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Infections caused by filamentous fungi in patients with hematologic malignancies. A report of 391 cases by GIMEMA Infection Program

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Background and Objectives. To evaluate the clinical characteristics of patients with hematologic malignancies developing a filamentous fungal infection (FFI) and to define the prognostic factors for their outcome.

Design and Methods. A retrospective study, conducted on patients admitted to 14 Hematology divisions of tertiary care or university hospitals, participating in the GIMEMA Infection Program, over a ten-year period (1988-1997). The study included patients with hematologic malignancies and a histologically and/or microbiologically proven or probable FFI.

Results. We included 391 patients (male/female: 262/129, median age 49 years) with hematologic malignancies (225 acute myeloid leukemia, 67 acute lymphocytic leukemia, 30 chronic myeloid leukemia, 22 non-Hodgkin's lymphoma, 12 myelodysplastic syndrome, 10 aplastic anemia, 7 Hodgkin's disease, 8 chronic lymphocytic leukemia, 5 multiple myeloma, and 5 hairy cell leukemia) who developed a proven FFI. Eighty percent of the patients had been neutropenic for an average of 14 days before the infection, and 71% had an absolute neutrophil count lower than 0.5×10^9/L at the time of FFI diagnosis. The primary sites of infection were: lungs (85%), nose and paranasal sinus (10%), and other sites (5%). The diagnosis was made while still alive in 310 patients (79%), and at autopsy in the remaining 81 patients (21%). Chest X-ray was diagnostic in 77% of patients with pulmonary FFI, while computed tomography (CT) scan of the thorax was positive in 95% of cases. A significant diagnostic advantage for CT scan was observed in 145 patients who had both a chest X-ray and CT scan. *Aspergillus* was identified as the cause of FFI in 296 patients, *Mucorales* in 45 patients, *Fusarium* in 6 patients and other filamentous fungi species in 4 patients, while in a further 40 patients no agent was identifiable. The overall mortality rate three months after the diagnosis of FFI was 74%, and fungal infection had been the cause of death in 51% of patients.

Interpretation and Conclusions. Our retrospective study shows that FFI still remains a life-threatening complication in neutropenic patients. Despite appropriate treatment, half of the patients die due to this complication. The use of glucocorticoids and recovery from neutropenia are the most important prognostic factors. *Mucorales* infections are associated with a significantly poorer prognosis than those due to *Aspergillus* spp.

Key words: filamentous fungi infection, Aspergillus, leukemia, hematologic malignancies.

The incidence of deep fungal infections in patients with malignancies has increased dramatically over the past decades. The majority of these infections occur in patients with hematologic malignancies, particularly acute myeloid leukemia (AML).[1-5] This increase has been attributed to several factors: impairment of host defenses due to intensive cytotoxic therapy, including blood stem cell transplantation, ablative radiation therapy, use of corticosteroids or cyclosporine, as well as the underlying hematologic malignancy.[6-16]

Until recently, *Candida spp*. were the most frequent cause of invasive fungal infection in neutropenic patients. Nevertheless, various autopsy studies have shown that the incidence of *Candida*

infections has remained substantially stable over the years, while that of filamentous fungi (FFI) is progressively increasing.[17,18]

Aspergillus spp. represent the main cause of FFI, but emerging opportunistic fungal pathogens (i.e. *Fusarium spp.*) have been reported over the last 20 years.[2,10,19-21] In this retrospective analysis, we reviewed the clinical features and their prognostic relevance in 391 patients with hematologic malignancies and a proven or probable FFI.

Design and Methods

Fourteen Hematology Departments from tertiary care or university hospitals participated in this study. Between January 1, 1988 and December 31, 1997, we examined the clinical characteristics of patients with hematologic malignancies and a documented microbiological and/or histologic diagnosis of FFI.

