Prophylaxis of invasive aspergillosis with voriconazole or caspofungin during building work in patients with acute leukemia

Amélie Chabrol,[1] Lise Cuzin,[1] Françoise Huguet,[2] Muriel Alvarez,[1] Xavier Verdeil,[3] Marie Denise Linas,[4] Sophie Cassaing,[4] Jacques Giron,[5] Laurent Tetu[6] Michel Attal,[2] and Christian Récher[2]

[1]Department of Infectious Diseases, [2]Department of Clinical Hematology, [3]Department of Epidemiology, [4]Department of Parasitology and Mycology, [5]Department of Medical Imaging, and [6]Department of Pulmonary Diseases, Toulouse University Hospital, Toulouse, France

ABSTRACT

Background
Invasive aspergillosis is a common life-threatening infection in patients with acute leukemia. The presence of building work near to hospital wards in which these patients are cared for is an important risk factor for the development of invasive aspergillosis. This study assessed the impact of voriconazole or caspofungin prophylaxis in patients undergoing induction chemotherapy for acute leukemia in a hematology unit exposed to building work.

Design and Methods
This retrospective cohort study was carried out between June 2003 and January 2006 during which building work exposed patients to a persistently increased risk of invasive aspergillosis. This study compared the cumulative incidence of invasive aspergillosis in patients who did or did not receive primary antifungal prophylaxis. The diagnosis of invasive aspergillosis was based on the European Organization for Research and Treatment of Cancer/Mycosis Study Group criteria.

Results
Two-hundred and fifty-seven patients (213 with acute myeloid leukemia, 44 with acute lymphocytic leukemia) were included. The mean age of the patients was 54 years and the mean duration of their neutropenia was 21 days. Eighty-eight received antifungal prophylaxis, most with voriconazole (n=74). The characteristics of the patients who did or did not receive prophylaxis were similar except that pulmonary antecedents (chronic broncho-pulmonary disorders or active tobacco use) were more frequent in the prophylaxis group. Invasive aspergillosis was diagnosed in 21 patients (12%) in the non-prophylaxis group and four (4.5%) in the prophylaxis group (P=0.04). Pulmonary antecedents, neutropenia at diagnosis and acute myeloid leukemia with high-risk cytogenetics were positively correlated with invasive aspergillosis, whereas primary prophylaxis was negatively correlated. Survival was similar in both groups. No case of zygomycosis was observed. The 3-month mortality rate was 28% in patients with invasive aspergillosis.

Conclusions
This study suggests that antifungal prophylaxis with voriconazole could be useful in acute leukemia patients undergoing first remission-induction chemotherapy in settings in which there is a high-risk of invasive aspergillosis.

Key words: invasive aspergillosis, antifungal prophylaxis, building work, voriconazole, acute leukemia, caspofungin.

Citation: Chabrol A, Cuzin L, Huguet F, Alvarez M, Verdeil X, Linas MD, Cassaing S, Giron J, Tetu L, Attal M, and Récher C. Prophylaxis of invasive aspergillosis with voriconazole or caspofungin during building work in patients with acute leukemia. Haematologica 2010;95:996-1003. doi:10.3324/haematol.2009.012633

Manuscript received on June 16, 2009. Revised version arrived on November 3, 2009. Manuscript accepted on November 19, 2009.

Correspondence:
Christian Récher,
Service d'Hématologie,
CHU de Toulouse, Hôpital Purpan, place du Dr Baylac,
31059 Toulouse cedex, France.
E-mail: recher.c@chu-toulouse.fr

Introduction

Invasive fungal infections due to *Aspergillus* species are associated with a high rate of morbidity and mortality in immunocompromised patients. Prolonged neutropenia, use of steroids or immunosuppressive agents, and hematopoietic stem-cell or solid-organ transplantation are classically associated with a high-risk of developing invasive aspergillosis (IA).[1] Until recently, treatment of IA with available antifungal agents (mainly amphotericin B) resulted in an unacceptably high rate of mortality. Amphotericin B was also associated with general and renal toxicity precluding prolonged administration. Several new antifungal agents including voriconazole, liposomal amphotericin B and caspofungin have recently been developed for the treatment of IA. These drugs have an acceptable toxicity profile and are much better tolerated than amphotericin B. Moreover, these new compounds induce a higher response rate compared to amphotericin B and improve the outcome of patients with IA.[2,3]

Acute leukemias are a heterogeneous group of diseases characterized by an accumulation of transformed hematopoietic progenitor cells from both myeloid and lymphoid lineages, leading to bone marrow failure and profound neutropenia, which is further worsened by the intensive chemotherapy currently used for induction of complete remission. The duration of neutropenia in leukemic patients is frequently more than 21 days; this is now longer than in patients undergoing hematopoietic stem-cell transplantation, in whom the use of peripheral stem cells has dramatically improved recovery from neutropenia. Moreover, in contrast with hematopoietic stem cell transplantation, induction chemotherapy for acute leukemia is not always performed in laminar airflow units. Thus, patients with acute myeloid leukemia (AML) or acute lymphocytic leukemia (ALL) remain at high risk of developing IA. The incidence of proven or probable IA in acute leukemia is around 6% but can reach 50% in situations in which there is a risk of *Aspergillus* propagation and dissemination, such as during building/construction work.[4-7] Mortality from IA ranges from 40-60% in such patients.[8] Early recognition of IA is an important clinical challenge. New diagnostic tools such as fungal antigen detection or early computed tomography scans offer the possibility of initiating antifungal therapy earlier in the course of the disease. However, despite these new diagnostic methods, and because 40-50% of patients do not respond to antifungal therapy,[2,3] IA remains an important cause of morbidity and mortality in leukemic patients, leaving open the issue of primary prophylaxis. A recent study assessing posaconazole in primary prophylaxis in patients with myelodysplastic syndrome or acute myeloid leukemia treated by intensive chemotherapy showed promising results, with the prophylaxis reducing the incidence of invasive fungal infections and improving overall survival.[9]

