Risks and outcome of fungal infection in neutropenic children with hematologic diseases

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In this retrospective study, we report the results of antifungal treatments (AFTs) in febrile neutropenic episodes in patients with acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) and aplastic anemia (AA) in our center. From January 2004 to December 2005, a total of 52 patients and 221 febrile neutropenic episodes were evaluated. AFT was started in 96 (43%) of the 221 episodes. Amphotericin B and fluconazole were used in 44 (46%) and 52 (54%) febrile neutropenic episodes, respectively. Microbiologically or histopathologically evident fungal infections were detected in 35 of 96 febrile neutropenic episodes. The mortality rate due to fungal infection was higher in patients with AA (7/8 patients) and AML (7/12 patients) than in ALL patients (1/32). Mortality for the whole group was 28%. When the mortality rate was compared between the two treatment groups (amphotericin B vs fluconazole), mortality was significantly higher in patients receiving amphotericin B [n=14 (93%) and n=1 (7%), respectively].

Key words: children, fungal infection, neutropenic fever.

With the use of intensive chemotherapy, invasive fungal infections have become more frequent in the neutropenic periods of childhood hematologic disorders, and they remain an important cause of mortality and morbidity in these patients in the recent years. The majority of reported fungal infections are caused by *Candida albicans*, but infections due to non-albicans Candida species and Aspergillus species have become increasingly important causes of infection in neutropenic patients in the last two decades[1-3]. Despite particular tests and imaging methods that allow earlier detection of invasive fungal infections, diagnosis remains difficult. Empirical treatment is considered to be the most important factor in therapy success in patients who are at risk for fungal infection. Prolonged neutropenia and hospitalization, increased use of antibiotics, hospitalization in hospitals under construction, and lack of reliable tests for detecting invasive fungal infections have enhanced the use of empirical antifungal treatment (AFT). The incidence of proven or probable yeast infections can reach up to 24% among patients with leukemia, and reported mortality from candidiasis ranges from 40% to 50%[4,5]. Mortality incidences of 70% or higher have been reported due to aspergillosis or zygomycosis in adult patients, which is smiliar to pediatric studies[6-10]. The incidence of fungal infections, prognostic factors and the rates of clinical success with different kinds of AFTs are not well known in the childhood period. In the presented retrospective study, we report the results of AFTs in febrile neutropenic episodes in patients with acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) and aplastic anemia (AA) over a period of two years in our center.

Material and Methods

The study period covered 24 months, from January 2004 to December 2005. In this period, 52 patients (32 ALL, 12 AML, 8 AA) with 221 febrile neutropenic episodes were

evaluated retrospectively. Patients ranged in age from 8 months to 204 months (median: 96 months). AFT was started in 96 (43%) of the total 221 febrile neutropenic episodes (Table I). The results of AFT were analyzed and compared according to the antifungal drug, indication (empiric, definitive or radiological), beginning of the AFT, and duration of fever and neutropenia.

Statistical Analysis

Statistical comparison was performed using the SPSS statistical program. The results are presented as mean ± standard deviation and/or median (range) of both the resistant and non-resistant cases. The statistical significance was determined using the Mann-Whitney U test for independent samples.

Table I. Characterization of Patients During Neutropenic Episodes

Median age, years (range)	96 months (8-204 months)
Underlying disorder	
ALL	32
AML	12
AA	8
Number of neutropenic episodes (n)	221
Number of AFT-administered neutropenic episodes (n)	96
Mean ANC (mm^3)	105±49
Duration of neutropenia (mean) (days)	29.3±15.6

ALL: Acute lymphoblastic leukemia. AML: Acute myeloid leukemia. AA: Aplastic anemia. AFT: Antifungal treatment. ANC: Absolute neutrophil count.

Of the antifungal drugs, fluconazole was administered at a dose of 5 mg/kg/day orally and/or intravenously (iv), and liposomal amphotericin B at a dose of 1-3 mg/kg/day iv. Both antifungal drugs were added to the systemic antibiotic therapy if the fever persisted after modification of antibacterial therapy for a minimum of four days; if fungal infection was suspected clinically or radiologically; or if fungal organism was demonstrated in a culture or biopsy specimen from the involved site. The choice of empirical AFT is mainly based on the suspected pathogens. However, expectation of deep and prolonged neutropenia and hospitalization, construction in the hospital, and/or history of previous neutropenic fever and of previously documented fungal infections were determining factors.

