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ORIGINAL ARTICLE

Clinical characteristics and outcome of invasive fungal infections in pediatric acute myeloid leukemia patients in a medical center in Taiwan

Gu-Lung Lin, Hsiu-Hao Chang*, Chun-Yi Lu, Chung-Ming Chen, Meng-Yao Lu, Ping-Ing Lee, Shiann-Tarng Jou, Yung-Li Yang, Li-Min Huang, Luan-Yin Chang*

Department of Pediatrics, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan

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KEYWORDS
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Abstract *Background:* Invasive fungal infection (IFI) causes significant morbidity and mortality in patients with hematological malignancies, especially those with acute myeloid leukemia (AML), recurrent acute leukemia, high-risk acute lymphoblastic leukemia, and after allogeneic hematopoietic stem cell transplantation. The study aimed to investigate the clinical characteristics and outcome of IFIs in pediatric AML patients in a medical center in Taiwan.
Methods: We performed retrospective chart reviews. We enrolled pediatric AML patients who were admitted to National Taiwan University Hospital between January 2005 and December 2014. IFI was defined according to the European Organization for Research and Treatment of Cancer/Mycosis Study Group 2008 consensus criteria.
Results: In total, 78 patients were included for analysis. Twenty two episodes of IFIs were identified in 16 patients. The incidence for IFIs was 20.5% (16/78), and no specific trend of increase or decrease was observed through the study period ($p = 0.374$). *Candida* species caused the majority (59.1%) of IFIs. Prolonged neutropenia and elevated alanine aminotransferase and creatinine values were factors associated with IFIs ($p < 0.001$, $p < 0.001$, and $p = 0.001$, respectively). Patients with endotracheal intubation or inotropes usage had a higher probability of developing IFIs ($p < 0.001$ and $p = 0.001$, respectively). The overall mortality of IFIs was 53% (8/15) over 10 years, and patients with pulmonary aspergillosis had the highest mortality (80%).

* Corresponding authors. Number 8, Chung-Shan South Road, Taipei 10041, Taiwan.
E-mail addresses: changhh2001@ntu.edu.tw (H.-H. Chang), lychang@ntu.edu.tw (L.-Y. Chang).

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Conclusion: IFIs continue to pose significant morbidity and mortality in pediatric AML patients, and patients with other hematology-oncology cancers. Recognition of factors associated with IFIs may help us early identify IFIs and promptly initiate antifungal therapy.

Introduction

Invasive fungal infection (IFI) causes significant morbidity and mortality in both adult and pediatric cancer patients. Patients with acute myeloid leukemia (AML), recurrent acute leukemia, high-risk acute lymphoblastic leukemia, and after allogeneic hematopoietic stem cell transplantation (HSCT) are especially at a high risk (incidence of about 10% or higher) for developing IFIs.[1]

The introduction of more intensive and new chemotherapeutic agents has been accompanied by stronger immunosuppression and consequently increased the rate of IFIs. Nonculture-based lab techniques such as β-glucan and galactomannan assays, lateral-flow device, and fungus polymerase chain reaction (PCR) have been developed; however, they still have some limitations and are not all standardized. Despite the advances in nonculture-based techniques and antifungal regimens, timely diagnosis and management of IFI are still challenging.[2,3]

The findings of pulmonary aspergillosis in computed tomography imaging are often less typical in children (particularly those younger than 5 years) than those in adults. Nodules or fluffy masses, mass-like lesions, or even nontypical pulmonary infiltrates may be indicative of pulmonary aspergillosis.[1] This also makes early diagnosis of pulmonary aspergillosis more difficult.

There are many studies investigating the characteristics of IFIs in patients with hematological malignancies, but only few focusing on the pediatric group. Local epidemiology of IFIs is of great importance for developing antifungal strategies to treat different at-risk populations and for preventing IFIs.[4] Reviewing the literature, there are few data characterizing IFIs in patients with hematological malignancies in Taiwan. Lai et al[5] reported that the incidence of IFI was 35.4% in children with persistent febrile neutropenia (fever for over 96 hours) from January 1, 1999 to December 31, 1999. Liu et al[6] demonstrated that the incidence of proven and probable IFIs was 7.4% in adult patients undergoing allogeneic HSCT between 2002 and 2013. Yang et al[7] reported that the incidence of proven and probable invasive mold diseases was 17.9% in AML patients undergoing first remission-induction chemotherapy during 2010–2014. Wang et al[8] found that the IFI incidence per 1000 admitted leukemic children increased from 5.8 in 1997 to 25.9 in 2002. None of them focused on IFIs in pediatric AML patients. Therefore, the study aimed to investigate the clinical characteristics and outcome of IFIs in pediatric AML patients in a tertiary medical center in northern Taiwan.