Cases were defined using modifications of the *Mycoses Study Group Criteria*.[22] Proven FFI was defined as follows: a) histo/cytopathologic findings of filamentous fungi, associated with tissue damage, even in the absence of microbiological isolation. The observation of branched septate hyphae was considered generically diagnostic for hyalohyphomycosis (i.e. aspergillosis, fusariosis), while that of broad non-septate or rarely septate hyphae with branches occurring at right angles were considered diagnostic for infection by *Mucorales*; b) positive culture obtained from a normally sterile site in absence of possible contamination.

Probable FFI was defined as the isolation of filamentous fungi from a non-sterile site (bronchoalveolar lavage, one sample; sputum, two samples; nasal swab, two samples), associated with clinical signs of invasive FFI (pneumonia, cavitary infiltrates or nodules, sinusitis). Radiologic findings such as halo signs or air crescent signs on the chest X-ray or CT-scan were also considered criteria for the diagnosis. Patients with a radiologically or clinically suspected FFI, in the absence of pathologic or microbiological isolation, despite positive *Aspergillus* antigenemia, were not included in this study.

The clinical records of the enrolled patients were examined. All the participating centers received a form to be completed and sent to the co-ordinating center for each case of suspected, probable or proven FFI during the study period. The centralized data-base was constructed by assigning a number to every case and transforming all the variables into code. At the end of the study, all the centers were asked to add, for each patient signaled, any new data useful for examination. The following patients' characteristics were taken into consideration: place of birth and occupation, type and stage of hematologic malignancy, clinical signs, symptoms and site of infection, radiologic findings, laboratory data [i.e. neutropenia (neutrophil count $<1\times10^9$/L), microbiological isolates], treatment received, cause of death, autopsy findings. Death due to FFI was defined as occurring in the presence of microbiological, histologic, or clinical evidence of active fungal infection. Data from the episode of FFI and follow-ups at 3 and 6 months were collected.

Statistical methods

Potential prognostic indicators predicting the outcome of FFI were analyzed by the χ^2 test and the factors significantly associated in the univariate analysis were included in a logistic regression model to analyze their independent role. The Cox proportional-hazard model was used for multivariate assessment of the relative risk of death during infection, after adjustment for potentially confounding factors. The two-tailed test of significance at $p<0.05$ was used to determine statistical significance.

Results

Among patients with hematologic malignancies, 449 episodes of FFI were reported by the 14 participating divisions of hematology during the study period. Fifty-eight episodes were excluded from the study because the diagnosis of FFI was based only on clinical evidence. The 391 evaluable patients had an age ranging between 12 and 84 years (median 49 years), 262 were male and 129 were female. Two hundred and thirty-seven patients (62%) had a proven FFI, while 154 (38%) had a probable FFI. Overall, most FFI (222 episodes, 56.7%) were documented in patients undergoing first treatment for their hematologic malignancy.

Table 1 shows the underlying disease and the treatment phase at the time of FFI diagnosis. Acute leukemia was diagnosed in 75% of cases (292 patients), AML being the most frequent malignancy (225 patients, 57.5%). The overall incidence of FFI in patients with adult acute leukemia (over 12 years of age) registered by the participating centers was 6.5% (292 FFI /4448 new cases of acute leukemia). The lack of a common national register for hematologic malignancies other than acute leukemia makes it impossible to calculate the incidence of FFI in such pathologies.

Risk factors for FFI prior to diagnosis

None of the patients included in this study had a previous history of FFI. The FFI had been preceded by chemotherapy in 355 patients (90%), while

Table 1. Hematologic malignancy and phase of treatment of 391 cases of FFI.

	AML	*ALL*	*NHL*	*HD*	*CML*	*MM*	*CLL*	*Aplasia*	*MDS*	*HCL*	*Total (%)*
First induction	151	39	10	4	8	3	3	/	3	1	222 (57)
Re-induction	24	16	3	/	/	/	/	/	/	/	43 (11)
Consolidation	20	3	1	/	/	/	/	/	/	/	24 (6)
Maintenance	2	/	/	/	3	/	2	/	/	/	7 (2)
Autologous BMT	7	2	/	2	2	1	/	1	/	/	15 (4)
Allogeneic BMT	3	3	1	/	13	1	/	1	1	/	23 (6)
Salvage	7	3	6	1	2	/	1	/	1	/	21 (5)
Immunosuppression	/	/	1	/	1	/	/	5	/	1	8 (2)
None	11	1	/	/	1	/	2	3	7	3	28 (7)
Total	225	67	22	7	30	5	8	10	12	5	391 (100)