Construction work during hospital renovation close to hematology units has been identified as an important cause of environmental *Aspergillus* contamination and is associated with an increased incidence of nosocomial IA.[6,9-11] Environmental control measures, including high-efficiency particulate air (HEPA) filtration, are not always sufficient.[12] Since 2003, there have been recurrent periods of construction work in the area around our hematology department, leading to an aspergillosis outbreak. Indeed, while there were fewer than six cases of IA per year in our hematology unit during the early 2000s, we identified eight cases between July and October 2003. These cases of proven or probable IA were contemporary with construction work in the building. The responsibility of the construction work in this outbreak was indicated by the chronology and microbiological studies of air and contact samples showing the presence of *Aspergillus fumigatus*, *flavus* and *niger* during this period. After this first outbreak, more building work took place inside or near our building and is expected to continue for at least 5 years.

The aim of this study was to assess the impact of primary prophylaxis with voriconazole or caspofungin in acute leukemia patients undergoing intensive chemotherapy for remission-induction in a conventional unit without laminar air flow during a period of construction work.

Design and Methods

Patients

All consecutive patients undergoing first intensive induction chemotherapy for AML or ALL in the Department of Hematology, Toulouse University Medical Center, between May 2003 and January 2006 were included. Patients with relapsing disease, consolidation treatment for complete remission, palliative treatment, or prior IA were excluded. AML patients who were 60 years old or younger were treated according to the AML-2001 trial from the GOELAMS,[13] whereas those over 60 years old were treated according to the BGMT 95 protocol.[14] Patients with Philadelphia-negative ALL were treated according to the GRAALL-2003 protocol,[15] those with ALL-L3 (Burkitt type) were treated according to the adapted LMB protocol,[16] and Philadelphia-positive ALL patients were treated according to the DIV protocol.[17] Systematic use of granulocyte colony-stimulating factor (G-CSF) was restricted to AML patients over 60 years old and ALL patients. Karyotype risk groups were defined according to the US Southwest Oncology Group criteria.[18]

Primary prophylaxis of invasive aspergillosis

All rooms in our hematology unit are protected by HEPA filters without any laminar air flow room. In general, two physicians were in charge of the patients with acute leukemia and the decision to initiate prophylaxis was first taken according to the knowledge of planned building work or environmental *Aspergillus* contamination, respiratory disease history or tobacco use, and neutropenia at diagnosis. IA prophylaxis was started on the first day of chemotherapy and was discontinued in the following situations: neutrophil recovery (neutrophil count greater than 0.5×10^9/L), introduction of empirical antifungal therapy, proven or probable invasive fungal infection, drug-related adverse event, or death. The main agent used for IA prophylaxis was oral voriconazole (400 mg *bid* on day 1 and then 200 mg *bid*). Intravenous caspofungin (70 mg on day 1 and then 50 mg/day) was administered to patients with liver abnormalities at diagnosis (aspartate aminotransferase and alanine aminotransferase levels more than 2.5 times the upper limit of normal, or bilirubin level more than 1.5 times the upper limit of normal) or in patients with contraindications to azoles, such as possible drug-drug interactions. Most patients who did not receive IA prophylaxis were given fluconazole (200 mg/day). No other anti-microbial prophylaxis was used.

Data collection, definitions and imaging review

Clinical, biological, microbiological and radiological parameters were recorded retrospectively for 258 consecutive acute leukemia patients by the same clinician (AC) on a standardized data collection form. Specific data known to influence IA outcome such as pulmonary antecedents (defined as chronic broncho-pulmonary disor-

ders or active tobacco use), duration of neutropenia, use of steroids (more than 1 mg/kg for at least 1 week), G-CSF use, and complete remission of underlying leukemia, were carefully recorded. Neutropenia was defined as a neutrophil count less than 0.5×10^9/L. All diagnoses of proven, probable or possible IA were validated by an expert group including two infectious disease clinicians, two mycologists, one pneumologist, one radiologist experienced in the diagnosis of IA, and one hematologist. The revised criteria of the European Organization for Research and Treatment of Cancer/Mycosis Study Group classification were used to define IA.[19] The criteria were divided into those related to clinical, mycological, host factor and histopathological findings. The *clinical criteria* for lower respiratory tract fungal disease were the presence of one of the following three signs on computed tomography: dense, well-circumscribed lesion(s) with or without a halo sign, air-crescent sign, and cavity; the clinical criteria for fungal tracheobronchitis were tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis; those for fungal sinonasal infection were imaging showing sinusitis plus at least one of the following three signs: acute localized pain (including pain radiating to the eye), nasal ulcer with black eschar, extension from the paranasal sinus across bony barriers, including into the orbit; while those for central nervous system infection were the presence of one of the following two signs: focal lesions on imaging, meningeal enhancement on magnetic resonance imaging or computed tomography. The *mycological criteria* were: (i) the presence of fungal elements indicating *Aspergillus* in direct microscopy or the presence of *Aspergillus* in culture, from sputum, bronchoalveolar lavage fluid, bronchial brush, or sinus aspirate samples; (ii) galactomannan antigen detected in plasma, serum, bronchoalveolar lavage fluid, or cerebrospinal fluid; (iii) β-d-glucan detected in serum. The *host factor criteria* were: (i) a recent history of neutropenia (neutrophil count less than $<0.5\times10^9$/L for more than 10 days) temporally related to the onset of the fungal disease; (ii) prolonged use of corticosteroids at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for more than 3 weeks. The *histopathological criteria* were: (i) identification of *Aspergillus* hyphae from a needle aspiration or biopsy specimen with evidence of associated tissue damage; (ii) a positive culture result for a sample obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with infection, excluding bronchoalveolar lavage fluid, cranial sinus cavity specimen, and urine. According to these definitions, three levels of diagnostic certainty were defined for IA: (i) proven, a case with at least one histopathological criterion; (ii) probable, a case with at least one clinical criterion, one mycological criterion and one host factor criterion; and (iii) possible, a case with at least one clinical criterion and one host factor criterion. The expert group was unaware of the use of IA prophylaxis at the time of data analysis.

