Invasive fungal diseases in children with hematologic disorders

Hematolojik hastalıklı çocuklarda invazif fungal infeksiyonu

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Abstract

Objective: Fungal infection is a significant problem, causing of infective deaths of leukemic patients. The situation in developing countries is not well documented. The purpose of this study was characterizing IFD by analyzing data retrospectively to determine the incidence, predisposing factors, diagnostic methods, efficacy of treatment, and the outcome in pediatric patients with hematological disorders.

Materials and Methods: There were 160 children with leukemia (22 AML, 129 ALL) and 9 with aplastic anemia (AA). The diagnostic criteria for IFD were defined according to the EORTC/MSG, 2008. IFD was classified as proven or probable. Empiric antifungal treatment with L-AmB was commenced by day 5-7 of persistent fever. Patients with invasive aspergillosis (IA) who were refractory to primary treatment were commenced on voriconazole (VCZ). Salvage therapy as combination of VCZ and caspofungin was given to those with progressive infection.

Results: The incidence of IFD was found 23 (14.3%). 19 with leukemia (14 ALL, 5 AML) and 4 with aplastic anemia were diagnosed as IFD. IA was the dominant cause of infection (n=17) and the rest (n: 6) had candidiasis. Ten children had "proven" infection and 13 children were defined as "probable". The most frequent site of infection was lungs. In our series, the most frequently used diagnostic methods were clinical findings (100%) and radiologic methods (84%). The success rate of treatment for candidiasis and IA were found 60%, 71% respectively. IFD related death rate was found 30%.

Conclusion: IFD is still a major morbidity and mortality reason in children with hematologic disorders. However, the availability of new antifungal treatments and diagnostic tests will improve the survival rates in these children.

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Key words: Hematologic disorders, invasive fungal infection, children

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Özet

Amaç: Fungal infeksiyonlar lösemili hastalarda infeksiyonlardan ölümlerde önemli bir sorundur. Bu sorun gelişmekte olan ülkelerde iyi dökümente edilmemiştir. Bu çalışmanın amacı, geriye dönük olarak çocuk hastalarda görülen hematolojik hastalıklardaki invazif fungal hastalık (İFH) insidansını, predispozan faktörleri, tanı metodları, tedavi etkiliği ve sonuçlarını incelemektir.

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Yöntem ve Gereçler: Merkezimizde Ocak 2003 ve Aralık 2008 tarihleri arasında tedavi edilen 160 lösemili (22 AML, 129 ALL) ve 9 aplastik anemili olgu çalışmaya alındı. EORTC-MSG 2008 kriterlerine göre olgulara olası veya kesin İFH tanısı kondu. Antibiyotik tedavisine rağmen 5 günden uzun süren ateş yüksekliği saptanan olgulara ampirik antifungal tedavi olarak L-AmB başlandı. İnvasive aspergillozis (IA) saptanan olgularda tedavi voriconazole (VCZ) değiştirildi. Kurtarma tedavisinde VCZ ve kaspofungin kombinasyonu kullanıldı.
Bulgular: Çalışmamızda İFH %14,3 (n: 23) bulundu. 19 lösemili(14 ALL, 5 AML) ve 4 aplastik anemili olguda IFH tanısı kondu. IA en sık rastlanan enfeksiyon oldu (n: 17), diğer 6 olguda kandidiazis saptandı. On olguya kesin ve 13 olguya kuvvetli olası enfeksiyon tanısı kondu. En sık tutulan enfeksiyon bölgesi akciğerdi. Çalışmamızda, en sık kullanılan tanı yöntemleri; klinik bulgular (%100) ve radyolojik metodlar (%84) olarak saptandı. Tedavide başarı oranları kandidiasis ve IA'da %60 ve %71 bulundu. İFH ile ilişkili ölüm oranı %30 saptandı.
Sonuç: Sonuç olarak, İFH hala hematolojik hastalıklı olgularda ciddi mortalite morbitide sebebidir. Ancak yeni antifungal ilaçlar ve tanı yöntemleri ile yaşam oranları artmaktadır. *(Turk J Hematol 2009; 26: 190-6)*
Anahtar kelimeler: Hematolojik hastalıklar, çocuk, invazif mantar infeksiyonu

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Introduction

Invasive fungal diseases (IFD) are a serious cause of morbidity and mortality in immunocompromised children, especially those with hematological malignancies and bone marrow failure [1-3]. The incidence has dramatically increased in recent decades [4]. Various factors account for this increased frequency. Of these factors, dose-intensive regimens causing profound neutropenia and mucosal barrier damage, widespread use of broad spectrum antibiotics are important. Moreover, the placements of indwelling central venous catheters, hematopoietic stem cell transplantation (HSCT) render these children more vulnerable to fungal infections [5]. Although, there has been a great improvement in the survival rates of children with leukemia and other hematological disorders, IFD still remains a life-threatening problem [6,7]. The pediatric data about IFD is scarce and the situation in developing countries is not well documented. The purpose of this study, therefore, was to investigate the incidence of IFD, risk factors, diagnostic methods, efficacy of treatment and the outcome in pediatric patients with hematological disorders treated in a single center.