AML = acute myeloid leukemia; ALL = acute lymphoid leukemia; NHL = lymphoma; HD = Hodgkin's disease; CML = chronic myeloid leukemia; MM = multiple myeloma; CLL = chronic lymphocytic leukemia; Aplasia = aplastic anemia; MDS = myelodysplastic syndrome; HCL = hairy cell leukemia; BMT = bone marrow transplantation.

28 (7%) had not received any treatment during the previous 3 months either because not indicated (e.g. myelodysplastic syndromes) or due to their poor performance status. In 5 patients with aplastic anemia the immunosuppressive treatment was only cyclosporine; 3 further patients with hairy cell leukemia (HCL), chronic myeloid leukemia (CML), and non-Hodgkin's lymphoma (NHL) received only α-interferon. Before the diagnosis of FFI, 311 patients (80%) had been neutropenic (absolute neutrophil count [ANC] lower than 0.5×10^9/L) for a median of 14 days (range 1-250). At the diagnosis of FFI, 277 patients (71%) had an ANC lower than 0.5×10^9/L, and 221 patients (57%) had an ANC lower than 0.1×10^9/L. Broad-spectrum antibiotics (β-lactam plus an aminoglycoside with or without a glycopeptide) had been administered to 311 patients (80%) for a median of 11 days (range 1-60). A clinically suspected or microbiologically documented bacterial infection preceded FFI in 156 cases (40%). In particular, a bacteremia was diagnosed in 55 cases (30 Gram positive, 22 Gram negative, and 3 polymicrobial).

Antifungal prophylaxis was administered to 313 patients (80%). Oral non-absorbable polyenes, amphotericin B (amB) or nystatin were used in 141/391 cases (36%); systemic prophylaxis, with fluconazole, 150-300 mg/daily, or itraconazole, 200-400 mg/daily, had been employed in 109 and 41 cases, respectively (38%). Other types of prophylaxis were employed in 22 cases [aerosol-administered amB, 6 cases; low doses (<0.5 mg/kg/day) i.v. amB, 10 cases; ketoconazole, 6 cases]. The median duration of prophylaxis before FFI was 20 days (range 1-100).

Two hundred and twenty-five patients (57%) had been treated with steroids, using a median equivalent dose of 6-methylprednisolone of 60 mg daily (range 20-120), for a median of 20 days (range 1-370) and a median total dose of 875 mg (range 48-24,000). In the majority of cases, steroids were administered as part of the treatment of chemotherapy or during the transplant procedure. Considering other known risk factors, 45 patients (13%) had diabetes and 18 patients (4%) had undergone a recent surgical procedure. Nineteen patients (4.8%) developed FFI despite protective isolation in laminar airflow or hepa-filtered rooms.

Clinical presentation

Sites of fungal infection are illustrated in Table 2. Primary sites of infection were defined as those where the earliest and/or major signs or symptoms of disease appeared, and secondary sites were those following dissemination. In 7 patients the primary site of infection was not identified, because the infection presented with multiple localizations. Table 3 illustrates signs and symptoms according to the infected sites. Fever, with a median temperature of 38.4°C at presentation, was the most frequent symptom (80% of episodes). Twenty-nine patients were asymptomatic and diagnosis was made only at autopsy.

Diagnostic procedures

A diagnosis of FFI was made *in vivo* in 310 patients (79%), while in 81 patients (21%) the clinical suspicion of FFI was confirmed at autopsy. Table 4 reports the main diagnostic procedures employed and the percentage of positive results, according to infected sites. CT scan of the chest had a higher efficacy than standard chest X-ray (94.5% vs.

Table 2. Sites of fungal infection (*in vivo* or at autopsy).

