Invasive aspergillosis in patients with acute myeloid leukemia: a SEIFEM-2008 registry study

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ABSTRACT

Background
The aim of this study was to evaluate prognostic factors, treatments and outcome of invasive aspergillosis in patients with acute myeloid leukemia based on data collected in a registry.

Design and Methods
The registry, which was activated in 2004 and closed in 2007, collected data on patients with acute myeloid leukemia, admitted to 21 hematologic divisions in tertiary care centers or university hospitals in Italy, who developed proven or probable invasive aspergillosis.

Results
One hundred and forty cases of invasive aspergillosis were collected, with most cases occurring during the period of post-induction aplasia, the highest risk phase in acute myeloid leukemia. The mortality rate attributable to invasive aspergillosis was 27%, confirming previous reports of a downward trend in this rate. Univariate and multivariate analyses revealed that the stage of acute myeloid leukemia and the duration of, and recovery from, neutropenia were independent prognostic factors. We analyzed outcomes after treatment with the three most frequently used drugs (liposomal amphotericin B, caspofungin, voriconazole). No differences emerged in survival at day 120 or in the overall response rate which was 71%, ranging from 61% with caspofungin to 84% with voriconazole.

Conclusions
Our series confirms the downward trend in mortality rates reported in previous series, with all new drugs providing similar survival and response rates. Recovery from neutropenia and disease stage are crucial prognostic factors. Efficacious antifungal drugs bridge the period of maximum risk due to poor hematologic and immunological reconstitution.

Key words: aspergillosis, acute myeloid leukaemia, antifungal treatment.

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Introduction

The epidemiology of invasive aspergillosis has changed significantly over the last two decades. Patients with acute myeloid leukemia (AML) are most frequently affected, with a 10% incidence during post-induction or consolidation aplasia.[1-3] Although the severity and duration of neutropenia remain the major risk factors, the incidence of invasive aspergillosis has also increased after immunosuppressive therapy, such as alemtuzumab, infliximab or fludarabine-based chemotherapy.[4-6] The aspergillosis-attributable mortality rate (AMR) in AML is generally around 30-40%. In two consecutive multicenter studies we observed that the AMR decreased from 48% in 1987-1998 to 38.5% in 1999-2003.[2,7,8]

There was, therefore, the need for an observational registry to identify emerging risk factors, stratify patients according to risk and assess the efficacy of anti-fungal agents as they are introduced into clinical practice. The present study charted the incidence and outcome of invasive aspergillosis in Italian patients with AML from 2004 to 2007, identified factors influencing outcome and determined whether prescribed treatment influenced outcome.

Design and Methods

A prospective registry investigation was conducted in 21 tertiary care centers or university hospitals in Italy from January 2004 through December 2007. Inclusion criteria were development of invasive aspergillosis in AML patients. Exclusion criteria were: (i) allogeneic and autologous stem cell transplant procedures; (ii) a history of invasive aspergillosis; and (iii) end-stage AML (patients with relapsed/resistant AML after two or more chemotherapy regimens). Only infections that were classified as "proven" or "probable" were included in this analysis.[9]

Each participating center completed a questionnaire eliciting the following data: age, gender, AML stage (onset, first relapse/resistance, complete remission), neutropenia (severe if count was $<0.5\times10^9$/L and moderate if $0.5\text{-}1.0\times10^9$/L) and its duration (<10 *versus* ≥10 days), antifungal prophylaxis, site of infection, diagnostic microbiology, (direct microscopy, cultures, galactomannan assay), imaging and histology (*in vivo* and *post mortem*), granulocyte colony-stimulating factor (G-CSF) administration, neutrophil transfusions, empirical/pre-emptive therapy, first or second line targeted therapy, oral antifungal maintenance therapy, and outcome.

"Empirical" therapy was started in patients with clinical signs and symptoms of infection, without any pathogen identified or any radiological sign. "Pre-emptive" treatment was initiated in patients with persistent fever and imaging-documented pneumonia or acute sinusitis. In patients with compatible radiological signs and microbiological tests allowing identification of the pathogen and in those with histopathological evidence of an aspergillosis, "targeted" therapy was initiated.