Methods

Patients and institution

There were 160 children with leukemia (22 AML, 129 ALL) and 9 with aplastic anemia admitted to the pediatric hematology department between January 2003 and December 2008. In this study, we evaluated only the patients diagnosed as proven and probable IFD. Each patient was evaluated and those with colonization were excluded. Children with ALL and AML were treated according to ALL- BFM 95 and AML-UK-MRC 12 studies, respectively [8,9].

Patients during the phase of neutropenia were allocated in one ward in separate rooms with single bed without high efficiency particulate air filtration. They had restricted access to visitors and other particular behavioral measures: such as a ban on plants or flowers and hand washing.

Diagnostic Studies

Diagnostic works-ups for IFD included collection of blood, urine cultures at the onset of fever, serum galactomannan (GM) levels twice weekly (Platelia® Aspergillus; Bio-Rad Laboratories, France). A positive result was based on two consecutive samples with a GM index of 0.5 or above [10]. High resolution computed tomography (HRCT) on the 4-7th day of fever was performed. Additional examinations (e.g., abdominal ultrasonography, sinus or cranial computerized tomography, tissue biopsy) were performed if indicated.

Definition of IFD

We identified patients who developed proven or probable IFD using standardized definitions set by the revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) Consensus Group in 2008 [11]. According to this group, proven IFD requires histopathology, cytopathology or direct microscopic examination showing hyphal invasion or yeast cells, or a positive culture from a radiological abnormal site that is normally sterile excluding bronchoalveolar lavage fluid, a cranial sinus cavity specimen, and urine. Probable IFD is defined by mycological evidence in a patient with a host factor and clinical features.

Treatment

Empiric antifungal treatment liposomal amphotericin B (L-AmpB) 3 mg/kg/day were commenced by day 5-7 of persistent fever that did not respond to broad-spectrum antibacterial therapy. Liposomal amphotericin B (5mg- 8mg/kg/day) was given to those with non-responded of 3 mg/kg/day. Patients with invasive aspergillus (IA) who were refractory to primary treatment based upon clinical, radiological and serological progression were commenced on voriconazole (VCZ). Salvage therapy as combination of VCZ and caspofungin for IA were given to those with suggestive progressive signs and/or symptoms and persistent neutropenic fever.

The criteria for responses to antifungal therapy; therapeutic success was classified as complete, partial, stable and progression of fungal disease, each defined according to EORTC/MSG [12].

Results

The Incidence and Patient's demographics:

IFD was identified in 23 (n:23/160; 14.3%) cases (F/M; 12/11). The incidence in leukemic children and AA was 12.5% [14 ALL (10%), 5 AML (22%)] and 44.4% (n:4), respectively. The mean age was 7.8±5.3 years (range 2.4-17.2 years, median age 6,4 years). For 3 patients with AA, a concomitant

Table 1. The Clinical Symptoms of Children

Clinical symptoms	n: 23 (%)
Persistent fever	23 (100%)
Cough	10 (43%)
Dyspnea	4 (17%)
Pleural pain	3 (13%)
Hemoptysis	3 (13%)
Focal neurological signs	4 (17%)
Tachypnea	7 (30%)
Headache	4 (17%)

bacterial infection was diagnosed. Klebsiella pneumonia (n:2) and Stenotrophomonas maltophilia (n:1) were isolated from their blood cultures (patients 1, 14 and 17). IFD was more frequent (n: 5/22; 22%) in AML than (n: 14/129; 10%) in ALL. Of the 19 leukemic patients, 11 were in induction therapy, 3 had bone marrow relapse receiving induction therapy and the others were in complete remission at consolidation phase. All of the children with aplastic anemia (n:4) was diagnosed as severe AA with total leukocyte count below 500/mm^3. None had fanconi. Of them, 3 received immunosuppressive (antithymocyte globulin, cyclosporine and corticosteroid) therapy. The other one was on the waiting list for HSCT with no therapy. IFD rate in these children was 44.4% (n:4/9), higher than the acute leukemia group. All patients' clinical symptoms were given in Table 1.

Risk Factors

All children had a compromised immune function owing to underlying hematologic diseases. Nine-teen with leukemia were receiving intensive chemotherapy when IFD occurred. Of them, 14 children with ALL had taken steroids longer than 10 days. Five leukemic children were on relapse therapy. Of the AA patients, 3 were receiving immunosuppressive therapy. All children were suffering from long-term neutropenia (<500 mm^3) longer than 10 days. These children were not on fungal prophylaxis. None of them was stem cell transplant recipient and had central venous catheter. All patients were on broad spectrum antibiotics when the diagnosis was made.

Clinical, radiological and microbiological factors

Host, clinical and microbiological factors were demonstrated in Table 2. All children had persistent fever longer than 5 to 7 days. Thorax HCT findings related to IFD were determined in 15 children. The radiographic findings on thorax HRCT related to IA were shown in Table 2.