Methods

Patients

A retrospective study was conducted. We enrolled all pediatric patients (aged $<$ 18 years) who were diagnosed with AML after 2005 and admitted to the pediatric hematology-oncology ward at National Taiwan University Hospital between January 1, 2005 and December 31, 2014. The hospitalizations for chemotherapy and treatment of infectious diseases were included for analysis, and those for other purposes, such as bone marrow examination, were excluded. The treatment courses during and after HSCT were also excluded. Each patient had been followed since he/she was admitted to our hospital due to AML for the first time until he/she received HSCT, was 18 years old, lost to follow-up, died, or end of follow-up (December 31, 2014).

In total, 87 patients were enrolled, of which 9 were excluded because 6 of them underwent HSCT and 3 of them did not receive chemotherapy. Therefore, 78 patients were included for analysis. There were 480 hospitalizations and 496 courses of chemotherapy in the 78 patients. All the medical records were reviewed individually and thoroughly. We gathered detailed information of IFIs. We recorded the state of leukemia, chemotherapy protocol and agent, and duration and severity of neutropenia (if any) in each course of chemotherapy. We also collected endotracheal intubation status, inotropes usage, and the peak values of alanine transaminase (ALT) and creatinine of each patient during each hospitalization and used them to represent the patient's respiratory, cardiovascular, hepatic, and renal functions, respectively.

We classified the data into two groups, IFI and No-IFI groups. IFI group included the patients with IFIs and the hospitalizations and courses of chemotherapy when IFIs were identified, whereas No-IFI group included those patients without IFIs and those IFI-free hospitalizations and courses of chemotherapy. Physicians made treatment strategies for patients with AML, according to the protocols proposed by Taiwan Pediatric Oncology Group. When patients had neutropenic fever, we gave them broad-spectrum antibiotics, such as ticarcillin/clavulanate, piperacillin/tazobactam, or cefepime, plus gentamicin or amikacin. Meropenem, vancomycin, or fluoroquinolone was used in specific condition. Antifungal prophylaxis was not routinely administered during the study period. Only four (5%) patients had antifungal prophylaxis (1 with voriconazole and 3 with fluconazole). The study was approved by the hospital review board.

Definitions

IFIs are those infections where fungi have invaded deep into the tissues and have caused systemic symptoms and prolonged illness.[9] We used the consensus criteria of European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) proposed in 2008 to define IFIs and to classify them into three categories—proven, probable, and possible.[10] Proven IFI requires that a fungus be detected by culture or pathohistological analysis of blood or a sample obtained from a sterile site. Probable IFI requires lesions on imaging indicative of fungal infection and mycological evidence, which encompasses not only culture and pathohistological analysis of a sample taken from a nonsterile site but also indirect tests, such as galactomannan or β-glucan assays. Possible IFI only requires lesions on imaging indicative of fungal infection without mycological evidence. According to EORTC/MSG criteria, IFIs are classified into fungemia, pulmonary aspergillosis, sinonasal infection, and disseminated candidiasis (retinal exudates or small, target-like abscesses in internal organs, such as liver, spleen, or kidney).

The laboratory diagnostic techniques in National Taiwan University Hospital comprised traditional culture, microscopic examination, histopathology, and galactomannan assay. β-Glucan assay, lateral-flow device, and fungus PCR were not available.

Neutropenia was defined as a neutrophil count $< 500/\mu L$. The duration of neutropenia in each course of chemotherapy was collected. If another course of chemotherapy was started before neutropenia recovered, the duration of neutropenia would include more than one course of chemotherapy. In the situation, we only counted it once. In patients with refractory diseases or severe bone marrow suppression, neutropenia might not recover before they died. Then, we assumed the date of death to be the date of recovery of neutropenia. Consequently, there were 470 data of duration of neutropenia in 496 courses of chemotherapy.

The patient's age was defined as the age at the first diagnosis of AML. Patients who died within 1 month after the diagnosis of IFIs were considered to have died of IFI. The IFI-attributable mortality was determined by subtracting the crude mortality of the patients without IFIs from the crude mortality of the patients with IFIs.[11]

Statistics

Differences of categorical variables between IFI and No-IFI groups were evaluated using Fisher's exact or Chi-square tests. We also used the Chi-square test for linear trend of incidence and mortality. Mann—Whitney U test was used to determine the differences of continuous variables between the two groups. Survival analysis was made by Kaplan—Meier survival curve and the log-rank test. A p value < 0.05 was considered to be statistically significant.