Additional information included in the questionnaire were: dates of symptom onset, diagnosis, start of antifungal therapy (empirical, pre-emptive, targeted), and death, with attending physicians and/or pathologists defining causes of death as aspergillosis or 'other' with or without aspergillosis. Death was defined as occurring early (<6 weeks) or late (>6 weeks) after the diagnosis of invasive aspergillosis.[10] Each patient was followed-up for a minimum of 120 days.

Diagnostic work-ups, which were practically identical in participating centers, included: nasal, pharyngeal, and rectal swabs at the time of admission; blood cultures and chest X-rays at onset of fever; galactomannan assays twice a week, and computed tomography (CT) scan on the 4th to 7th day of fever. Additional examinations (e.g., abdominal ultrasound scan, sinus or brain CT, skin biopsy, bronchoalveolar lavage, fundus examination) were performed as required.

At the end of data collection all clinical, diagnostic and therapeutic data were reviewed by two independent, blinded physicians and the current European Organization for Research and Treatment of Cancer/Mycosis Study Group consensus criteria were thus applied to define invasive aspergillosis.[9]

Two end-points were used to assess outcome: (i) aspergillosis-AMR on day 120, according to criteria proposed by Wingard *et al.*;[10] death due to causes other than invasive aspergillosis were excluded from the survival analysis; and (ii) first-line antifungal therapy response rate after a minimum of 7 days of therapy. Failure was defined as no clinical improvement and/or change of first-line targeted drug, according to the clinicians' decision.

Statistical analysis

Univariate analysis was performed using the χ^2 test with the following independent variables: sex, age, year of observation, AML stage, site of infection, severity and duration of neutropenia, neutrophil recovery, antifungal prophylactic agent and route of administration (topical *versus* systemic), antifungal therapy, first and second line targeted therapy, microbiological data, etiological agent, radiological and histological data, G-CSF administration, neutrophil transfusions, maintenance antifungal therapy, outcome and participating center. Variables for which data sets were incomplete were not included. Multivariate analysis was performed using a logistic regression model in which goodness of fit was assessed with the Hosmer and Lemeshow test.[11] The model included only variables with a univariate *P* value of less than 0.25, applying the stepwise-with-backward-elimination method. Adjusted odds' ratios (OR) and 95% confidence intervals (CI) were calculated. *P* values less than 0.05 were considered statistically significant. The analyses were performed using SPSS software for Windows, version 13.0.

Two different end-points were defined for the univariate and multivariate analyses: outcome on day 120 and response to first-line antifungal therapy, respectively.

Results

During the study period (2004-2007), 152 cases of invasive aspergillosis in patients with AML were observed in 21 participating centers. Of these, 140 met the required criteria and were included in the present analysis. Twelve patients were excluded because they were in the terminal phase of AML (n=3), had a diagnosis of possible aspergillosis (n=2) or had undergone allogeneic hematopoietic stem cell transplantation (n=7).

The patients ranged from 14 to 79 years old (median, 57 years). The male-to-female ratio was 1.8:1. Of the 140 cases of invasive aspergillosis, 85 (60%) occurred during aplasia after first-line chemotherapy, 4 (3%) after consolidation in patients who had obtained complete remission and 51 (36%) after treatment for refractory or relapsed AML. The mean period between symptom onset and diagnosis of invasive aspergillosis was 12 days (range, 1-85).

The lung was the most commonly affected site (126/140; 90%). Six patients had disseminated invasive aspergillosis (≥3 sites involved). Severe neutropenia was

present at the onset of invasive aspergillosis in 130/140 patients (93%). Six patients (4%) became neutropenic after clinical evidence of invasive aspergillosis. Neutrophil count normalized in 105/136 evaluable patients (77%) (Table 1).

Cases of probable invasive aspergillosis predominated over the histologically proven cases (105 *versus* 35; 75% *versus* 25%). For two of the proven infections (1%), the diagnosis was formulated at autopsy. *Aspergillus* spp. subtypes were identified in 55/140 of the cases of invasive aspergillosis (39%), with *A. fumigatus* (56%) being the most common (Table 1).

Treatment

Antifungal prophylaxis was administered to 121/140 patients (86%). The systemic route was chosen in 101 patients (72%) for a mean of 20 days (range, 2-90). Itraconazole was given to 67% of cases, for a mean of 22 days. Fluconazole was prescribed for 33% for a mean of 16 days (Table 2).