	Primary (%)	*Secondary (%)**	*Total (%)*
Lung	321 (82.1)	11 (2.8)	332 (84.8)
Nose	23 (5.9)	16 (4)	39 (9.9)
Sinus	21 (5.4)	31 (7.9)	52 (13.3)
Bowel	4 (1)	5 (1.2)	9 (2.2)
Blood	4 (1)	3 (0.8)	7 (1.7)
Skin	3 (0.8)	8 (2)	11 (2.7)
Orbit	3 (0.8)	3 (0.8)	6 (1.5)
CNS	3 (0.8)	34 (8.7)	37 (9.4)
Oral cavity	1 (0.2)	1 (0.2)	2 (0.5)
Liver	0	6 (1.5)	6 (1.5)
Myocardium	1 (0.2)	3 (0.8)	4 (1)
Middle ear	0	1 (0.2)	1 (0.2)
Kidney	0	2 (0.5)	2 (0.5)
Spleen	0	5 (1.2)	5 (1.2)
Multiple at diagnosis°	7 (1.8)	0	7 (1.7)

**Some patients had two or more involved sites. °Patients had more than 2 involved sites and it was not possible to identify the primary site.*

Table 3. Signs and symptoms of the patients at the diagnosis by site of infection.

Sign/symptom	*N*	*%*
Pulmonary localization	332	
Fever	269	81
Cough	235	71
Chest pain	153	46
Dyspnea	144	43
Hemoptysis	24	7
Pneumothorax	5	1.5
Sino-nasal localization	91	
Fever	63	69
Facial edema	77	85
Nasal obstruction	74	81
Facial pain	52	57
Rhinorrhea	33	36
Epistaxis	7	8
Palate destruction	4	4
Central nervous system involvement	37	
Fever	15	40
Cerebral hemorrhage	18	49
Hemiplegia	7	19
Ptosis	4	11
Epilepsy	2	5
Gastro-intestinal tract	9	
Fever	6	67
Abdominal pain	4	44
Diarrhea	8	89
Rectorrhagia	1	1
Asymptomatic	22	
Total	469*	

**more sites infected in the same patient.*

76.7% respectively) in patients with pulmonary FFI. Among 143 patients who underwent both investigations, with no more than 5 days elapsing between the two (median 3 days), CT scan was positive in 135 patients (94%) and X-ray in 124 patients (87%) (O.R. 2.59, 95%C.I. 1.03-6.70; $p<0.02$). Seven of 11 patients with a negative X-ray and a positive CT scan had a peripheral pulmonary lesion, while 4 patients had a retro-cardiac infection. Ten of these patients had a negative chest X-ray and positive CT scan of the chest on the same day. No differences in documenting single or multiple lung lesions were found between X-ray and CT scan. The presence of a cavitation was demonstrated in 5% of X-rays (12 patients) and 13% of CT scans (18 patients). Bronchoalveolar lavage (BAL) culture was positive in 80 out of 128 examinations (62%). In 48 patients with a negative BAL, diagnosis was made following surgery in 13 patients and at autopsy in 16 patients. FFI was documented in the sputum in 15 cases, by histology of tissues other than the lungs (2 cases) and by culture of pleural effusion (2 cases), performed after BAL.

Table 4. Radiological and microbiological procedures used to diagnose FFI in respect of the main sites involved.

	% Performed	*Positive/performed*	*% Sensitivity*
Pulmonary localization (no. 332)			
Chest X-ray	98	251/327	77
Single lesion		117	
Multiple lesions		134	
Chest CT	44	138/146	94
Single lesion		63	
Multiple lesions		75	
Broncho-alveolar lavage culture	38	80/128	62
Surgical specimens	18	40/51	87
Sputum	72	139/241	58
Nasal swab	56	61/191	32
Brain localization (no. 37)			
Cerebral CT	65	21/24	88
Surgical specimens	8	2/3	66
Sino-nasal localization (no. 58)*			
Cranial CT	50	25/29	86
Nasal swab	71	35/41	85
Surgical specimens	19	11/11	100
Serology			
Antigen		29/90	32
Antibody		2/50	4
Cutaneous biopsy		12/12	100
Bowel resection		1/1	100
Autopsy specimen	28	106/111	96

**33 patients had both nasal and sinus localizations.*

Table 5. Etiological agents of 391 cases of FFI.