Results

Incidence

Over the 10-year study period, 78 (46 male and 32 female) patients were included for the analysis. The study cohort was followed for a median of 1.6 years (range, 0—9.7 years) and contributed 213.3 person-years. The median age of the cohort was 9.9 years (range, 0.1—17.7 years). The French-American-British (FAB) classification of AML and demography of the cohort are listed in Table 1.[12] AML with maturation (M2) was the most frequent subtype (33.3%). The FAB subtype of AML of each patient had no association with the probability to develop IFI ($p = 0.357$).

In total, 22 episodes of IFIs were identified in 16 patients during the study period (Table 2). Five patients had multiple IFIs over times, three had candidemia, followed by disseminated candidiasis, and one had disseminated candidiasis at different sites and in different periods. The only one with three episodes of IFIs had two sinonasal infections with an interval of more than 2 years and one hepatic candidiasis.

The overall incidence of IFIs, including proven, probable, and possible IFIs, in pediatric AML patients was 20.5% (16/78) or 103 cases per 1000 person-years. If we only considered proven and probable IFIs, the incidence of IFIs was 11.5% (9/78) or 47 cases per 1000 person-years. The incidences of invasive yeast infections and invasive mold infections were 12.8% (10/78) and 9% (7/78), respectively.

The biennial incidence of IFIs is shown in Figure 1. No specific trend of increase or decrease was observed in the incidence of total IFIs through the study period ($p = 0.374$). The incidence of proven/probable IFIs seemed reduced after 2007, but it did not reach statistical significance ($p = 0.281$). The pediatric hematology-oncology ward was moved to a newly constructed medical building in November, 2008. The incidence of invasive aspergillosis decreased from 14.3% between 2005 and 2008 to 7% between 2009 and 2014, and it was not statistically significant either ($p = 0.430$).

Types of IFIs

Five proven IFIs were documented; four of them were fungemia, and one of them was hepatosplenic candidiasis (Patient 9, Figure 2), diagnosed by microscopic detection of hyphae in aspirated liver abscess. Two of them were caused by *Candida tropicalis*, one by *C. albicans*, and one by *Trichosporon asahii*. Five probable IFIs were recorded; two of them were pulmonary aspergillosis (diagnosed by CT plus galactomannan assay, Patient 6, Figure 3), and three were sinonasal infections. Twelve possible IFIs were recorded. Nine of them were disseminated candidiasis, and three were pulmonary aspergillosis.

Nine patients were diagnosed with disseminated candidiasis, and the incidence was 11.5% (9/78). Spleen was the most common site of involvement (37.5%), followed by liver (31.3%) and kidney (18.8%). Four patients (44.4%) had multiple foci (Table 2). CT and/or magnetic resonance imaging were utilized in all nine patients, and the detection rate was 100%. Abdominal ultrasonography was arranged in six (66.7%) of the nine patients, and four (66.7%) revealed the lesions. Gallium 67 scintigraphy was performed in three (33.3%) of the nine patients, and it detected all the lesions.

During the study period, *Candida* species (59.1%) caused the majority of IFIs, followed by *Aspergillus* species (36.4%) and *T. asahii* (4.5%). However, *Aspergillus* species accounted for 50% of causative pathogens if we excluded

Table 1 Demography and characteristics of IFI and No-IFI groups in pediatric AML patients

Characteristics	IFI[a]	No-IFI[a]	Total	*p*
Age (y)	*N* = 16	*N* = 62	*N* = 78	
Median	12.4	9.0	9.8	0.102
Range	0.4–17.5	0.1–17.7	0.1–17.7	
Sex	*N* = 16	*N* = 62	*N* = 78	
Male	12	34	46	0.167
Female	4	28	32	
FAB classification	*N* = 16	*N* = 62	*N* = 78	
M0	0	1	1	0.357
M1	2	2	4	
M2	6	20[b]	26	
M3	0	6	6	
M4	3	8	11	
M4Eo	0	2	2	
M5	2	12	14	
M6	1	2	3	
M7	0	7	7	
Others[c]	2	2	4	
Duration of neutropenia (day)	*n* = 18	*n* = 452	*n* = 470	
Median	32	11	11	< 0.001
Range	2-65	0-98	0-98	
Alanine transaminase, U/L[d]	*n* = 19	*n* = 457	*n* = 476	
Median	160	56	57	< 0.001
Range	35–679	8–1801	8–1801	
Creatinine, mg/dL[e]	*n* = 19	*n* = 460	*n* = 479	
Median	1.00	0.60	0.60	0.001
Range	0.30–7.71	0.20–5.70	0.20–7.71	
Endotracheal intubation, no.[f]	*n* = 19	*n* = 458	*n* = 477	
Yes	5	13	18	< 0.001
No	14	445	459	
Inotropes usage, no.[f]	*n* = 19	*n* = 458	*n* = 477	
Yes	6	24	30	0.001
No	13	434	447	