Therapy was empirical in 87/140 patients (62%) and pre-emptive in 41 (29%). The remaining 12 patients received only targeted therapy (9%). The mean period between symptom onset and the start of empirical/pre-emptive treatment was 6 days (range, 1-19). The difference between times to treatment was not significant (symptoms to empirical therapy, 1-18 days, mean 5 days; symptoms to pre-emptive therapy 1-19 days, mean 6 days). Liposomal amphotericin B (L-AmB), caspofungin, and voriconazole were most frequently prescribed as empirical/pre-emptive treatment and in 81/121 patients (67%) the drug used empirically or pre-emptively was confirmed as the targeted therapy. Targeted antifungal therapy was administered to 136 patients. Combined therapy was given as first-line targeted therapy in 22/136 patients (16%). A sequential schedule was used in 16 of them (73%) (i.e. adding a second antifungal drug to pre-existing therapy). Various different drugs were combined, as shown in Table 2. Second-line rescue therapy was successful in 10/15 patients who received it.

To hasten neutrophil recovery, 93/140 patients (66%) received G-CSF; granulocyte transfusions were given to only two patients. Two patients underwent surgery in addition to chemotherapy. Oral antifungal maintenance therapy with voriconazole, itraconazole or posaconazole was given to 93/106 responders (88%) for a mean of 61 days (range, 4-250).

Outcome

The overall mortality rate on day 120 was 33% (47/140), with no significant inter-center differences. Death was due to invasive aspergillosis or occurred in its presence in 38 patients (AMR, 27%). The mean time to death was 35 days (range, 2-117 days) (Table 3). Most early deaths were due to invasive aspergillosis (20/140, 14%) or occurred in the presence of invasive aspergillosis (18/140, 13%), while other causes predominated for late deaths. The mean time to death due to invasive aspergillosis was 22 days (range, 3-58 days). The mean time in cases with invasive aspergillosis was 37 days (range, 2-117 days) and in those without invasive aspergillosis was 62 days (range, 15-110 days).

Univariate analysis showed that outcome was significantly influenced by AML stage as well as duration of, and recovery from, neutropenia (Tables 1 and 2). Indeed, among the patients with invasive aspergillosis, those with relapsed/resistant AML had a worse prognosis than those in remission (43% *versus* 19%, P=0.002). Neutropenia persisting for 10 or more days was associated with a two-fold increase in AMR (31% *versus* 15% among those with neutropenia recovery in less than 10 days, P= 0.05). The probability of AMR for patients who did not recover from neutropenia was 90% compared with 7% among those in whom neutropenia was overcome, P<0.001). G-CSF administration shortened the time to neutrophil recovery (15 *versus* 25 days) but did not affect AMR (Figure 1). Other parameters, such as disease extension or *Aspergillus* spp. did not influence outcome. The outcome of patients with "proven" or "probable" invasive aspergillosis was almost identical (AMR, 29% *versus* 27%) (Table 1). Multivariate analysis confirmed that recovery from neutropenia and AML stage were independent prognostic factors.

No antifungal drug conferred a clear survival advantage. Although combined therapy was not associated with better survival, combination therapy with L-AmB and caspofungin reduced AMR to 12.5%.

Table 1. Univariate analysis of 140 cases of invasive aspergillosis.

	N. of cases (%)	N. of deaths (AMR %)	*P* value
Year of observation			
2004	31 (22%)	7 (23%)	
2005	29 (21%)	12 (41%)	0.27
2006	56 (40%)	14 (25%)	
2007	24 (17%)	5 (21%)	
AML stage			
1st induction therapy	85 (61%)	16 (19%)	
Complete remission	4 (3%)	0	<0.001
Relapse/resistance	51 (36%)	22 (43%)	
Site of infection			
Lung	126 (90%)	32 (25%)	
Sinuses	6 (4%)	2 (33%)	0.38
Disseminated[1]	6 (4%)	2 (33%)	
Other[2]	2 (2%)	2 (100%)	
Certainty of diagnosis			
Proven	35 (25%)	10 (29%)	0.82
Probable	105 (75%)	28 (27%)	
Aspergillus subtype[3]			
A. flavus[4]	14 (25.5%)	4 (29%)	
A. fumigatus	31 (56%)	11 (35%)	0.71
A. niger	8 (14.5%)	2 (25%)	
A. terreus	2 (4%)	0	
Previous neutropenia			
Yes	130 (93%)	34 (26%)	0.34
No	10 (7%)	4 (40%)	
Severity of neutropenia			
Moderate	7 (5%)	2 (29%)	0.88
Severe	123 (95%)	32 (26%)	
Mean duration of neutropenia			
<10 days	40 (31%)	6 (15%)	0.05
≥10 days	90 (69%)	28 (31%)	
Recovery from neutropenia[5]			
Yes	105 (77%)	7 (7%)	<0.001
No	31 (23%)	28 (90%)	