Building construction or renovation work and environmental monitoring of Aspergillus contamination during the study period

Data on building work (type, period and location with regards to the position of the hematology ward) were provided by the administrative section of the hospital. Only building work considered to increase the risk of *Aspergillus* dissemination, with regards to the type and location, were taken into account according to the Assistance Publique-Hôpitaux de Paris scale.[20,21] The risk levels of hospital building work were expressed as a score taking into account the type and location of the work: score 1 for work taking place outdoors and at a distance; score 2 for minor renovation work taking place indoors on another floor; score 3 for work taking place outdoors in the proximity or with a prevailing wind; score 4 for minor renovation work indoors on the same floor or major renovation work on another floor; and score 5 for major renovation work on the same floor. The building work considered in this study had a score of 3 or more. Air samples were collected using a Bio-Impactor 100-8 (AES, Bruz, France) and cultured in a maltose agar medium [2% malt extract (Merck) with agar (Biorad)]. The plates were impacted with a 0.5 m^3 sample of air. Surfaces were sampled by contact methods with 25 cm^2 Petri dishes (Rodac) and cultured in Sabouraud dextrose agar with lecithin and polysorbate 80 sterile pack plates (Becton Dickinson). All plates were incubated at 25°C for 5 days and examined daily. Moulds were identified by their macroscopic and microscopic appearance after lactophenol cotton blue staining. Samples were considered positive for *Aspergillus* species if there was one or more colony-forming unit (CFU)/m^3 for air samples and one or more CFU/100 cm^2 for surface samples. Sampling was conducted every 3 months, although additional samples could be taken after Nosocomial Infection Committee notification.

Statistical analysis

The primary end-point was the cumulative incidence of proven, probable or possible IA. The secondary end-points included the cumulative incidence of other invasive fungal infections, use of empirical antifungal treatment, overall survival, survival at 100 days after chemotherapy, IA-specific survival, mean duration of hospitalization, cumulative incidence of adverse events, and costs of antifungal therapy. Overall survival was measured from the time of diagnosis to death (failure) or time of the last follow-up (censored). Comparability between the two groups (patients receiving or not receiving primary prophylaxis) was assessed at baseline. Continuous numerical data were compared using ANOVA, and categorical data using the χ^2 test. Cumulative incidence rates were compared using log-rank analysis. Relations between baseline characteristics, including the use of primary prophylaxis, and IA were also tested in univariate analyses by ANOVA and the χ^2 test. All variables significantly related to IA by univariate analysis were included in a multiple logistic regression model to assess their independent relation with IA. Median survival times were compared using the Kaplan-Meier method. All statistical analyses were performed using SAS V9 (SAS Institute Inc., Cary, NC, USA).

Results

Characteristics of the patients with acute leukemia at baseline

Of the 258 patients who underwent first intensive induction chemotherapy during the study period, one already had IA at diagnosis and was, therefore, excluded. Among the 257 patients included in the final analysis, 213 (83%) had AML and 44 (17%) had ALL. At diagnosis of leukemia, 55 (21%) patients were neutropenic, 146 (57%) received G-CSF as part of their induction chemotherapy regimen, and the median duration of neutropenia was 21 days. Prednisone (1 mg/kg for 7 or more days) was used in 59 (23%) patients. Eighty-eight patients received aspergillosis primary prophylaxis (APP+) and 169 did not (APP-). The baseline characteristics of the patients, divided into two groups according to whether or not they received prophylaxis, are shown in Table 1. The two groups had similar characteristics except for pulmonary antecedents which were significantly more frequent in the APP+ group (32% *versus* 20%, P=0.03). For the 88 patients in the group that received prophylaxis, the median duration of prophylaxis was 23 days (range, 5-47 days). IA prophylaxis consisted of voriconazole in 74/88 patients (84%) or caspofungin in 14/88 (16%). Among the 169 patients in

the APP- group, 75% were given fluconazole and 25% received no antifungal prophylaxis.

Building construction/renovation work during the study period and environmental monitoring

Building work that increases the risk of IA took place regularly throughout the study period. Indeed, the total duration of building work was 473 days, representing 48% of the study period, with a clear increase in 2005 compared to in 2003 and 2004 (Figure 1). Assessment of both air and surfaces in the unit showed that several samples were positive for *Aspergillus* species (defined as more one or more CFU per m^3 and per 100 cm^2 for air and surface samples, respectively) (Table 2). In contrast, *Aspergillus* contamination had been detected only twice in the year preceding the study (from May 2002 to April 2003), with samples positive for *Aspergillus ochraceus* and *Aspergillus ornatus* (Table 2).

Tolerance of invasive aspergillosis prophylaxis

Among the 74 patients who received voriconazole, ten patients (14%) had drug-related adverse events (six had increases in transaminase levels more than five times the upper limit of normal, three had confusion/hallucinations, and one developed sinus bradycardia) leading to treatment discontinuation. A comparison of the two groups of patients (APP+ *versus* APP-) showed no significant difference in severe hepatic or renal grade 3/4 events, or occurrence of bacteremia (Table 3). No severe toxicity was observed with caspofungin.