[a] IFI group contained 16 patients (22 episodes of IFIs), 19 hospitalizations, and 18 data of duration of neutropenia, whereas No-IFI group contained 62 patients, 461 hospitalizations, and 452 data of duration of neutropenia. *N* denotes patient number and *n* denotes episode or hospitalization number.
[b] One patient was diagnosed with AML, M2 initially, but it changed to M5 when AML relapsed.
[c] Others included two patients with M6/7, one with secondary MDS, and one with MDS with AML change.
[d] The levels of alanine transaminase were not tested in four hospitalizations in No-IFI group.
[e] The levels of creatinine were not tested in one hospitalizations in No-IFI group.
[f] Information about conditions of endotracheal intubation and inotropes usage was incomplete in three hospitalization in No-IFI group.
AML = acute myeloid leukemia; FAB = French-American-British; IFI = invasive fungal infection; M4Eo = acute myelomonocytic leukemia with bone marrow eosinophilia; MDS = myelodysplastic syndromes.

possible IFIs and were followed by *Candida* species (40%) and *T. asahi* (10%). *C. tropicalis* was responsible for two patients with invasive candidiasis, and *C. albicans* for one. There was no identification of *Candida* at the species level in the remaining six patients with invasive candidiasis. Table 2 gives the detailed diagnosis, treatment, and outcome of the 22 episodes in the 16 patients with IFIs.

Associated factors and predisposing factors

IFI group consisted of 16 patients (22 episodes of IFIs), 19 hospitalizations (3 patients were diagnosed with 2 different IFIs in 1 hospitalization), and 18 data of duration of neutropenia (1 duration of neutropenia included 2 hospitalizations), whereas No-IFI group consisted of 62 patients, 461 hospitalizations, and 452 data of duration of neutropenia. Table 1 illustrates analysis of clinical factors in pediatric AML patients. Prolonged neutropenia and elevated ALT and creatinine values were factors associated with IFIs ($p < 0.001$, $p < 0.001$, and $p = 0.001$, respectively). Patients with endotracheal intubation or inotropes usage had a higher probability of developing IFIs ($p < 0.001$ and $p = 0.001$, respectively). There was a trend for older patients to develop IFIs ($p = 0.102$).

The neutrophil nadirs of almost all patients were 0 during each course of chemotherapy, but the durations of neutropenia were quite different in two groups. The durations of neutropenia in IFI group were significantly longer than those in No-IFI group (median duration, 32 days and 11 days, respectively, $p < 0.001$). On the date of identification

Table 2 Detailed diagnosis, treatment, and outcome of 22 episodes of IFIs in 16 pediatric AML patients