*AMR: aspergillosis-attributable mortality rate. [1]≥3 involved sites [2]one bowel + one orbit [3]identified in 55/140 patients only; [4]three cases were due to multiple agents (*A.flavus + A.fumigatus*) [5]included six patients who became neutropenic after the onset of aspergillosis.*

Efficacy of anti-fungal therapy

Four patients died while receiving empirical treatment, leaving 136 patients evaluable for response to treatment. Efficacy was assessed by the success of first-line therapy only. Of the 136 evaluable patients, 93 (68%) had a good response. Univariate and multivariate analyses with good response as the end-point confirmed that AML stage and recovery from neutropenia were prognostic factors.

When comparing the drugs most frequently used for targeted therapy, the response rate ranged from 61% with caspofungin to 84% with voriconazole (Figure 2), perhaps because a higher percentage of patients recovered from neutropenia in the voriconazole group (Table 4). Despite this, no significant differences emerged in efficacy in either univariate (P=0.09) or multivariate (P=0.3) analysis.

Table 2. Univariate analysis of anti-fungal prophylaxis and therapy in 140 cases of invasive aspergillosis.

	N. of cases (%)	N. of deaths (AMR %)	*P* value
Antifungal prophylaxis			
Topical	20 (14%)	4 (20%)	0.55
Systemic	101 (72%)	30 (30%)	
None	19 (14%)	4 (21%)	
Systemic anti-*Aspergillus* prophylaxis			
Yes[1]	68 (67%)	23 (34%)	0.19
No	33 (33%)	7 (21%)	
Empirical or pre-emptive therapy			
Yes	128 (91%)	34 (26%)	0.61
No	12 (9%)	4 (33%)	
Empirical versus pre-emptive therapy[2]			
Empirical	87 (68%)	25 (28%)	0.61
Pre-emptive	41 (32%)	10 (24%)	
Empirical/pre-emptive drug[2]			
Caspofungin	27 (21%)	8 (30%)	
L-AmB	54 (42%)	12 (22%)	
Voriconazole	25 (20%)	6 (24%)	0.61
d-AmB	14 (11%)	6 (43%)	
Other[3]	8 (6%)	2 (25%)	
First line therapy [4]			
Same as empirical	84 (67%)	19 (23%)	0.46
Different	42 (33%)	12 (29%)	
Drug in first line target therapy [5]			
d-AmB	6 (5%)	1 (17%)	
L-AmB	37 (27%)	9 (24%)	
Caspofungin	28 (21%)	9 (32%)	
Voriconazole	38 (28%)	7 (18%)	
Posaconazole	2 (1%)	0	0.79
Other[6]	3 (2%)	3 (100%)	
Combined	22 (16%)	5 (23%)	
L-AmB + caspofungin	8	1 (12.5%)	
L-AmB + voriconazole	8	2 (25%)	
Caspofungin + voriconazole	6	2 (33%)	
G-CSF administration			
Yes	93 (66%)	24 (26%)	0.62
No	47 (34%)	14 (30%)	
Oral maintenance therapy[7]			
Yes	93 (88%)	5 (5%)	0.39
No	13 (12%)	0	
Oral antifungal drug[7]			
Voriconazole	72 (78%)	3 (4%)	
Itraconazole	18 (19%)	2 (11%)	0.46
Posaconazole	3 (3%)	0	

AMR: aspergillosis-attributable mortality rate; d-AmB: deoxycolate amphotericin B; L-AmB: liposomal amphotericin B; G-CSF: granulocyte colony-stimulating factor [1] all patients received itraconazole [2] performed in 128 patients only [3] itraconazole (n = 6 patients), posaconazole (n = 1), fluconazole (n = 1) [4] related to 126 patients only (patients treated with target therapy only and early deaths excluded) [5] performed in 136 patients only (4 early deaths) [6] amphotericin B in lipid complex (2 patients), itraconazole (n = 1) [7] patients who died while receiving i.v. treatment were excluded.

