is taking place or has just been completed.[5–9] This has been associated with the increase of dust in the air, which facilitates the spread of the fungal particles present.

In the period of September 2003–March 2004 major renovation work was carried out in a Florentine haematology unit, thus increasing the risk of fungal, particularly *Aspergillus*, infections, mainly in patients with acute leukaemia during severe neutropenia related to aggressive chemotherapy.

The aim of this study was to monitor the fungal burden for two purposes: (i) to highlight possible risk



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situations for patients; (ii) to correlate any *Aspergillus* infections with the fungal burden.

## Patients and methods

### Patients

From November 2003 to January 2005, two haematology wards on the first floor of a Florentine hospital were monitored to make both a qualitative and quantitative evaluation of fungal burden in the air. In this period, 115 haematologic patients were admitted to the wards, 97 of whom (84%) were with acute myeloid leukaemia (AML) or acute lymphoblastic leukaemia (ALL).

Patients with acute leukaemia, during the phase of neutropenia, were allocated in one ward where the rooms [multiple-bed rooms without high efficiency particulate air (HEPA) filtration] had restricted access to visitors and other particular behavioural measures: a ban on opening windows and on plants or flowers, measures adopted by the medical and nursing personnel and visitors, such as masks, caps, shoe coverings, sterile gloves and hand-washing.

In the other ward there were non-neutropenic patients with haematological diseases allocated in rooms that had neither restricted access nor particular behavioural measures. All acute leukaemic patients received an aggressive anti-neoplastic regimen for remission–induction, consolidation or relapse/refractory disease and were examined to evaluate the incidence of nosocomial infections. Neutropenia was defined as absolute neutrophil count (ANC) less than 1000 cells $\mu l^{-1}$ >10 days and severe neutropenia as ANC < 100 cells $\mu l^{-1}$. Fever was defined as a single measurement >38.5 °C on one occasion or >38 °C on two or more occasions within 12 h, not related to underlying disease, chemotherapy or blood infusion.

The characteristics of patients with acute leukaemia during the different phases of intensive chemotherapy, and classification of febrile episodes are shown in Table 1.

Standard antifungal prophylaxis was performed with oral azoles (itraconazole 400 mg $day^{-1}$) and stopped when antifungal therapy was started. Febrile episodes were classified according to EORTC criteria. The diagnosis of proven or probable or possible IPA was based on clinical, radiographic and microbiological data according to established criteria[10]: (i) clinical factors included neutropenic patients (<500 neutrophils $\mu l^{-1}$ for >10 days); (ii) persistent fever despite broad-spectrum antibacterial therapy; (iii) radiographic evidence included plain radiography or a computed tomography scan with the presence of a halo or an air-crescent sign; (iv) microbiologic evidence, when available, was reviewed and included culture and/or microscopic evaluation (cytology or histopathology) or antigen, in bronchoalveolar-lavage fluid (BAL), blood samples, induced sputum, transbronchial biopsy, fine-needle pulmonary aspirations and open lung biopsies.

**Table 1** Clinical characteristics of the 97 patients with acute leukaemia

| Characteristics | *n* | Per cent |
|---|---|---|
| Age (years) | | |
| Mean | 49 | |
| Range | 17–77 | |
| Sex | | |
| Male | 57 | 59 |
| Female | 40 | 41 |
| Underlying disease | | |
| AML | 84 | 87 |
| ALL | 13 | 13 |
| CVC | 74 | 76 |
| Initial absolute neutrophil count (ANC) | | |
| <100 cells $\mu l^{-1}$ | 37 | 38 |
| 100–500 cells $\mu l^{-1}$ | 45 | 46 |
| 500–1000 cells $\mu l^{-1}$ | 15 | 16 |
| Days of neutropenia | | |
| Mean duration | 15 | |
| Range | 11–40 | |
| Status of underlying disease | | |
| Remission–induction | 58 | 60 |
| Consolidation | 24 | 25 |
| Relapse/Refractory | 15 | 15 |
| Prophylaxis | | |
| Antibacterial prophylaxis | 89 | 91 |
| Antifungal prophylaxis | 84 | 87 |
| Classification of febrile episodes | | |
| MDI | 45 | 46 |
| CDI | 16 | 18 |
| FUO | 36 | 37 |

AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; MDI, microbiologically documented infections with and without bacteraemia; CDI, clinically documented infections; FUO, fever of undetermined origin; CVC, central venous catheter.