Table 1. Baseline characteristics of the 257 patients with acute leukemia.

	APP+ (n=88)	APP- (n=169)	P
Age [years, mean (range)]	56 (17-79)	53 (15-79)	ns
Sex [men:women]	51:37	96:73	ns
Zubrod performance status [mean]	1.17	1.13	ns
Pulmonary antecedent [n (%)]	28 (32)	34 (20)	0.03
Underlying disease = AML [n (%)]	71 (81)	142 (84)	ns
Favorable risk* [n (%)]	5 (6)	28 (17)	ns
Intermediate risk* [n (%)]	39 (44)	64 (38)	
Unfavorable risk* [n (%)]	22 (25)	39 (23)	
Failure* [n (%)]	5 (6)	11 (6)	
Underlying disease = ALL [n (%)]	17 (19)	27 (16)	
B [n (%)]	9 (10)	10 (6)	ns
T [n (%)]	4 (4.5)	5 (3)	ns
Philadelphia [n (%)]	4 (4.5)	7 (4)	
Burkitt [n (%)]	0	5 (3)	
Bone marrow blasts (%) [mean (range)]	63 (0-100)	59 (0-98)	
Hemoglobin level (g/L) [mean (range)]	99 (57-160)	94 (19-172)	ns
Platelets ($\times 10^9$/L) [mean (range)]	103 (5-965)	86 (6-619)	ns
WBC count ($\times 10^9$/L) [mean (range)]	43 (0.3-357)	37 (0.7-343)	ns
Initial neutropenia [n (%)]	14 (16)	41 (24)	ns
Use of growth factors [n (%)]	54 (61)	92 (54)	ns
Use of corticosteroids >7 days** [n (%)]	22 (25)	37 (22)	ns
Duration of neutropenia [mean (range)]	22 (0-77)	21 (0_52)	ns

*APP+: aspergillosis primary prophylaxis; APP-: no aspergillosis primary prophylaxis; AML: acute myeloid leukemia; ALL: acute lymphocytic leukemia; WBC: white blood cell; *AML cases were classified according to cytogenetics; **corticosteroids ≥1 mg/kg/day for ≥7 days.*

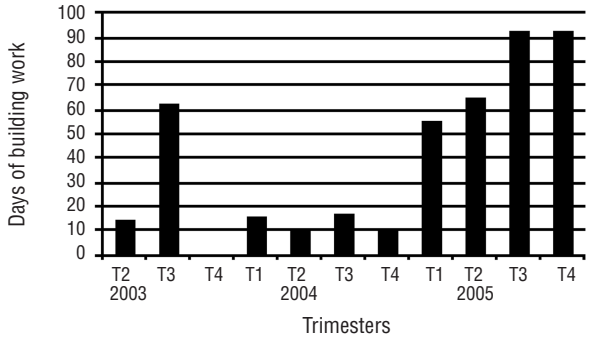

Figure 1. Timetable of building work during the study period.

Table 2. Environmental contamination.

Date	Type	Location	Results*
25/08/02	Air	Hallway	2 CFU *A. ochraceus*
16/01/03	Surface	Patient's room	4 CFU *A. ornatus*
28/05/03	Air	Nursery	2 CFU *A. fumigatus*
28/05/03	Air	Patient's room	2 CFU *A. flavus*
28/05/03	Air	Patient's room	4 CFU *A. fumigatus*
28/05/03	Air	Hallway	6 CFU *A.niger* 10 CFU *A. fumigatus*
28/05/03	Air	Patient's room	6 CFU *A. fumigatus*
28/05/03	Air	Nursery	4 CFU *A. niger* 4 CFU *A. fumigatus*
20/06/03	Air	Hallway	2 CFU *A. niger*
31/07/03	Air	Nursery	2 CFU *A. flavus*
25/09/03	Air	Nursery	6 CFU *A. niger*
26/09/03	Surface	Patient's room	2 CFU *A. niger*
26/09/03	Surface	Patient's room	6 CFU *A. niger*
19/09/03	Air	Hallway	2 CFU *A. niger*
19/09/03	Air	Nursery	2 CFU *A. niger*
24/02/04	Surface	Patient's room	4 CFU *A. niger*
23/03/04	Air	Patient's room	2 CFU *A. fumigatus*
28/07/04	Surface	Patient's room	4 CFU *A. ustus*
12/08/04	Surface	Hallway	2 CFU *A.flavus*
09/09/04	Air	Nursery	2 CFU *A. niger*
14/09/04	Air	Nursery	6 CFU *A. niger* 2 CFU *A. fumigatus*
14/09/04	Air	Nursery	8 CFU *A. niger* 2 CFU *A. fumigatus*
06/10/04	Air	Hallway	2 CFU *A. niger*
06/10/04	Surface	Patient's room	4 CFU *A. niger*
06/10/04	Surface	Patient's room	4 CFU *A. niger*
06/10/04	Air	Hallway	2 CFU *A. niger*
06/10/04	Air	Nursery	2 CFU *A. glaucus*
05/10/04	Air	Hallway	2 CFU *A. niger*
06/10/04	Air	Patient's room	4 CFU *A. niger*
10/08/05	Air	Nursery	1 CFU *A. fumigatus*