Discussion

Invasive aspergillosis is one of the most serious complications in patients with hematologic malignancies.[2,3] Since past studies frequently focused on groups of patients with marked diversity in risk, disease, disease stage, and type of transplant they were unable to provide clear conclusions about the impact of individual risk factors on outcome.[12-13] To avoid such confounding factors, the present analysis focused only on patients with AML who received standard chemotherapy and who had proven/probable invasive aspergillosis.

Our study confirms that the lung is the most frequent site of invasive aspergillosis, probably due to inhalation of spores playing a primary role in colonization. Most of our patients developed invasive aspergillosis after the first course of chemotherapy. In healthy subjects macrophages and polymorphonuclear leukocytes are effective defenses against the ubiquitous *Aspergillus*.[14] However, in patients with leukemia, the diseased white blood cells impair immune responses, facilating fungal colonization which becomes manifest with neutropenia, mucosal damage and immunosuppression due to induction chemotherapy.[15] Advances in the diagnosis of this infection (e.g. through the galactomannan assay or high resolution CT scanning) have increased the number of cases of *in vivo* proven inva-

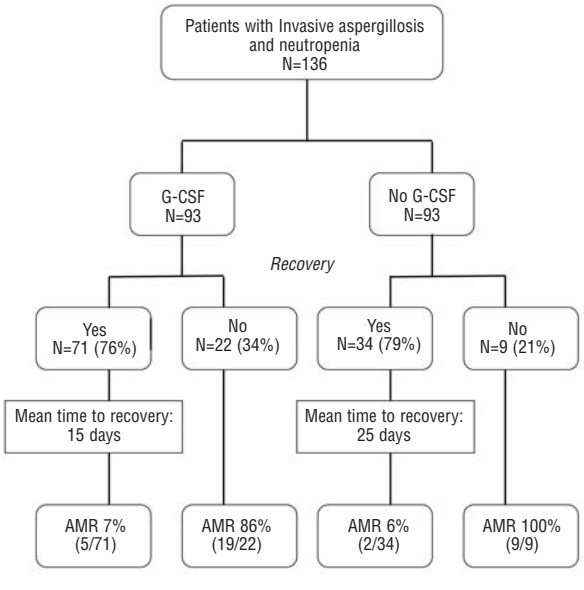

Figure 1. Granulocyte-colony stimulating factor (G-CSF) does not affect attributable mortality rate (AMR) or probability of neutrophil recovery, but does shorten recovery times.

sive aspergillosis compared to the number in previous SEIFEM studies,[2,8] while aggressive prescription of empirical/pre-emptive therapy has greatly reduced the rate of dissemination to very few cases. Consequently, unlike others, we are unable to comment on the prognostic impact of dissemination.[16-19]

Neutropenia emerged as a crucial variable in influencing outcome. Over the years clinicians have focused on managing neutropenia by administering G-CSF.[20] The present study showed that G-CSF shortened the period of neutropenia but had no effect on outcome because 34% of patients did not respond to it. Although another approach to neutropenia management is granulocyte transfusions, the use of this strategy in our series was limited to anecdotal cases, probably because of the scarce and divergent clinical evidence of its efficacy. To date, there have been no clinical trials balancing efficacy and adverse reactions in invasive aspergillosis.

The prognostic significance of certainty of diagnosis has not yet been clearly defined. In our analysis the outcome of patients with "proven" invasive aspergillosis was identical to that of patients with "probable" infection. In other recent studies, patients with proven invasive aspergillosis had a worse outcome,[21] or, in complete contrast, a better outcome than patients with probable invasive aspergillosis.[22]

Standard anti-fungal prophylaxis was based on fluconazole or itraconazole, given that posaconazole had not been approved for prophylaxis in Italy when the study was on-going. About two-thirds of the patients in our series developed invasive aspergillosis despite prior anti-*Aspergillus* prophylaxis. The use of systemic prophylaxis is still debated since its efficacy is uncertain and breakthrough infections with non-*fumigatus* strains are often feared.[23] In our series *A. fumigatus* was confirmed as the most frequent causative species of aspergillosis, independently of whether prophylaxis was systemic or not.