### Methods

Air samples were taken twice a month between 10:00 and 12:00 A.M. in at least one room of each ward, along the corridors of the wards, along the corridor between the wards and outside the building (270 total air samples). To take the samples we used the Surface Air Systems device (SAS; PBI International, Milan, Italy), which allows direct impact of the air aspirated onto the microbiological culture medium. For this study Sabouraud dextrose agar (Difco, Becton-Dickinson, Sparks,

MD, USA) mixed with chloramphenicol 0.05 mg $ml^{-1}$, penicillin 500 U $ml^{-1}$ and streptomycin 0.5 mg $ml^{-1}$ contained in 'contact' type plates was used.

Two air samples were taken at every point examined, one plate was incubated to 22 °C to grow the total mycotic flora and another plate was incubated to 37 °C to grow the thermophilic mycotic flora (especially *A. fumigatus* and other *Aspergillus* species).

After 10 days of incubation, the colonies were counted. Calculating the most probable number of colony forming units (CFU) according to the conversion table provided with the instrument, we reached the concentration for $m^3$ of air ($X$) with the following formula: $X = \text{CFU} \times 1000\ l^{-1}$ of air drawn.

Identification of the filamentous fungi was made directly from the isolation media, on the basis of both the macroscopic (colour, dimension, aspect) and microscopic (fructification) characteristics of the colony. For the yeasts no species identification was made. Comparison of the quantitative data was made through variance analysis.

## Results

From November 2003 to January 2005 the mean total fungal concentration (culture at 22 °C) was significantly higher outside the building (mean 323.55 CFU $m^{-3}$) compared with the restricted access rooms (52.74 CFU $m^{-3}$, $P < 0.001$). Moreover, the fungal burden was higher, although not significantly, outside the building (mean 323.55 CFU $m^{-3}$) in comparison with the corridors (mean 220.79 CFU $m^{-3}$ along the corridors of the wards and 273.58 CFU $m^{-3}$ along the corridor between the wards) and to the non-restricted access rooms (194.31 CFU $m^{-3}$) (Table 2).

*Cladosporium* was the most frequently isolated genus (60.41% of the total isolated colonies), followed by *Penicillium* (19.24%), *Alternaria* and other Dematiaceae (2.8%), *Aspergillus* (2.27%), *Paecilomyces* (1.84%), other hyphomycetes (13.44%), yeasts (1.65%).

**Table 2** Mean fungal concentration (CFU $m^{-3}$) in haematology wards and outside

| | Total fungal concentration (22 °C) | Total fungal concentration (37 °C) |
|---|---|---|
| Restricted access rooms | 52.74 | 2.4 |
| Non-restricted access rooms | 194.31 | 7.9 |
| Corridor inside the wards | 220.79 | 11.2 |
| Corridor between the wards | 273.58 | 16.67 |
| Outside | 323.55 | 17.58 |

In cultures at 37 °C a lower total fungal concentration compared with that found in cultures at 22 °C was observed, but even at this temperature the scalarity of the total fungal burden, which was the highest outside and minimum inside the restricted access rooms, was evident (Table 2). The *Aspergillus* genus was the most isolated (33.8% of the total colonies) while other hyphomycetes and yeasts (Dematiaceae, Mucoraceae, *Chrysonilia, Chrysosporium, Geotrichum, Penicillium, Schizophyllum, Scopulariopsis, Trichoderma* and so on) were isolated occasionally. The *Cladosporium* genus was never found.

The highest *Aspergillus* concentration was found along the corridor between the two haematology wards (10.32 CFU $m^{-3}$), while low concentrations were detected outside the building (3.93 CFU $m^{-3}$), along the corridors of the wards (3.04 CFU $m^{-3}$) and in the non-restricted access rooms (3.09 CFU $m^{-3}$). *Aspergillus* concentration was 0.53 CFU $m^{-3}$ in the restricted access rooms (with a highly significant difference compared with the corridor between the wards, $P < 0.001$) (Table 3).

*Aspergillus niger* was the most frequently isolated species (35.08% of the total *Aspergillus* colonies), followed by *A. fumigatus* (33.18%), *A. flavus* (7.85%), *Aspergillus nidulans* (3.8%), *A. terreus* (2.3%), *Aspergillus clavatus* (0.75%) and other species not identified (17.04%).