**Results are expressed as CFU/m^3 for air samples and CFU/100 cm^2 for surface samples.*

Incidence of invasive aspergillosis

IA was diagnosed in 25 patients (9.7%); the diagnosis was considered proven in 3, probable in 17 and possible in 5. The characteristics of these patients are shown in Table 3. The cumulative incidence of IA in AML and ALL patients was 10% (22/213) and 6.8% (3/44), respectively. Of note, 11/22 (50%) AML patients with IA had high-risk disease according to cytogenetic features. In contrast, no IA was diagnosed in AML patients with low-risk cytogenetics. Patients with IA had a median age of 59 years (range, 17–75), were neutropenic at the diagnosis of acute leukemia in 44% of cases, and had a 31-day mean duration of neutropenia after chemotherapy. The delay between the first day of chemotherapy and the diagnosis of IA was 17 days. IA involved the lungs (n=22), sinuses (n=1), brain (n=1), or was disseminated (n=1). Bronchoalveolar lavage was performed in all patients with suspected pulmonary IA and was positive for *Aspergillus* in 38% of cases. Chest computed tomography scans showed halo or crescent signs in 70% of cases of pulmonary IA. The unique cases of sinus and brain IA were diagnosed after surgical biopsy and at autopsy, respectively. Galactomannan antigen (Platelia® *Aspergillus*; Biorad, France) tests were positive (detection limit of 0.5 ng/mL) in 68% of cases. The cumulative incidence of IA was significantly lower in the APP+ group (n=4) than in the APP- group (n=21) (4.5% and 12.4%, respectively; P=0.04) (Table 3 and Figure 2). Empirical antifungal treatment was given to 15 patients (17%) in the APP+ group and 71 patients (42%) in the APP- group (P<0.0001). The mean duration of empirical treatment did not differ significantly between the two groups; 15 days (APP+ group) *versus* 13 days (APP- group). Three other invasive fungal infections occurred in the APP- group (1.8%): two cases of *Geotrichum capitatum* fungemia and one of *Candida krusei*. One of these three patients had received fluconazole. No other invasive fungal infections occurred in the APP+ group; in particular, no cases of zygomycosis were diagnosed during the study.

Outcome of patients with invasive aspergillosis

Of the 25 patients who developed IA, six died early (a median of 16 days after the diagnosis of IA). All deaths were related to IA although disease status was unknown in four patients. Two patients were refractory to induction chemotherapy and four died from IA before evaluation of the leukemic disease. Of the other 19 patients who developed IA, 13 achieved first complete remission and followed specific anti-leukemic treatment with secondary IA prophylaxis. The 3-month mortality rate was 28% and the median overall survival of IA patients was significantly shorter than that of patients without IA (215 *versus* 782 days; P=0.0008) (Figure 3).

Survival

There was no significant difference in 100-day survival between the two groups (83% in the APP+ group and 82% in the APP- group). The 1-year survival rate was 53% in the APP+ group and 65% in the APP- group (P=ns) (Figure 3). There was also no significant difference when the 213 AML patients were analyzed separately (*data not shown*).

Table 3. Occurence of invasive aspergillosis, survival and adverse events in acute leukemia patients receiving or not receiving aspergillosis primary prophylaxis.

	APP+ (n=88)	APP- (n=169)	*P*
Proven, probable or possible IA, N (%)	4 (4.5)	21 (12.4)	0.04
Proven or probable IA, N (%)	3 (3.4)	17 (10)	0.059
100-day survival	83%	82%	ns
Other invasive fungal infection, N	0	3	
Empirical antifungal treatment, N (%)	15 (17)	71 (42)	< 0.001
ALT/AST elevation (grade 3 or 4) (%)	11	9	ns
Alkaline phosphatase elevation (grade 3 or 4) (%)	0	3	ns
Serum creatinine elevation (grade 3 or 4) (%)	1	0.5	ns
Bacteremia (%)	18	22	ns

APP+: aspergillosis primary prophylaxis; APP-: no aspergillosis primary prophylaxis; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

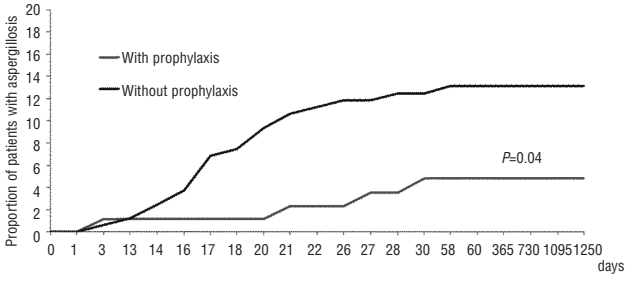

Figure 2. Cumulative incidence of invasive aspergillosis according to treatment arms.

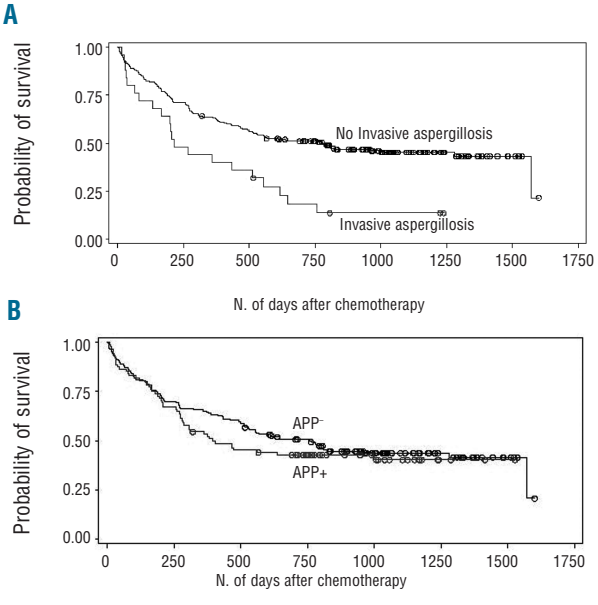

Figure 3. (A) Kaplan-Meier probability of overall survival after remission-induction chemotherapy, according to the diagnosis of invasive aspergillosis. (B) Kaplan-Meier probability of overall survival after remission-induction chemotherapy, according to whether patients received primary prophylaxis against aspergillosis or not.