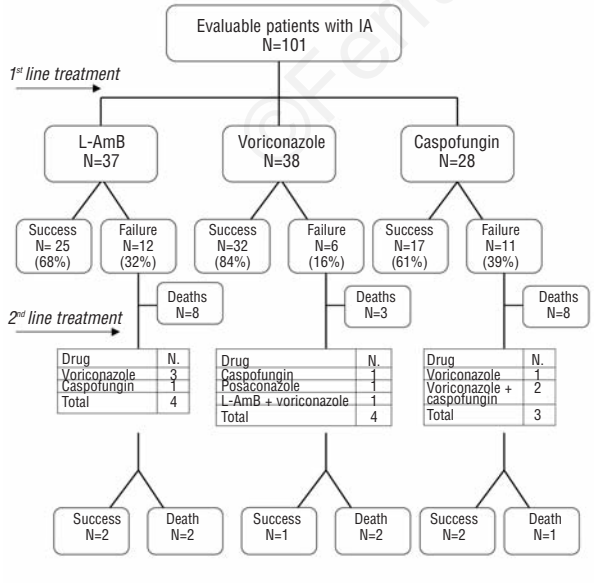

Figure 2. First- and second-line antifungal targeted therapy and responses (focused on liposomal amphotericin B, voriconazole and caspofungin) in invasive aspergillosis.

As demonstrated by univariate and multivariate analyses, empirical and pre-emptive therapies produced similar outcomes, probably because there was little or no delay in starting pre-emptive antifungal therapy. Indeed, targeted therapy was begun within a mean of 8 days. After the definitive diagnosis of invasive aspergillosis, the empirical/pre-emptive treatment was not changed in 81% of patients, because they were considered clinically stable.

In the present cohort the 27% AMR confirmed the downward trend in mortality rates. Most invasive aspergillosis-related deaths occurred within the first 6 weeks of the onset of symptoms, when patients were most vulnerable, whereas other causes predominated for later deaths.

In analyzing response to first-line targeted therapy, this study focused particularly on the three most frequently employed drugs (L-AmB, caspofungin and voriconazole). The response rate to standard- or high-dose L-AmB,

Table 3. Causes of death over time in 47 patients.

Cause of death	Number (%)	N. of deaths <6 weeks* (%)	N. of deaths >6 weeks* (%)	*P* value
Invasive aspergillosis	20/47 (43%)	18/33 (55%)	2/14 (14%)	0.02
Other with invasive aspergillosis	18/47 (38%)	12/33 (36%)	6/14 (43%)	n.s.
Other without invasive aspergillosis	9/47 (19%)	3/33 (9%)	6/14 (43%)	0.007

**According to Wingard* et al.[10]

Table 4. Comparison among the three groups of patients in terms of principal epidemiological characteristics.

	L-AmB N. of cases: 37	Caspofungin N. of cases: 28	Voriconazole N. of cases: 38	*P* value
AML phase				
Onset	21 (57%)	16 (57%)	25 (66%)	
Relapse/resistance	16 (43%)	10 (36%)	11 (29%)	0.67
Complete remission	0	2 (7%)	2 (5%)	
Site of infection				
Lung	34 (92%)	27 (96%)	34 (89%)	
Sinuses	2	0	3	0.57
Multiple[1]	1	1	0	
Other	0	0	1	
Previous neutropenia				
Yes	35 (95%)	26 (93%)	37 (97%)	0.68
No	2	2	1	
Mean duration of previous neutropenia				
<10 days	12 (32%)	11 (39%)	10 (26%)	0.53
≥10 days	25 (68%)	17 (61%)	28 (74%	
Recovery from neutropenia				
Yes	28 (77%)	19 (68%)	33 (87%)	0.06
No	8	9	5	
Success (response rate)	25 (68%)	17 (61%)	32 (84%)	0.09
Survivors at day 120 (Survival rate)	27 (73%)	19 (68%)	33 (87%)	0.15

AML: acute myeloid leukemia, G-CSF: granulocyte colony-stimulating factor. [1]≥3 involved sites.

caspofungin or voriconazole was already reported to range from 32% to 53%.[24-30] The percentage of success reported in those studies is lower than that observed in our registry, which ranged from 64% to 82%. This could be explained by the very restrictive criteria for response evaluation in pivotal trials. However, when we compared the AMR reported in the present study with that of the afore-mentioned trials, the results are comparable. These observations suggest that the efficacy of antifungal agents is probably higher in clinical practice than in randomized trials.