No.	Age/Sex	Diagnosis	Categories	Locations	Pathogen	First-line therapy (day)	Salvage or continuous therapy (day)	Oral therapy	Total therapy duration (day)	Outcome (day[a])
1	14.5/F	Disseminated candidiasis	Possible	Liver, spleen, kidney	Possible *Candida*	AMB-D (131)	—	—	—	Alive
	15.1/F	Disseminated candidiasis	Possible	Subphrenic area	Possible *Candida*	LAMB (92)	—	VRC	305	Alive
2	1.9/M	Disseminated candidiasis	Possible	Liver, spleen	Possible *Candida*	FLC (40)	AMB-D (129)	—	169	Died (174)
3	1.2/M	Disseminated candidiasis	Possible	Spleen	Possible *Candida*	AMB-D (2)	—	—	2	Loss to follow-up
4	10.7/M	Sinonasal infection	Probable	Sinus	*Aspergillus flavus*	AMB-D (9)	—	—	9	Alive
5	14.8/F	Sinonasal infection	Probable	Sinus	*Aspergillus* spp.	AMB-D (4)	VRC (46)	VRC	281	Died (960)
	17.1/F	Sinonasal infection	Probable	Sinus	*Aspergillus nidulans*	VRC (62)	LAMB (31)	—	—	Died (147)
	17.2/F	Disseminated candidiasis	Possible	Liver	Possible *Candida*	LAMB (31)	VRC (37)	VRC	145	Died (78)
6	7.2/M	Pulmonary aspergillosis	Probable	Lung	*Aspergillus* spp.	AMB-D (2)	—	—	2	Died (2)
7	17.2/M	Pulmonary aspergillosis	Possible	Lung	Possible *Aspergillus*	AMB-D (36)	—	FLC	155	Died (161)
8	16.2/M	Fungemia & disseminated candidiasis	Proven & Possible	Blood, kidney	*Candida tropicalis*	CAS (69)	VRC (47)	FLC	277	Alive
9	12.1/M	Disseminated candidiasis	Proven	Liver, spleen	*Candida* spp.	FLC (43)	—	FLC	359	Died (625)
10	1.9/M	Fungemia & disseminated candidiasis	Proven & Possible	Blood, spleen	*Candida albicans*	AMB-D (5)	FLC (60)	FLC	186	Alive
11	13.0/M	Fungemia & disseminated candidiasis	Proven & Possible	Blood, spleen, kidney, lung and pleura	*Candida tropicalis*	FLC (2)	CAS (26)	FLC	392	Alive
12	13.9/F	Pulmonary aspergillosis	Probable	Lung	*Aspergillus* spp.	FLC (3)	VRC (19)	—	22	Died (22)
13	17.9/F	Disseminated candidiasis	Possible	Liver	Possible *Candida*	CAS (33)	MFG (27)	FLC	430[b]	Alive
14	13.0/M	Fungemia	Proven	Blood	*Trichosporon asahii*	AMB-D (8)	VRC (58)	VRC	135	Died (138)
15	11.1/M	Pulmonary aspergillosis	Possible	Lung	Possible *Aspergillus*	VRC (13)	POS (6)	FLC	51	Alive
16	16.2/M	Pulmonary aspergillosis	Possible	Lung	Possible *Aspergillus*	VRC (18)	—	VRC	356	Died (361)

[a] Duration from diagnosis of IFI to death.
[b] Still in use as of January 20, 2016.
AML = acute myeloid leukemia; IFI = invasive fungal infection; AMB-D = amphotericin B deoxycholate, CAS = caspofungin; F = female; FLC = fluconazole; LAMB = liposomal amphotericin B; M = male; MFG = micafungin; POS = posaconazole; VRC = voriconazole.

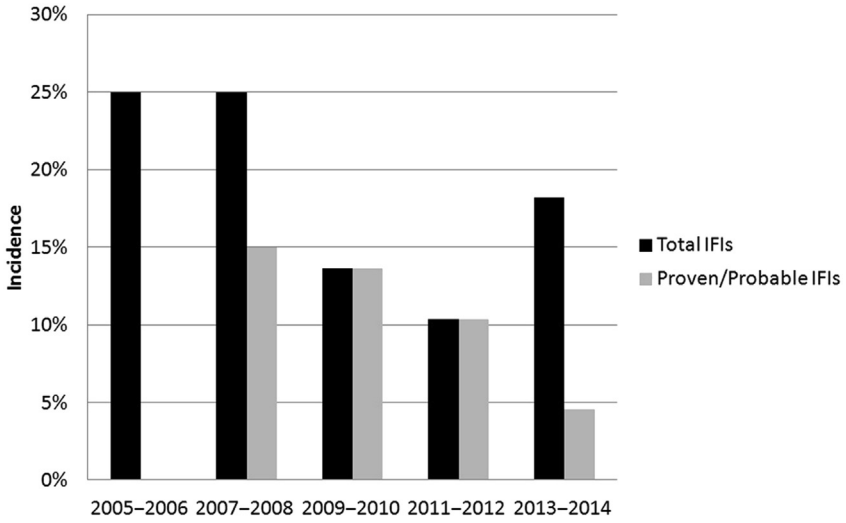

Figure 1. The biennial incidences of total and proven/probable invasive fungal infections (IFIs). No proven or probable IFIs were diagnosed during 2005—2006. No specific trend of increase or decrease was observed in the incidence of total IFIs through the study period ($p = 0.374$). The incidence of proven/probable IFIs seemed reduced after 2007, but it did not reach statistical significance ($p = 0.281$).