Risk factors for invasive aspergillosis

Age, performance status, type of leukemia (high-risk cytogenetics AML *versus* others), neutropenia at the time of diagnosis of acute leukemia, neutropenia duration, use of corticosteroids (for 7 days or more), pulmonary antecedents, response to chemotherapy, and IA prophylaxis, were assessed for their potential correlation with IA. AML with high-risk cytogenetics, neutropenia at diagnosis, neutropenia duration, pulmonary antecedents and IA prophylaxis were significantly correlated with IA in univariate analysis and were included in the Cox model for multivariate analysis. Neutropenia at diagnosis, pulmonary antecedents and high-risk cytogenetics AML were positively correlated with IA occurrence whereas IA prophylaxis was an independent protective factor (Table 5).

Discussion

This retrospective, single-center study shows that primary prophylaxis for IA using highly active antifungal agents is a valuable approach in acute leukemia patients undergoing first intensive chemotherapy in the presence of building work near hematology wards. The use of voriconazole (or caspofungin) significantly reduced the cumulative incidence of IA from 12.4% to 4.5% (P=0.04). The incidence of IA in patients in the group that did not receive primary prophylaxis was particularly high compared to that in the most recent studies (6.1-7.3%)[4,8] reflecting environmental contamination which has been associated with the occurrence of IA among hematology patients.[12] Since *Aspergillus* spores can be readily found in the environment for a long time after dissemination,[22] it is likely that most, if not all, patients had been exposed to a significant risk.

Current guidelines recommend several preventive measures that aim to decrease the rate of IA by reducing exposure of neutropenic patients to *Aspergillus* spores. Well-documented measures include HEPA filtration and positive air pressure rooms in high-risk units, cleaning of surfaces in patients' rooms, regular environmental surveillance, and

Table 4. Characteristics of the 25 acute leukemia patients who developed invasive aspergillosis.

N.	Age (years)	Acute leukemia	Prophylaxis	Aspergillosis*	Time to diagnosis (days)	Microbiological results	Scan results	Survival (months)
1	68	HR-AML	APP-	Probable pulmonary	13	GM + BAL+ (*A. fumigatus*)	Halo sign	6
2	38	B-ALL	APP-	Proven cerebral	14	GM + Autopsy +	Cerebellar mass	<1 **
3	41	HR-AML	APP-	Probable pulmonary	24	GM +	Halo sign	20
4	32	HR-AML	APP-	Probable pulmonary	14	GM +	Halo sign	<1 **
5	73	HR-AML	APP-	Probable pulmonary	15	Sputum +	Air crescent sign	16
6	55	AML	APP-	Probable pulmonary	15	GM +	Halo sign	20
7	67	AML	APP-	Possible pulmonary	14	GM +	Consolidation	24
8	51	HR-AML	APP-	Probable pulmonary	15	GM + BAL + (*A. fumigatus*)	Halo sign	5
9	64	HR-AML	APP-	Probable pulmonary	20	GM +	Halo sign	17
10	61	AML	APP-	Probable pulmonary	15	GM +	Halo sign	Alive
11	17	AML	APP-	Proven pulmonary	27	GM + Biopsy + (*A. flavus*)	Halo sign	Alive
12	79	T-ALL	APP-	Probable diffuse	14	GM + BAL+ (*A. terreus*)	Halo sign cerebral abscess	<1 **
13	21	AML	APP-	Possible pulmonary	5		Macronodules	8
14	67	AML	APP-	Probable pulmonary	14	Sputum + (*A. fumigatus*)	Consolidation	<1 **
15	70	HR-AML	APP-	Probable pulmonary	18	GM +	Macronodules	3
16	75	AML	APP-	Probable pulmonary	14	GM +	Macronodule	10
17	72	B-ALL	APP-	Probable pulmonary	18	GM + BAL+	Halo sign	5
18	72	HR-AML	APP-	Proven sinusal	14	Biopsy + (*A. terreus*)	Ethmoidal lysis	Alive
19	70	AML	APP-	Probable pulmonary	18	GM +	Halo sign	11
20	53	AML	APP-	Possible pulmonary	16		Halo sign	Alive
21	69	HR-AML	APP-	Possible pulmonary	56	GM +	Consolidation	<1 **
22	59	AML	Voriconazole	Probable pulmonary	23	GM +	Halo sign	5
23	68	HR-AML	Voriconazole	Probable pulmonary	16	GM +	Halo sign	5
24	61	HR-AML	Voriconazole	Probable pulmonary	31	BAL +	Micronodules	2
25	74	AML	Caspofungin	Possible pulmonary	3		Halo sign	<1 **

*AML: acute myeloid leukemia; HR-AML: high-risk AML cytogenetics; ALL: acute lymphocytic leukemia; APP-: no aspergillosis primary prophylaxis; GM: galactomannan; BAL: bronchoalveolar lavage *According to EORTC criteria. ** Death due to IA.*

wearing of high-efficiency masks by neutropenic patients.[22] However, the ability of these measures to prevent IA is probably not sufficient in the context of hospital building work. Thus, our results suggest that primary IA prophylaxis with well-tolerated antifungal agents such as voriconazole could be of benefit in reducing the incidence of IA in hematology units exposed to construction/renovation work.