Interestingly, in our analysis no significant differences emerged between response rates to three commonly used drugs. The trend towards to better response in patients who received voriconazole might have depended on the larger number of patients who recovered from neutropenia. However, one should also consider that a higher number of cases could have made the differences statistically significant.

Antifungal treatments have often been combined in recent years in order to exploit potential synergies and a broader spectrum activity and to prevent resistance. However, in the absence of prospective studies in patients with hematologic disorders this type of expensive and potentially toxic therapy should be reserved for rescue therapy.[31] Surprisingly, 16% of our patients with invasive aspergillosis received two-drug, often sequential, combination therapy as first-line treatment. The most efficacious antifungal combination appeared to be L-AmB and caspofungin, which may merit further evaluation in randomized clinical trials.

In conclusion, advances in diagnosis and the availability of a larger antifungal armamentarium for the treatment of invasive aspergillosis in patients with hematologic malignancies have contributed to reducing fungus-related mortality.[8] Neutropenia and depressed immunity to fungi are still major risk factors[14,32,33] and, until recovery of these biological deficiencies is hastened, we recommend the current practice of aggressive empirical and pre-emptive therapy for invasive aspergillosis in patients with AML.

Authorship and Disclosures

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References

1. Caira M, Girmenia C, Fadda RM, Mitra ME, Picardi M, Van Lint MT, et al. Invasive fungal infections in patients with acute myeloid leukemia and in those submitted to allogeneic hemopoietic stem cell transplant: who is at highest risk? Eur J Haematol. 2008; 81(3):242-3.
2. 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.
3. Herbrecht R, Natarajan-Amé S, Letscher-Bru V, Canuet M. Invasive pulmonary aspergillosis. Semin Respir Crit Care Med. 2004; 25(2):191-202.
4. Baddley JW, Stroud TP, Salzman D, Pappas PG. Invasive mold infections in allogeneic bone marrow transplant recipients. Clin Infect Dis. 2001; 32(9):1319-24.
5. Klastersky J. Adverse effects of the humanized antibodies used as cancer therapeutics. Curr Opin Oncol. 2006;18(4):316-20.
6. Martin SI, Marty FM, Fiumara K, Treon SP, Gribben JG, Baden LR. Infectious complications associated with alemtuzumab use for lymphoproliferative disorders. Clin Infect Dis. 2006;43:16–24.
7. Pagano L, Girmenia C, Mele L, Ricci P, Tosti ME, Nosari A, et al. Infections caused by filamentous fungi in patients with hematologic malignancies. A report of 391 cases by GIMEMA Infection Program. GIMEMA Infection Program; Gruppo Italiano Malattie Ematologiche dell'Adulto. Haematologica. 2001;86(8):862-70.
8. Pagano L, Caira M, Picardi M, Candoni A, Melillo L, Fianchi L, et al. Invasive aspergillosis in patients with acute leukemia: update on morbidity and mortality–SEIFEM-C Report. Clin Infect Dis. 2007; 44(11):1524-5.
9. Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer; Mycoses Study Group of the National Institute of Allergy and Infectious Diseases. Clin Infect Dis. 2002; 34(1):7-14.
10. Wingard JR, Ribaud P, Schlamm HT, Herbrecht R. Changes in causes of death over time after treatment for invasive aspergillosis. Cancer 2008; 112(10):2309-12.
11. Hosmer DW, Lemeshow S. Applied Logistic Regression. New York: Wiley & Sons 1989.
12. Nivoix Y, Velten M, Letscher-Bru V, Moghaddam A, Natarajan-Amé S, Fohrer C, et al. Factors associated with overall and attributable mortality in invasive aspergillosis. Clin Infect Dis. 2008; 47(9):1176-84.
13. Kohno S. High mortality in invasive aspergillosis: what we need to know for determination of poor prognosis and next countermeasures. Clin Infect Dis. 2008; 47(9):1185-7.
14. Feldmesser M. Role of neutrophils in invasive aspergillosis. Infect Immun. 2006; 74(12):6514-6.
15. Pagano L, Fianchi L, Leone G. Fungal pneumonia due to molds in patients with hematological malignancies. J Chemother. 2006; 18(4):339-52.
16. Gottfredsson M, Steingrímsdóttir H. Disseminated invasive aspergillosis in a patient with acute leukaemia. Acta Biomed. 2006; 77 (Suppl 2):10-3
17. Keven K, Sengul S, Memikoglu O, Soypacaci Z, Ustuner E, Cakmak A, et al. Fatal outcome of disseminated invasive aspergillosis in kidney allograft recipients. Med Mycol. 2008; 46(7):713-7.
18. Patterson TF, Kirkpatrick WR, White M, Hiemenz JW, Wingard JR, Dupont B, et al. Invasive aspergillosis. Disease spectrum, treatment practices, and outcomes. I3 Aspergillus Study Group. Medicine. 2000; 79(4):250-60.
19. Zwitserloot AM, Warris A, van't Hek LG, van Die LE, Verweij PE, Mavinkurve-Groothuis AM. Disseminated aspergillosis in an adolescent with acute lymphoblastic leukemia. Pediatr Blood Cancer. 2008; 51(3):423-6.
20. Clark OA, Lyman GH, Castro AA, Clark LG, Djulbegovic B. Colony-stimulating factors for chemotherapy-induced febrile neutropenia: a meta-analysis of randomized controlled trials. J Clin Oncol. 2005; 23(18):4198-214.
21. Reuter S, Kern W, Zenz C, Kern P. Prognostic factors for invasive aspergillosis in patients with haematological malignancies. Scand J Infect Dis. 2009;41(6-7):483-90.
22. Denning DW, Ribaud P, Milpied N, Caillot D, Herbrecht R, Thiel E, et al. Efficacy and safety of voriconazole in the treatment of acute invasive aspergillosis. Clin Infect Dis. 2002; 34(5):563-71.
23. Verweij PE, Mellado E, Melchers WJ Multiple-triazole-resistant aspergillosis. N Engl J Med. 2007; 356(14):1481-3.
24. Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE, Oestmann JW, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. Invasive Fungal Infections Group of the European Organisation for Research and Treatment of Cancer and the Global Aspergillus Study Group. N Engl J Med. 2002; 347(6):408-15.
25. Candoni A, Mestroni R, Damiani D,