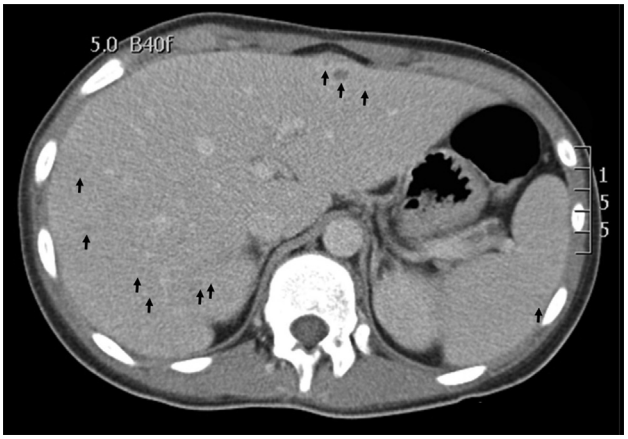

Figure 2. Computed tomography scan of the abdomen (Patient 9). It revealed multiple nodules with decreased enhancement at the liver and spleen (arrows).

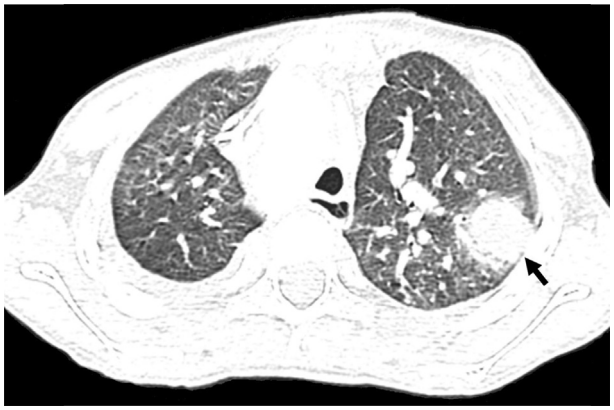

Figure 3. Computed tomography scan of the chest (Patient 6). It revealed diffusely patchy, air-space lesions at bilateral lungs, and fluid accumulation within the right pleural cavity. A nodular lesion (about 2 cm) with irregular margin was at the left upper posterior lung field (arrow).

of IFIs, the neutrophil counts were less than 100/μL in 50% of patients.

More than half (59.1%) of IFIs were diagnosed in patients with a large leukemic cell burden, i.e., newly diagnosed AML or resistant diseases (Table 3). The proportion rose to 80% if we excluded possible IFIs; 40% (4/10) of patients were with newly diagnosed AML and 40% (4/10) with resistant diseases. Patients who underwent chemotherapy of high intensity, namely induction or salvage chemotherapy, accounted for 54.5% of those with IFIs.

None of the patients with IFIs received systemic antifungal prophylaxis. Patients received systemic broad-spectrum antibiotics of more than 2 weeks before acquiring IFIs in 17 (77.3%) of 22 episodes of IFIs. Furthermore, 5 (31.3%) of 16 patients with IFIs had ever stayed in ICU within 1 month before developing IFIs, 9 (56.3%) of 16 patients required total parenteral nutrition (TPN) before having IFIs, and 6 (66.7%) of 9 patients required TPN before contracting invasive candidiasis. On the day of identification of IFI, 59.1% of patients had fever. The median duration from chemotherapy to the diagnosis of IFI was 30.5 days, ranging from 5 days to 115 days (Table 3).

Treatment and outcome

The antifungal agents used to treat IFIs are listed in Table 2. Amphotericin B deoxycholate (47.1%) was the most common antifungal agent we used initially to treat IFIs, followed by fluconazole (23.5%). The most frequent continuation or salvage antifungal agent was voriconazole (41.7%), followed by liposomal amphotericin B (16.7%), amphotericin B deoxycholate (8.3%), fluconazole (8.3%), posaconazole (8.3%), caspofungin (8.3%), and micafungin (8.3%). The oral maintenance agents were fluconazole