*Aspergillus niger* and *A. fumigatus* were isolated especially along the corridor between the wards (3.97 CFU $m^{-3}$ and 2.98 CFU $m^{-3}$, respectively), *A. flavus* along the corridors inside the wards (1 CFU $m^{-3}$). In the restricted access rooms, *A. niger* and *A. fumigatus* (0.31 CFU $m^{-3}$ and 0.09 CFU $m^{-3}$, respectively) were isolated rarely. *Aspergillus flavus* was never found at this site (Table 3).

*A. fumigatus* was isolated in particular between January and July 2004 (Table 4).

**Table 3** Mean *Aspergillus* concentration (CFU $m^{-3}$) in haematology wards and outside (culture at 37 °C)

| | *Aspergillus* genus | *Aspergillus niger* | *Aspergillus fumigatus* | *Aspergillus flavus* |
|---|---|---|---|---|
| Restricted access rooms | 0.53 | 0.31 | 0.09 | 0 |
| Non-restricted access rooms | 3.09 | 1.09 | 1.53 | 0.11 |
| Corridor inside the wards | 3.04 | 0.54 | 0.98 | 1.00 |
| Corridor between the wards | 10.32 | 3.97 | 2.98 | 0.60 |
| Outside | 3.93 | 1.32 | 1.42 | 0.10 |

 

**Table 4** *Aspergillus fumigatus* concentration (CFU $m^{-3}$) in the haematology wards and outside the hospital during the monitored period

| Monitoring date | Season | Restricted access rooms | Non-restricted access rooms | Corridor inside the wards | Corridor between the wards | Outside |
|---|---|---|---|---|---|---|
| 12/11/03* | Autumn 03 | 0 | 0 | 0 | 0 | 0 |
| 27/11/03* | | 0 | 0 | 0 | 4.17 | 0 |
| 11/12/03* | | 0 | 0 | 0 | 0 | 0 |
| 14/01/04* | Winter 03–04 | 0 | 0 | 0 | 8.33 | 0 |
| 28/01/04* | | 0 | 4.88 | 0 | 16.67 | 0 |
| 11/02/04* | | 0 | 0 | 0 | 0 | 3.38 |
| 25/02/04* | | 0 | 0 | 0 | 0 | 16.18 |
| 09/03/04* | | 0 | 0 | 0 | 0 | 0 |
| 23/03/04* | Spring 04 | 0 | 0 | 0 | 8.33 | 0 |
| 06/04/04 | | 0 | 0 | 4.00 | 0 | 0 |
| 05/05/04 | | 0 | 12.58 | 0 | 4.17 | 0 |
| 19/05/04 | | 1.99 | 0 | 4.17 | 8.33 | 0 |
| 09/06/04 | | 0 | 0 | 4.17 | 0 | 0 |
| 30/06/04 | Summer 04 | 0 | 3.57 | 0 | 0 | 6.65 |
| 07/07/04 | | 0 | 0 | 8.33 | 0 | 3.58 |
| 10/09/04 | | 0 | 0 | 0 | 0 | 0 |
| 22/09/04 | Autumn 04 | 0 | 3.57 | 0 | 0 | 0 |
| 05/10/04 | | 0 | 0 | 0 | 0 | 0 |
| 04/11/04 | | 0 | 0 | 0 | 0 | 0 |
| 01/12/04 | | 0 | 7.53 | 0 | 12.5 | 0 |
| 18/01/05 | Winter 04–05 | 0 | 0 | 0 | 0 | 0 |

*In this period renovation work was in progress.

This species was found mainly along the corridor between the wards where it was isolated almost exclusively in the period coinciding with or immediately after the renovation work taking place between September 2003 and March 2004. There were positive samples along the corridor inside the wards only in spring and summer 2004. In the restricted access rooms, *A. fumigatus* was isolated only one time (spring 2004). In the non-restricted access rooms it was found occasionally in all seasons. Outside this species was isolated only four times (in winter and summer 2004) (Table 4).

From November 2003 to January 2005, 15 atypical lung infiltrates were observed in chest tomography in patients with acute leukaemia. They were classified as proven/suspected invasive fungal infections in seven cases (7%), as shown in Table 5. All patients were febrile and with severe neutropenia.

The three cases of proven aspergillosis took place in the period of January–February 2004; the other cases between March and December 2004 (Table 5).