Blood cultures were drawn daily during fever. Pulmonary and sinus computerized tomography (CT) scans were performed based on clinical indications.

In this study, fungal disease was classified as microbiologically documented (demonstrating fungal organism in a culture or biopsy specimen), radiologically documented, or possible according to the clinical findings of the patients.

Results

Antifungal drugs were added to the therapy in 96 febrile neutropenic episodes. Of the 96 febrile neutropenic episodes, 63 (65.6%) were in ALL, 21 (21.9%) in AML and the remaining 12 (12.5%) in AA patients.

Initial absolute neutrophil counts (ANC) were not statistically different between ALL, AML and AA patients ($162/mm^3$, $84/mm^3$ and $69/mm^3$, respectively) ($p>0.05$). Duration of the neutropenic period was defined as the period (days) from the first day of ANC $\leq 0.5\times10^9$/L to the first day of ANC $>0.5\times 10^9$/L, and it was statistically longer in AA patients (47.3 days) compared to ALL (19.7 days) and AML (20.9 days) patients. Just as the duration of neutropenia was shortest in ALL patients (19.7 days), the duration of fever was shorter in the ALL group than in the AML and AA groups (mean 7.6 days vs 13.8 days and 15.7 days, respectively; $p<0.05$). Granulocyte colony-stimulating factor (G-CSF) was given in 43 (68%), 7 (33%) and 12 (100%) neutropenic episodes in ALL, AML and AA patients, respectively.

Amphotericin B and fluconazole were used in 44 (45.8%) and 52 (54.2%) febrile neutropenic episodes, respectively. Amphotericin B was

selected more frequently in AA patients (10/12 neutropenic episodes) than in ALL and AML patients (25/63 and 9/21, respectively; $p<0.05$). When amphotericin B usage was based on clinical indications, it was used in 14 (74%), 5 (26%) and 0 (0%) episodes in ALL, AML and AA patients, respectively; based on microbiological indications, these figures were 5 (45%), 1 (10%) and 5 (45%) episodes; and based on radiological indications, 6 (43%), 3 (21%) and 5 (36%) episodes, respectively (Table II). Amphotericin B was used empirically more often in cancer patients than AA patients ($p<0.05$). On the other hand, there was no difference between empirical or microbiologically evident-based fluconazole usage in the three groups of patients. The length of AFT was also not significantly different according to the three indications.

Microbiologically or histopathologically evident fungal infections were detected in 35 of 96 febrile neutropenic episodes. In the ALL group, *Aspergillus flavus* was detected in the paranasal sinus aspirates in 4 neutropenic episodes and *Aspergillus fumigatus* was detected in the nasal smear of 1 patient. In the AA group, *A. flavus* was detected in both blood and sinus aspirates in 1 neutropenic episode and *A. fumigatus* was positive in the sinus aspirate of 1 patient. No Aspergillus infection was detected in AML patients.

Candida species were documented in 28 febrile neutropenic episodes; 1 was *C. tropicalis* and the others were *C. albicans*. In the ALL group, Candida species were detected in the nasopharyangeal smear in 15 neutropenic episodes, in gastrointestinal samples in 4 episodes, in bronchoalveolar sample in 1 episode, and in vaginal drainage in 1 episode. Candida species were documented in the nasopharyngeal smear in 4 neutropenic episodes in patients with AML and in the urine in 3 episodes in patients with AA.

The diagnosis of fungal infection was based on radiology in 14 of 96 febrile neutropenic episodes (pulmonary CT scans [n=6], sinus CT scans [n=4], chest X-ray [n=3], and abdominal ultrasonography [n=1]).

The rate of mortality due to fungal infection was higher in patients with AA (7/8 patients) and AML (7/12 patients) than in ALL patients (1/32). Mortality in the whole group was

Table II. Results of AFT According to the Drug, Indications, Beginning of the AFT, and Duration of Fever and Neutropenia in ALL, AML and AA Patients

Primary diagnosis (96 febrile neutropenic episodes)	AFT		AFT indications					ANC (mean/mm^3)	Duration of neutropenia (mean/day)	Duration of AFT (mean/day)	Duration of fever (mean/day)	Exitus/ Alive
			Amphotericin B (n=44)			Fluconazole (n=52)						
	Amphotericin B (n=44)	Fluconazole (n=52)	Empirical (n=19)	Microbiological (n=11)	Radiological (n=14)	Empirical (n=28)	Microbiological (n=24)					
ALL (n=63)	25	38	14	5	6	17	21	162	19.7	19.3	7.6	1/31
AML (n=21)	9	12	5	1	3	9	3	84	20.9	21.1	13.8	7/5
AA (n=12)	10	2	0	5	5	2	0	69	47.3	25	15.7	7/1
p	<0.05			<0.05		≥0.05		≥0.05	<0.05	≥0.05	<0.05	<0.05

AFT: Antifungal treatment. ALL: Acute lymphoblastic leukemia. AML: Acute myeloid leukemia. AA: Aplastic anemia. ANC: Absolute neutrophil count.

determined as 28%. When the mortality rate was compared between the two treatment groups, it was significantly higher in patients receiving amphotericin B [n=14 (93%), n=1 (7%), respectively].