Table 3 Clinical features of 22 episodes of IFIs

Disease status	
Newly diagnosed	7 (31.8)
Complete remission	7 (31.8)
Partial remission	2 (9.1)
Resistant disease[a]	6 (27.3)
Chemotherapy	
Induction[b]	8 (36.4)
Post remission[c]	8 (36.4)
Salvage[d]	4 (18.2)
Palliative[e]	2 (9.1)
Type of IFI	
Disseminated candidiasis	10 (45.5)
Pulmonary aspergillosis	5 (22.7)
Fungemia	4 (18.2)
Sinonasal infection	3 (13.6)
Neutrophil counts on the day of diagnosis, per μL	
Median	137.5
Range	0–9090
< 100/μL	11 (50)
< 500/μL	12 (54.5)
Duration of neutropenia (day)[f]	
Median	32
Range	2–65
Patients with persistent neutropenia	2 (12.5)
Duration from chemotherapy to diagnosis of IFI (day)	
Median	30.5
Range	5–115
Duration from neutropenia to diagnosis of IFI (day)	
Median	25.5
Range	−5–120
Duration of systemic antibiotics before diagnosis of IFI (day)	
Median	22.5
Range	−7–82
> 2 week	17 (77.3)

[a] Resistant diseases include refractory or relapsed AML.
[b] The regimens of induction chemotherapy were idarubicin (9 mg/m^2/day for 3 days) and cytarabine (100 mg/m^2/day for 7 days) [I3A7].
[c] There were different combinations of regimens in post remission chemotherapy. Three of them were high-dose cytarabine (1 g/m^2 every 12 hours for 8 or 10 doses) plus etoposide (100 mg/m^2/day for 5 days). Three of them were high-dose cytarabine plus mitoxantrone (8 or 10 mg/m^2/day for 4 days). Two of them were cytarabine (100 or 200 mg/m^2/day for 5 or 7 days) plus idarubicin (9 mg/m^2/day for 1 or 3 days). The dosing and duration were based on protocols.
[d] Four patients underwent salvage chemotherapy. One patient received fludarabine (30 mg/m^2/day for 5 days), high-dose cytarabine (2 g/m^2/day for 5 days), and granulocyte colony-stimulating factor (G-CSF) [FLAG]. Another received FLAG plus idarubicin (10 mg/m^2/day for 3 days). Another received FLAG plus gemtuzumab (6 mg/m^2/day for 2 days). The other received high-dose cytarabine (3 g/m^2/day for 2 days).
[e] The regimen of palliative chemotherapy was low-dose cytarabine (20 mg/m^2/day for 5 days).
[f] Excluding those with persistent neutropenia.
AML = acute myeloid leukemia, IFI = invasive fungal infection. Data are presented as *n* (%).

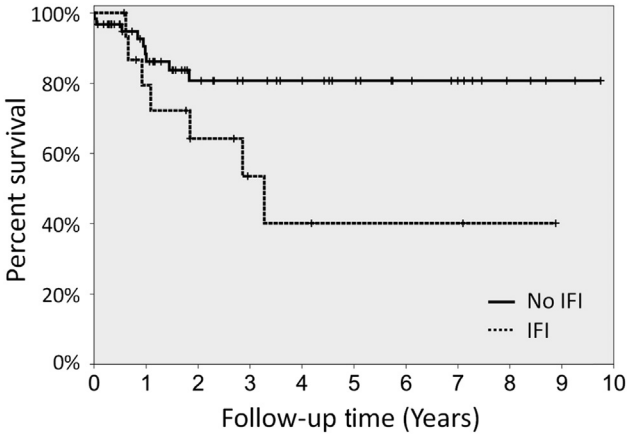

Figure 4. Overall survival after the diagnosis of acute myeloid leukemia with and without invasive fungal infection ($p = 0.033$).

(58.3%) and voriconazole (41.7%). Excluding those who did not complete antifungal therapy before they died, the median duration of antifungal therapy was 281 days (range, 9–430 days). Excluding the one who lost to follow-up, the duration of antifungal therapy had no association with the outcome ($p = 0.091$).

The overall survival was significantly worse in patients with IFIs than in those without IFIs ($p = 0.033$, log rank test, Figure 4). In 16 patients with IFIs, 1 lost to follow-up and 8 died during their treatment course (Table 2). Excluding the one lost to follow-up, the overall mortality of IFIs was 53% (8/15) over 10 years. Two of eight patients were considered to have died of IFIs (Patients 6 and 12). Both the patients were diagnosed with pulmonary aspergillosis, and the durations from the diagnosis of IFI to death were 2 days and 22 days, respectively. Among the other 62 patients without IFIs, 4 lost to follow-up and were excluded from analysis of mortality. Mortality of patients without IFIs was 25.9% (15/58). Thus, the IFI-attributable mortality was 27.4%.