	N	%
Hyalohyphomycetes*	40	10.2
Aspergillus	296	75.7
Aspergillus spp	102	34.5
Aspergillus fumigatus	108	36.5
Aspergillus flavus	58	19.6
Aspergillus terreus	15	5
Aspergillus niger	7	2.4
Aspergillus versicolor	4	1.4
Aspergillus glaucus	1	0.3
Aspergillus nidulans	1	0.3
Mucorales°	45	11.5
Mucorales spp	28	62.2
Mucor spp	10	22.2
Rhizopus spp	4	8.8
Cunninghammella	2	4.6
Absidia corymbifera	1	2.2
Fusarium spp	6	1.5
Scedosporium apiospermum/Pseudallescheria boydii	2	0.4
Scopulariopsis brevicaulis	1	0.2
Acremonium spp	1	0.2

**Observation at histo/cytopathology of branched septate hyphae.*
°Diagnosis on histologic or microbiological specimens.

Lung biopsy was performed in 51 patients (42 trans-bronchial, 11 trans-thoracic needle and 8 open lung biopsies). This procedure was positive in 78.4% of cases: 21 trans-bronchial (65.6%), 11 trans-thoracic (100%) and 8 open lung biopsies (100%).

Brain aspergillosis was suspected due to neurological signs in 37 patients and CT scan was compatible with fungal abscesses in almost all the cases it was performed (21/24, 87%). Cerebral biopsy was performed in 3 patients only and was positive in 2 of them. The diagnosis of central nervous system (CNS) FFI was confirmed at autopsy in 35 patients, including the patient with a negative CNS biopsy. All but one patient died within a few weeks from the onset of neurological signs.

Biopsy of sino-nasal tissues (11 cases) or of skin lesions (13 cases) was diagnostic in all cases examined. Interestingly, a localized bowel *Aspergillus fumigatus* infection was diagnosed at surgical debridement in one patient with intestinal obstruction. Histologic specimens (lung, central nervous system, sino-nasal, skin and bowel) were positive for FFI in 67 (75%) of 89 patients (23%). None of the patients had complications following the diagnostic procedure.

Serum *Aspergillus* antigenemia was studied in 90 patients using a latex agglutination assay of galactomannan and was positive (at least 2 times) in 29 cases (32%). In 27 patients (93%) the antigen was detected after the pulmonary infection had been diagnosed or suspected. Serology for *Aspergillus* was performed in 50 cases and was positive in 2 cases (4%).

Etiology

Table 5 reports the filamentous fungi identified as causal agent in 391 cases of FFI. Filamentous fungi were identified only histologically in 169 cases, in the absence of a positive culture of histologic specimens. However, on the basis of histo-microbiological findings, hyalo-hyphomycetes were identified in 40 cases, and *Mucorales* in 28 cases. *Aspergillus* was the agent of FFI most frequently isolated (296 cases; 76%) and of 194 types of *Aspergillus* isolates, *Aspergillus fumigatus* was the main agent, responsible for 53% (102 episodes) of infections. *Mucorales* were isolated in 11.5% of cases (45 episodes), and FFI were sustained by other filamentous fungi (*Fusarium spp., Scedosporium apiospermum/Pseudallescheria boydii, Scopulariopsis brevicaulis, Acremonium spp*) in a further 10 cases. The ratio *Aspergillus*/*Mucorales* was 6.9/1. No relationship was shown between clinical characteristics and outcome of infections due to the different *Aspergillus spp*. identified (*A. fumigatus, A. flavus,* and *A. terreus*).