**Table 5** Invasive pulmonary aspergillosis (IPA) cases during the monitored period

| Patient | Leukaemia | Diagnosis | Date diagnosis | Aetiology |
|---|---|---|---|---|
| 1 | ALL | Proven IPA | January 2004 | *Aspergillus fumigatus* |
| 2 | AML | Proven IPA | January 2004 | *A. fumigatus* |
| 3 | AML | Proven IPA | February 2004 | *A. fumigatus* |
| 4 | AML | Possible IPA | March 2004 | |
| 5 | AML | Possible IPA | April 2004 | |
| 6 | AML | Probable IPA | September 2004 | *Aspergillus* spp. |
| 7 | AML | Probable IPA | December 2004 | *Aspergillus* spp. |

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia.

## Discussion

The data obtained during this study show that the total fungal concentration (cultures at 22° and 37 °C) tends to increase from closed to open areas in the haematology wards, reaching a maximum outside the building. The low number of propagules found in the restricted access rooms of the ward may be the result of the level of environmental isolation (entrance limited to a minimum number of people, a ban on opening windows and so on).

The use of a double incubation temperature (22 ° and 37 °C) of the specimens allowed us to evaluate both the total fungal concentration (to monitor the degree of total fungal contamination) and the thermophilic

mycotic flora concentration (to better highlight thermophile species such as *A. fumigatus* and other *Aspergillus* species, which in a 22 °C culture could be inhibited by non-thermophile species, as *Cladosporium* and *Penicillium*, that are extremely abundant in the air or have a quicker growth rate).

The *Aspergillus* distribution in the different environments monitored does not seem to reflect what has been observed for the total fungal burden. In fact, the *Aspergillus* concentration within the building is, excluding the restricted access rooms, similar or greater than that observed outside.

The greatest *Aspergillus* concentration was found along the corridor between wards which was directly connected to the construction zone. So the dust that raised during the renovation, the humidity and temperature of a heated environment (the work took place in autumn–winter) may have contributed to the proliferation of these thermophile species.

In the restricted access rooms the total mycotic burden was always low and the *Aspergillus* burden was even lower. Thus we might think that the level of environmental isolation (entrance limited to a minimum number of people, a ban on opening windows and so on) was valid.

*Aspergillus niger* was the most frequently isolated species, but there were no cases directly ascribable to this species; the three case of certain aspergillosis were all attributed to *A. fumigatus.*

The correlation between the concentration of *A. fumigatus* in the air and cases of invasive aspergillosis has been recognised by various authors.[8,11,12] In particular, Arnow *et al.* [11] observed that the increase in the mean concentration of *A. fumigatus* and *A. flavus* was accompanied by a progressive increase in the incidence of aspergillosis. In fact, they noticed that during a pre-epidemic period the concentration of *A. fumigatus* and *A. flavus* had been less than 0.01 and 0.2 CFU $m^{-3}$, respectively while in an epidemic period the concentration rose to 1.1 and 2.2 CFU $m^{-3}$, respectively.

In our research, we found a high concentration of *A. fumigatus* along the corridor between wards, especially in the period coinciding with or immediately after the renovation work. On this site the mean concentration of *A. fumigatus* in the entire period observed (November 2003–January 2005) was 2.98 CFU $m^{-3}$ but, if we consider only the period November 2003–March 2004 (coinciding with the renovation work) and the two following months (April–May 2004), this reaches 4.17 CFU $m^{-3}$. *Aspergillus fumigatus* may have passed from the corridor between wards to places strictly contiguous such as the corridor inside the wards (mean concentration 0.98 CFU $m^{-3}$) and the rooms of non-neutropenic patients without restricted access (mean concentration of 1.53 CFU $m^{-3}$). Before the neutropenia phase related to chemotherapy, patients stay in rooms without restricted access and use the corridors inside and between the wards and thus may inhale these micro-organisms in these locations. Indeed, in our experience, the cases of proven aspergillosis coincide with the period of renovation work and with the period in which we have found the maximum concentration of *A. fumigatus* in the corridor.

The possibility of a correlation between *Aspergillus* concentration and cases of aspergillosis also seems to be confirmed by our earlier published study.[13] Indeed, in the years 1999–2000, during research conducted in the same haematology wards, we found extremely low concentrations of *A. fumigatus* (0.09 CFU $m^{-3}$ in the rooms, 0.21 CFU $m^{-3}$ along the corridors) and no case of invasive aspergillosis was documented. Moreover, in a previous clinical surveillance (unpublished data), performed in the period 2001–2002 on the same patient population (100 acute leukaemic patients) and in the absence of building construction work, we documented only two possible fungal pneumonias (2%).

In conclusion, this study suggests a possible relationship between environmental fungal contamination in haematologic patients and the incidence of invasive aspergillosis, and also underlines the importance of environmental surveillance.

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