Mortality of patients with invasive yeast infections (excluding the 1 who lost to follow-up) and invasive mold infections were 44% (4/9) and 71% (5/7), respectively. Patients with pulmonary aspergillosis tended to have a higher mortality than those with sinonasal infection, disseminated candidiasis, and fungemia (80%, 50%, 37.5% and 25%, respectively). Regarding different periods, mortality of patients with IFIs were 50% during 2005–2009 and 50% during 2010–2014 ($p = 1.000$). The IFI-attributable mortality was 29% during 2005–2009 and 27% during 2010–2014.

Discussion

In the current study, the overall incidences of all IFIs and proven/probable IFIs in pediatric AML patients were 20.5% and 11.5%, respectively. The incidence might be underestimated due to insufficient sensitivities of diagnostic techniques. Kami et al[13] reported that the sensitivities of blood cultures for diagnosis of invasive candidiasis and aspergillosis were only 21.3% and 1.1%, respectively, when compared with autopsy-proven infections. There were some previous studies about the incidence of IFIs in pediatric AML patients. Mor et al[14] reported that the incidences

of all IFIs and proven/probable IFIs were 39.4% (26/66) and 13.6% (9/66), respectively, during 1998—2006. Kobayashi et al[15] reported that the incidence of IFIs was 21.6% (11/51) during 1997—2006. Lehrnbecher et al[16,17] found that the incidence of proven/probable IFIs was 4.9%. Rosen et al[18] found that the incidence of all IFIs was 8.5% during 1991—2001. However, study designs, definitions of IFI, patient populations, and treatment protocols were different among studies; therefore, we could not draw direct comparisons between them.

Wang et al[8] reported that cutaneous lesions were found in 69% of children with leukemia and disseminated fungal infections during 1997—2002. In our current study, only 12.5% (2/16) of patients with IFIs had suspected cutaneous septic emboli. However, none of them received biopsy; therefore, there was no culture or pathohistological evidence. The lower rate of dermatologic manifestations of IFIs in our study might be due to less advanced infection and earlier treatment.

The incidence of disseminated candidiasis in pediatric AML patients was 11.5% in the current study. In our previous study during 1999—2009, the incidence of disseminated candidiasis was 5.4% (5/92), and the difference was not significant ($p = 0.171$).[19]

Mold infection was solely caused by *Aspergillus* species in our current study. It might be attributed to the intrinsic low incidence of pathogens other than *Aspergillus* species and the difficulties in identification of culprit pathogens. It might be also related to no antifungal prophylaxis in most of our patients.[20]

Predisposing factors for IFIs included large leukemic cell burdens, chemotherapy of high-intensity, TPN infusion, as well as fever. Prolonged neutropenia is a known risk factor for IFIs, and we had the same finding.[21] In our study, we found that there was a trend for older patients to get IFIs. Some studies demonstrated that older age was a risk factor for IFIs in pediatric patients with hematology-oncology disease or undergoing HSCT.[15,18,22] Host colonization by environmental fungi is important for and precedes invasive fungal infections. The chance of colonization is increasing with patient's age, and therefore, older patients tend to develop IFIs.[23]

Neofytos et al[24] identified baseline organ dysfunction (defined by creatinine and transaminase enzymes) as a significant risk factor for IFIs. Although our study used the peak values rather than the baseline data, we still found that patients with elevated ALT and creatinine levels had a higher probability of developing IFIs. Endotracheal intubation and inotropes usage were also factors associated with IFIs. Thus, patients with impaired respiratory, cardiovascular, hepatic, or renal functions were associated with development of IFIs.

Crude mortality was 53.3% in pediatric AML patients with IFIs. Mortality was higher in patients with invasive mold infections (71.4%), especially those with pulmonary aspergillosis (80%). Previous studies reported mortalities of 21.3—63.6%, but most of them included other cancer patients, not only AML patients.[14—18,25]

Our study has several limitations. First, it has the intrinsic limitations of a retrospective study. Second, it is a single-center study, and only focused on IFIs in AML patients. Thus, we cannot extend the results to general population. Third, our sample size was relatively small; therefore, the generated data may be somewhat over- or underestimated.

In conclusion, IFIs continue to pose significant morbidity and mortality in pediatric patients with AML and even other patients with hematology-oncology cancers. Recognition of factors associated with IFIs may help us early identify IFIs, promptly initiate antifungal therapy, and further improve the patient's outcome.

Conflicts of interest

All contributing authors have no conflicts of interest to declare.

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