Voriconazole is the recommended first-line treatment for IA.[2] In 2003, when voriconazole was first prescribed in our unit as prophylaxis to prevent IA, there were no data available on the efficacy and tolerance of this drug in patients with acute leukemia. In a recent randomized, phase III trial, prophylactic oral voriconazole showed a trend to decrease the incidence of lung infiltrates and hepatosplenic candidiasis in AML patients undergoing induction chemotherapy, and was safe and well-tolerated.[24] This trial was, however, stopped prematurely when another trial demonstrated reduced mortality in patients given antifungal prophylaxis with posaconazole,[9] thus rendering further randomization against placebo unethical. However, it is likely that voriconazole is also a valuable drug in this indication although we did not find any effect on overall survival in our cohort. In our study, voriconazole reduced the risk of IA by 75%, limited the prescription of empirical antifungal treatment from 42% to 17% of patients, and could have been associated with less other invasive fungal infections as the three fungal infections documented were all in the APP- group. Nevertheless, since the publication of the study by Cornely *et al.*, demonstrating the efficacy of posaconazole as primary IA prophylaxis in acute leukemia patients, we have used this latter drug in all patients undergoing intensive chemotherapy according to ECIL recommendations.[25]

As far as IA is concerned, each drug strategy aimed at decreasing the incidence of this rare infection raises issues of ecological, toxicity and economic costs. Several reports have described breakthrough fungal infections such as zygomycosis during voriconazole prophylaxis or treatment. These fungi are resistant to both voriconazole and echinocandins, and, although zygomycosis is documented less often than IA in leukemic patients and hematopoietic stem cell transplant recipients, there are recent reports suggesting that this infection has increased in incidence since the introduction of voriconazole. Two retrospective studies reported infections due to zygomycetes in 5/92 (5.4%) and 4/71 (5.6%) of hematopoietic stem cell transplant recipients given voriconazole as primary or secondary prophylaxis.[26] In our study, we did not observe any breakthrough zygomycosis during voriconazole treatment. However, it is particularly challenging to diagnose this invasive mycosis since bronchoalveolar lavage is usually unsuccessful and histological analysis of lung infiltrates or fungal polymerase chain reaction analyses were not performed for most of our patients.

The tolerance of prophylaxis was acceptable, with similar side-effects in the two groups. Voriconazole was withdrawn in 15% of cases, mainly because of mild hepatotoxicity. However, one case of sinus bradycardia linked to voriconazole must be mentioned because this side effect has never been described before. The bradycardia resolved after drug discontinuation and reappeared after reintroduction of voriconazole.

Although we studied a large, homogeneous population (all receiving remission-induction chemotherapy for acute leukemia only) in a particular context, our study has several limitations. This was a non-randomized, retrospective study and the selection of patients receiving primary IA prophylaxis could have introduced some bias. Moreover the duration, location and level of risk of the building work were variable through the study period. Nevertheless, case-by-case choice is likely to have selected high-risk patients in the prophylaxis group. This is reflected by the significant predominance of patients with pulmonary antecedents, which are known to be independent risk factors for IA, in the group that received prophylaxis.

Table 5. Multivariate analysis of risk factors for invasive aspergillosis.

	Odds ratio	P
Pulmonary antecedent	5.96 [2.31-15.33]	<0.001
Neutropenia at diagnosis of acute leukemia	4.06 [1.56-10.52]	<0.001
AML with high risk cytogenetics	3.55 [1.38-9.11]	0.008
Aspergillosis primary prophylaxis	0.25 [0.07-0.82]	0.02

Although IA remains a significant infection during treatment of acute leukemia, our results confirm that the actual mortality rate from IA is much lower than it was a decade ago. Recent studies have reported 3-month overall survival rates of 70.8% and 72% in IA patients treated with voriconazole and liposomal amphotericin B (Ambisome), respectively.[2,3] In our study, the 3-month mortality rate was similar (28%) but the mortality rate increased at 6 months (48%) probably due to IA but also to uncontrolled underlying disease. It is noteworthy that 50% of AML patients with IA had an adverse prognosis according to their cytogenetic features and most patients who died of IA-related complications were refractory to chemotherapy. Furthermore, the 33 AML patients with low-risk cytogenetics (of whom 28 did not receive prophylaxis) did not develop IA, suggesting that rapid and more effective disease control of leukemia is a determining factor in the incidence and outcome of IA. This is reflected by the significant adverse effect of high-risk cytogenetics AML in multivariate analysis of risk factors for IA.

This retrospective study suggests that antifungal prophylaxis with new agents such as voriconazole is safe and could be of interest in order to reduce the incidence of IA in hematology units exposed to recurrent building work/renovation. Our results demonstrate that acute leukemia patients could benefit from induction chemotherapy even in such a situation in which some clinicians are often reluctant to deliver intensive chemotherapy. Whether other antifungal agents such as posaconazole, liposomal amphotericin B or echinocandins are also effective is this situation remains to be determined. Prospective studies are needed to confirm the survival benefit of such an antifungal strategy and to assess its long-term ecological consequences.

Authorship and Disclosures

AC collected and analyzed data; LC performed statistical analysis; FH treated patients; XV, MDL, SC, JG, LT and MA analyzed data; CR designed the study, analyzed data and wrote the paper.

The authors reported no potential conflicts of interest.