Tiribelli M, Michelutti A, Silvestri F, et al. Caspofungin as first line therapy of pulmonary invasive fungal infections in 32 immunocompromised patients with hematologic malignancies. Eur J Haematol. 2005;75(3):227-33.
26. Viscoli C, Herbrecht R, Akan H, et al. Caspofungin as a first-line therapy of invasive aspergillosis in haematological patients. A study of the EORTC Diseases Group. J Chemother 2007, 19(3s): 36 (Abstract n° O.12).
27. 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.
28. Maertens J, Raad I, Petrikkos G, Boogaerts M, Selleslag D, Petersen FB, et al. Efficacy and safety of caspofungin for treatment of invasive aspergillosis in patients refractory to or intolerant of conventional antifungal therapy. Caspofungin Salvage Aspergillosis Study Group. Clin Infect Dis. 2004; 39(11):1563-71.
29. Walsh TJ, Raad I, Patterson TF, Chandrasekar P, Donowitz GR, Graybill R, et al. Treatment of invasive aspergillosis with posaconazole in patients who are refractory to or intolerant of conventional therapy: an externally controlled trial. Clin Infect Dis. 2007;44(1):2-12.
30. Maertens J, Glasmacher A, Herbrecht R, Thiebaut A, Cordonnier C, Segal BH, et al. Multicenter, noncomparative study of caspofungin in combination with other antifungals as salvage therapy in adults with invasive aspergillosis. Caspofungin Combination Therapy Study Group. Cancer. 2006;107(12):2888-97.
31. Bow EJ. Considerations in the approach to invasive fungal infection in patients with haematological malignancies. Br J Haematol. 2008; 140(2):133-52.
32. Roilides E, Lyman CA, Filioti J, Akpogheneta O, Sein T, Lamaignere CG, et al. Amphotericin B formulations exert additive antifungal activity in combination with pulmonary alveolar macrophages and polymorphonuclear leukocytes against Aspergillus fumigatus. Antimicrob Agents Chemother. 2002;46(6):1974-6.
33. Simitsopoulou M, Roilides E, Maloukou A, Gil-Lamaignere C, Walsh TJ. Interaction of amphotericin B lipid formulations and triazoles with human polymorphonuclear leucocytes for antifungal activity against Zygomycetes. Mycoses. 2008;51(2):147-54.