Treatment and outcome

Antifungal treatment was administered to 341 patients (87%) and was started empirically in 274 patients (81%) with persistent fever after 72 hours of intravenous broad spectrum antibiotics. Deoxycholate amB was employed in 285 cases (73%) at a median dose of 1 mg/kg/daily (range 0.6-1.6). The total median dose of amB was 15 mg/kg (range 0.50-100) for a median of 18 days (range 1-200). In 51 patients other antifungal drugs were added to or followed amB treatment [intravenous 5-fluocytosine (6 cases), oral itraconazole (39 cases), and ketoconazole (6 cases)]. Eighteen patients switched from amB to liposomal amB (L-amB) because of renal impairment (median daily dose: 2 mg/kg). In 56 cases (14%) FFI was initially treated with drugs other than amB: L-amB was given to 23 patients (median daily dose 2 mg/kg, range 1-3) for a median of 20 days (2-150) and a median cumulative dose of 18.5 mg/kg (4-44.2), oral itraconazole to 18 patients (400-600 mg/day) for a median of 31 days (8-200), and intravenous fluconazole to 15 patients (600-800 mg/day) for a median of 14 days (2-60). Granulocyte transfusions from family donors were given to 8 neutropenic patients whose fever was unresponsive to antifungal therapy. Only 1 of these

patients improved after transfusion. Ninety-six neutropenic patients (24%) received growth factors (rhG-CSF, 84 patients and rhGM-CSF, 12 patients). The use of growth factors was based on personal evaluation of physicians of each center.

The treatment resulted in disappearance of fever and improvement of clinical status in 164 patients (42%). The remaining 227 cases (58%) progressed. Two hundred and ninety patients (74%) died within 90 days after diagnosis of FFI: 83 patients (21%) because of progression of the underlying hematologic malignancies, 198 (51%) of FFI and 9 (2%) because of bacterial infection (septic shock caused by Gram-negative bacilli in 7 cases and bacterial pneumonia in 2 cases). Fifty patients (13%) did not receive any antifungal treatment. Forty-one died from FFI (82%), 6 patients (12%) died from malignancy and one patient (2%) from bacterial infection. In spite of the absence of antifungal treatment, 2 patients improved from FFI after a recovery of neutrophil count. Autopsy was performed in 111 patients. Ninety-six percent of patients showed findings consistent with active FFI.

Survival

Table 6 shows factors identified by univariate and multivariate analysis that correlated with a negative outcome of the infection. Patients dying within 5 days from the start of antifungal treatment were excluded from analysis. Older age, use of corticosteroids, an ANC lower than 0.1×10^9/L at the diagnosis of FFI, lack of recovery from aplasia, multiple pulmonary localizations of infection, *Mucorales* as agents of FFI, absence of treatment or use of fluconazole and a total cumulative dose of amB lower than 25 mg/kg negatively influenced the outcome of FFI. Other parameters such as sex, underlying hematologic disease (acute leukemia vs. all other diseases), stem cell transplantation, diabetes, use of growth factors, previous use of broad-spectrum antibiotics, nasal colonization, primary site of infection, antifungal prophylaxis and fever had no prognostic impact.

The multivariate analysis showed that the use of steroids (OR 1.98, 95%CI 1.13-3.48), *Mucorales* etiology (OR 3.54, 95%CI 1.51-8.32) and the lack of neutrophil recovery (OR 0.28, 95% CI 0.16-0.49) correlated significantly with death from FFI.

Table 6. Univariate and multivariate analysis of patients' characteristics in relation to fungemic death.