Discussion

During the past two decades, the prevalence of infections caused by non-albicans Candida species has increased, and in various surveys it was reported to account for more than 50% of episodes of fungemia[2,3,11,12]. The widespread use of azoles has been suggested as the main factor responsible for this challenging epidemiology. In this study, the documented infection rate was 36%, and *C. albicans* was found to be the most frequently documented fungi (77%). Seven of the 35 (20%) documented infections were Aspergillus, while non-albicans Candida was detected in 1 of the 35 (3%). Comparison of the two treatment groups revealed that the documented infection rate was higher (69%) in patients receiving fluconazole than in patients receiving amphotericin B (31%). Moreover, this study showed a significantly greater rate of documented invasive fungal disease in patients with AA (50%) who received amphotericin B compared with the other diagnoses (20% and 11% in ALL and AML, respectively). There is an apparent low incidence of aspergillosis in this study, which may be related to the difficulty in diagnosis. The incidence of aspergillosis is heightened in patients with AA, leading us to think that, as the period of neutropenia lengthens, the risk for fungal infections increases. The time between the onset of fungal infection and the inititiation of AFT is critical for recovery. The day of beginning AFT in patients with AA seemed later than in the other diagnoses clinically, but it was not statistically significant. On the other hand, depth of neutropenia influenced the risk of fungal infection, but there were no differences in ANCs of patients with ALL, AML and AA ($p \geq 0.05$).

Empirical treatment has been considered to be the most important factor in therapy success in patients who are at risk for fungal infection[13]. Beside its effects on reducing the mortality and morbidity and potential help to exclude diagnostic problems, empirical therapy would increase treatment-related costs and toxicity[14]. However, pre-emptive AFT is a new approach to the neutropenic patient with persistent fever, which is directed by a combination of radiological tests and non-culture based assays (high resolution CT [HRCT], Aspergillus polymerase chain reaction [PCR], galactomannan, etc.). This treatment may allow us to treat only high-risk patients and may decrease adverse reactions due to AFT. Therefore, preemptive strategies for invasive aspergillosis seem to be feasible and a more targeted approach[15,16]. Moreover, it was very recently shown that preemptive treatment increased the incidence of invasive fungal infection without increasing mortality and decreased the cost of AFT when compared with empirical treatment[17]. In this study, fluconazole and amphotericin B were given in 29% and 20% of febrile neutropenic episodes, respectively, as an empirical treatment. No renal toxicity due to liposomal amphotericin B or hepatotoxicity related with fluconazole was observed.

It was reported that G-CSF reduced the rate of febrile neutropenia, decreased hospitalization duration and reduced the documented infection rate, but that it did not affect infection-related mortality[18]. In our study, 68% of ALL patients received G-CSF, and we think that it diminished the duration of neutropenia and fever in this group. However, in the AA patients who received G-CSF, no reduction in duration of neutropenia, fever or mortality was observed, suggesting that G-CSF was ineffective in these patients due to the extent of defects in bone marrow and the microenvironment.

The mean mortality rate of invasive aspergillosis has been reported to be approximately 90% in allogeneic bone marrow transplant recipients and 50-70% in patients with leukemia[7-10,12,19-22]. In this study, 15 of 52 (28%) patients died, and fungal infection-related mortality rate was found to be 28% in the whole group that was receiving AFT. The efficacy of the AFT, as based on the prevention of fungal infection-related mortality, was significantly higher in patients receiving amphotericin B. Fluconazole was mostly given in patients with microbiologically documented infections; however, in the group of patients receiving amphotericin B, invasive fungal infection rate was higher and there was great diffuculty in documenting these fungal infections. Mortality rate was significantly higher in patients with AA (58%) than in those with ALL and AML. In this

group of patients, AFT should be initiated as soon as possible without radiologic and microbiologic evidence of infection.

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