References

1. Pfaller MA. Invasive fungal pathogens: current epidemiological trends. Clin Infect Dis. 2006;43(Suppl 1):S3-14.
2. Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE, Oestmann JW, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med. 2002;347(6):408-15.
3. Cornely OA, Maertens J, Bresnik M, Ebrahimi R, Ullmann AJ, Bouza E, et al. Liposomal amphotericin B as initial therapy for invasive mold infection: a randomized trial comparing a high-loading dose regimen with standard dosing (AmBiLoad trial). Clin Infect Dis. 2007;44(10):1289-97.
4. Pagano L, Caira M, Candoni A, Offidani M, Fianchi L, Martino B, et al. The epidemiology of fungal infections in patients with hematologic malignancies: the SEIFEM-2004 study. Haematologica. 2006;91(8): 1068-75.
5. Caillot D, Mannone L, Cuisenier B, Couaillier JF. Role of early diagnosis and aggressive surgery in the management of invasive pulmonary aspergillosis in neutropenic patients. Clin Microbiol Infect. 2001;7(Suppl 2):54-61.
6. Oren I, Haddad N, Finkelstein R, Rowe JM. Invasive pulmonary aspergillosis in neutropenic patients during hospital construction: before and after chemoprophylaxis and institution of HEPA filters. Am J Hematol. 2001;66(4):257-62.
7. Cornet M, Fleury L, Maslo C, Bernard JF, Brucker G. Epidemiology of invasive aspergillosis in France: a six-year multicentric survey in the Greater Paris area. J Hosp Infect. 2002;51(4):288-96.
8. Lin SJ, Schranz J, Teutsch SM. Aspergillosis case-fatality rate: systematic review of the literature. Clin Infect Dis. 2001;32(3):358-66.
9. Cornely OA, Maertens J, Winston DJ, Perfect J, Ullmann AJ, Walsh TJ, et al. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. N Eng J Med. 2007; 356 (4): 348-59.
10. Vonberg RP, Gastmeier P. Nosocomial aspergillosis in outbreak settings. J Hosp Infect. 2006;63(3):246-54.
11. Alberti C, Bouakline A, Ribaud P, Lacroix C, Rousselot P, Leblanc T, et al. Relationship between environmental fungal contamination and the incidence of invasive aspergillosis in haematology patients. J Hosp Infect. 2001;48(3):198-206.
12. Weber SF, Peacock JE Jr, Do KA, Cruz JM, Powell BL, Capizzi RL. Interaction of granulocytopenia and construction activity as risk factors for nosocomial invasive filamentous fungal disease in patients with hematologic disorders. Infect Control Hosp Epidemiol. 1990;11(5):235-42.
13. Tamburini J, Elie C, Bardet V, Chapuis N, Park S, Broët P, et al. Constitutive phosphoinositide 3-kinase/Akt activation represents a favourable prognostic factor in de novo acute myelogenous leukemia patients. Blood. 2007;110(3):1025-8.
14. Pigneux A, Perreau V, Jourdan E, Vey N, Dastugue N, Huguet F, et al. Adding lomustine to idarubicin and cytarabine for induction chemotherapy in older patients with acute myeloid leukemia: the BGMT 95 trial results. Haematologica. 2007;92(10):1327-34.
15. Huguet F, Leguay T, Raffoux E, Thomas X, Beldjord K, Delabesse E, et al. Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 study. J Clin Oncol. 2009;27(6):911-8.
16. Diviné M, Casassus P, Koscielny S, Bosq J, Sebban C, Le Maignan C, et al. Burkitt lymphoma in adults: a prospective study of 72 patients treated with an adapted pediatric LMB protocol. Ann Oncol. 2005;16(12): 1928-35.
17. Rea D, Legros L, Raffoux E, Thomas X, Turlure P, Maury S, et al. High-dose imatinib mesylate combined with vincristine and dexamethasone (DIV regimen) as induction therapy in patients with resistant Philadelphia-positive acute lymphoblastic leukemia and lymphoid blast crisis of chronic myeloid leukemia. Leukemia. 2006;20(3):400-3.
18. Slovak ML, Kopecky KJ, Cassileth PA, Harrington DH, Theil KS, Mohamed A, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group study. Blood. 2000; 96(13):4075-83.
19. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis. 2008;46(12):1813-21.
20. Bocquet P, Aggoune M, Aussant M, Rykner G, Brucker G. Aspergillose invasive nosocomiale et travaux hospitaliers: recommandations. Paris: Doin, 1993.
21. Berthelot P, Loulergue P, Raberin H, Turco M, Mounier C, Tran Manh Sung R, et al. Efficacy of environmental measures to decrease the risk of hospital-acquired aspergillosis in patients hospitalized in haematology wards. Clin Microbiol Infect. 2006;12(8):738-44.
22. Leenders AC, van Belkum A, Behrendt M, Luijendijk A, Verbrugh HA. Density and molecular epidemiology of Aspergillus in air and relationship to outbreaks of Aspergillus infection. J Clin Microbiol. 1999;37(6):1752-7.
23. ANAES. Hygienes 2000 ANAES. Prévention du risque aspergillaire chez les patients immunodéprimés (hématologie, transplantation). Hygiènes 2000;VIII(6): 336-501.
24. Vehreschild JJ, Bohme A, Buchheidt D, Arenz D, Harnischmacher U, Heussel CP, et al. A double-blind trial on prophylactic voriconazole (VRC) or placebo during induction chemotherapy for acute myelogenous leukaemia (AML). J Infect. 2007;55(5):445-9.
25. ECIL Maertens J. Primary antifungal prophylaxis in leukaemia patients. Eur J Cancer. 2007;S5:43-8.
26. Siwek GT, Pfaller MA, Polgreen PM, Cobb S, Hoth P, Magalheas-Silverman M, et al. Incidence of invasive aspergillosis among allogeneic hematopoietic stem cell transplant patients receiving voriconazole prophylaxis. Diagn Microbiol Infect Dis. 2006;55(3):209-12.