Characteristics	*Death from FFI/total (%)*	*Univariate analysis*	*Multivariate analysis*
Sex			
male	132/262 (50)	n.s.	
female	66/129 (51)		
Age			
<30	29/80 (36)		1.00
30-60	117/220 (53)	0.007	1.86 (0.92-3.75)
>60	53/91 (58)		2.14 (0.92-4.98)
Diabetes*			
yes	26/45 (50)	n.s.	
no	152/306 (58)		
Corticosteroids			
yes	125/225 (56)	0.024	1.98 (1.13-3.48)
no	73/166 (44)		1.00
Neutropenia at diagnosis			
$<0.1\times10^9$/L	126/221 (57)	0.01	1.00
$<0.5\times10^9$/L	22/56 (39)		0.70 (0.39-1.27)
$<1\times10^9$/L	16/34 (47)		1.00
Neutrophil recovery			
yes	63/166 (38)	0.0001	0.28 (0.16-0.49)
no	101/144 (70)		1.00
Growth factors			
yes	45/96 (47)	n.s.	
no	153/295 (52)		
Chest CT-scan			
single lesion	18/63 (29)	0.019	
multiple lesions	36/75 (48)		
Etiology			
Aspergillus spp.	144/296 (49)	0.002	1.00
Mucorales	33/45 (73)		3.54 (1.51-8.32)
Prophylaxis			
yes	158/313 (50)	n.s.	
no	40/78 (51)		
Therapy			
none/fluconazole	47/65 (72)	0.0002	1.00
AmB/itraconazole	154/326 (47)		0.92 (0.41-2.09)
AmB treatment°			
<25 mg/kg	93/184 (51)	0.012	
>25 mg/kg	22/67 (33)		

**Information was not available for 40 patients; °33 patients died within 5 days of beginning treatment and were excluded from the evaluation.*

Discussion

In this study we have enrolled the largest ever-collected series of proven FFI in patients with hematologic malignancies. At least 6.5% of patients developed FFI during the course of acute leukemia, and in particular acute myeloid leukemia was associated with the highest risk of FFI. Considering the retrospective nature of the study and the inclusion of only microbiologically and/or histologically documented cases, the real incidence of FFI in acute leukemia patients is probably significantly higher. We could not evaluate the incidence of documented FFI in malignancies other than leukemia during the study period because of the lack of a registry of other hematologic diseases.

Interestingly, most of the patients (57%) developed FFI following the first course of chemotherapy. It is well known that exposure to *Aspergillus* is almost universal and in immunocompetent subjects macrophages and polymorphonuclear leukocytes are an effective defense system.[23] Impairment of the immune response due to the underlying hematologic malignancy or the immunosuppressive therapy may play a role in the onset and diffusion of FFI, during the early phases of the treatment in colonized patients.[8] The risk of developing FFI has been reported to increase progressively with the duration of neutropenia, reaching a plateau of 70% among patients neutropenic for more than 34 days.[24] On the other hand, it is possible that the high incidence of FFI in acute myeloid leukemia patients is due to the fact that these patients have a prolonged hospitalization, particularly those who are undergoing first induction chemotherapy. The majority of our patients had a profound neutropenia and had had a prolonged neutropenic phase prior to the diagnosis of FFI. Corticosteroids are an additional important risk factor:[11,13] in our series corticosteroids were used by 57% of patients at a relatively high dose as part of the chemotherapy regimen or as treatment for graft-versus-host disease. Perhaps it would be helpful to reduce the use of corticosteroids whenever this is possible. Antifungal prophylaxis is widely employed in patients with acute leukemia but its real benefits and the proper schedule are still controversial.[25] We cannot comment on the role of FFI prophylaxis because only 12% of our patients received a drug active against *Aspergillus* (itraconazole or i.v. amB).

Our study clearly shows that lung and nasal sinuses are the most frequent sites of FFI, which correlates with the modality of transmission of infection. Signs and symptoms were various, but fever and pain at the site of infection were present in almost all patients. It is remarkable that 22 patients were completely asymptomatic and that their infection was diagnosed at autopsy. It has been reported that FFI develops in about 20% of patients with acute leukemia,[17,18] but the diagnosis can be proven in only 30% of them. Invasive procedures are frequently limited by the bad general condition of the patients or because of thrombocytopenia. Both invasive and non-invasive procedures allowed the diagnosis of FFI in a relatively high percentage (79%) of patients while alive. However, because of the retrospective nature of the study and the relatively low number of autopsies performed, these data may represent an overestimate of the real diagnostic power of the current procedures employed. Although our data cannot be used to evaluate the sensitivity and specificity of single diagnostic procedures, we observed a different yield of the diagnostic techniques in relation to the sites involved.

Radiological exams such as standard chest X-ray and early CT scan were almost routinely employed. As recently reported in different studies, tomography pictures characterized by a mass-like infiltrate with a surrounding halo of ground glass attenuation or by the air crescent sign compatible with pulmonary cavitation can be considered diagnostic of a pulmonary FFI.[26,27] We observed that in patients with lung involvement, who had both chest X-ray and CT scan performed almost simultaneously, CT scan gave a diagnostic advantage over the standard lung X-ray. Bronchoscopy with BAL is lately being frequently employed due to the low incidence of complications. However in the literature, the sensitivity of BAL in the diagnosis of FFI has a wide range, from 0 to 80%.[28-32] Taking into account the retrospective nature of our study and the diagnostic criteria of FFI adopted, the 62% sensitivity of BAL in our experience was higher than other published results. False negative cases were probably due to variables such as the presence of peripheral lesions not attainable by bronchoscopy, to a perilesional fibrous annulet preventing the passage of hyphae into the bronchial system, or due to early execution of BAL, with later positive culture of other biological samples. Surgical biopsies represented the most useful tool for the diagnosis of FFI. Due to the critical conditions of the patients, surgery was performed in our study in only 23% of patients.

Our work confirms data by Kami *et al*.[33] on the low sensitivity (32%) of *Aspergillus* galactomannan serum detection by latex agglutination tests. Antigenemia also showed a poor diagnostic value, confirming only documented diagnosis of Aspergillosis. *Aspergillus spp.* were responsible for 76% of all FFI, followed by *Mucorales* (11.5%). Histology was diagnostic for 10% of unspecified FFI, and other agents such as *Fusarium, Acremonium, Scedosporium,* and *Scopulariosis* were responsible for 2.5% of all documented infections. It is remarkable that a great number of infections due to *Mucorales* (13 episodes) were reported over a two-year period by one center and that the source of infection was never identified, although an epidemic could not be excluded.

To date, amB has been considered the drug of choice for the treatment of FFI.[34-36] The antifungal treatment was based on the experience of different participating centers. In the great majority of patients, amB (deoxycolate or rarely liposomal) and

in some cases itraconazole, was the first-line treatment. Only a few asymptomatic cases diagnosed by autopsy did not receive any therapy. The treatment, frequently started empirically, was effective in 42% of patients, while about 50% of patients died from FFI within 90 days of diagnosis. The univariate analysis showed that the prolonged use of amB was correlated with recovery from the infection. However, we cannot exclude that the correlation between the high cumulative dose of amB and the good outcome of patients was simply due to better clinical conditions and prolonged survival.

At univariate analysis an absolute neutrophil count $<0.1\times10^9$/L at diagnosis of FFI was associated with a poor survival, and, at multivariate analysis, persistent neutropenia was associated with a significantly lower probability of survival at three months from the diagnosis of FFI. Some studies recently suggested that the use of growth factors, to aid rapid recovery from aplasia and to increase the fungicidal activity of monocytes, associated with granulocyte transfusions collected from rhG-CSF-primed healthy volunteers, could support antifungal treatment and stimulate recovery from infection.[37] However, the use of growth factors was not associated with a statistically significant improvement of survival.

In conclusion, our study shows that patients with acute myeloid leukemia are at high risk of developing FFI. These infections occur during the first phases of the disease and despite prompt and timely antifungal therapy the outcome is strongly affected by the degree and duration of neutropenia. The appropriate use of diagnostic techniques is critical for the timely identification of infections. Future studies should focus on the prevention of environmental and host factors.

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LP and PR co-ordinated the study; LP, CG and LM wrote the paper; MET, LM, AC were responsible for the data analysis; all the other co-authors collected the clinical data; PM and ADF critically reviewed and approved the final version. The authors wish to thank Dr. D.W. Denning for useful suggestions and comments.

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Potential implications for clinical practice

New information on the most relevant infectious complication in patients with hematologic malignancies.

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Appendix

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