Mycological studies

Of the 10 children with proven IFD, 6 had candidemia and 4 had IA. Candida albicans was the most frequently isolated yeast from blood (n:4). The others, Candida krusei and tropicalis were detected in blood culture (patient 1, 4).

Invasive procedures for defining fungal infection could be done only in 5 children. Cranial magnetic resonance imaging (MRI) was performed in 2 cases due to abnormal neurological findings. Both showed mass lesions, suggesting tonsillary herniation. Children were taken to surgery and the masses were resected. In other two patients, sinus CT suggested IA, and aspergillus hypes were demonstrated taken by endonasal endoscopic biopsy. Aspergillus flavus was grown in cultures of these 4 children (patients 7,16,20,21). The fifth child had BAL due suspicion of IA in thorax HRCT. Aspergillus hypes were demonstrated in the BAL sample.

Treatment

Regarding antifungal treatment, all children empirically received first line therapy with L-AmpB. Eleven out of 23 (48%) patients were treated only with L-AmB. Dose ranged between 3 and 8 mg/kg/day. In 12 children, L-AmB had to be changed due to therapy failure. Of them, two children with candidiasis were successfully treated with caspofungin. Voriconazole was given to 10 children with IA due to progressive infection. Six of them were successfully treated VCZ monotherapy. Combined antifungal treatment as VCZ and caspofungin were required in the rest (n:5) in Table 3.

Outcome

Overall mortality in this cohort was 40% (n: 9/23). Of them, the cause of death was related to IFD in 7 (30%) children and resistant leukemia in 2 cases. The underlying disorders, treatments, site of IFD and definitions of IFD, antifungal therapy in those cured and lost ones are given in Tables 3 and 4, respectively. Two children following the treatment of cerebral IA had sequels as motor mental retardation (patient 7) and convulsion (patient 13).

Discussion

In our series where 12.5% of the children treated within the last 5 years for hematologic malignancies had documented IFD. The largest epidemiologic study in pediatric patients with hematologic malignancies, reported the overall 11-year incidence of fungal infections as high as 9%-10% [13]. There are two main published studies from Turkey, regarding IFD in children with cancer. Kaya Z et al. [14] reported the incidence of IFD within a 10 year period as 13.6% in 154 leukemic children receiving fluconazole prophylaxis. In another report, a high rate of fungal colonization (69.2%) was found in children receiving chemotherapy for leukemia and lymphoma [15]. In the same study, proven IFD was found 5.8%, of which was less than observed by the other reports. The authors attributed this low incidence to sample selection and failure to isolate fungi. In the current study, IFD rate for aplastic anemia was 44.4% (n:4/9), higher than the acute leukemia group. We have a very limited data for this group to make a conclusion. However, in the aplastic anemia studies consisted of both adults and children; fungal infection rate was reported as 7% [16]. The major risk factor for the development of invasive mycoses in this group was prolonged and severe neutropenia [17]. For our patients, all of them were neutropenic for longer than 10 days.

Although Candida spp. has been the main cause of IFD, the recent epidemiological data indicated that an increasing number of infections in immunocompromised patients are being caused by Aspergillus spp. [18-20]. In our data, aspergillosis was also the major cause of IFD and the incidence was found 74%. Additional risk factor for the high incidence at our

Table 2. The Patients Host, Clinical and Microbiological Factors

Patients	Suspected pathogen	Host Factors	Clinical Manifestations	Mycological Evidence	Definition
1	Candidiasis	Use of ATG[1],cyc[2],cs[3]		Blood Culture	Proven
2	Candidiasis	Neutropenia	Bull's eye lesion in spleen	Blood Culture	Proven
3	Candidiasis	Neutropenia		Blood Culture	Proven
4	Candidiasis	Neutropenia	Bull's eye lesion in spleen-liver	Blood Culture	Proven
5	Candidiasis	Neutropenia		Blood Culture	Proven
6	Candidiasis	Prolonged use of Cs		Blood Culture	Proven
7	IA[4]	Prolonged use of Cs	Focal lesion on Cranial MRI	Cytology, culture	Proven
8	IA	Prolonged use of Cs	Thorax HRCT (nodules*)	BAL[5] Cytology	Probable
9	IA	Neutropenia	Thorax HRCT (air cresent)	GM[6]+/Sputum cytology	Probable
10	IA	Neutropenia	Thorax HRCT (nodul)	GM+	Probable
11	IA	Prolonged use of Cs	Thorax HRCT (nodules, cavity)	GM+	Probable
12	IA	Prolonged use of Cs	Thorax HRCT (nodules)	GM+	Probable
13	IA	Neutropenia	Focal lesion on Cranial MRI, Thorax CT (nodules)	GM+	Probable
14	IA	Use of ATG,cyc,cs	Thorax HRCT (halo signs)	GM+/Sputum cytology	Probable
15	IA	Spesific monoclonal antibodies	Thorax HRCT (nodul)	GM+	Probable
16	IA	Neutropenia	Sinus CT and black eschar	Cytology, culture	Proven
17	IA	Use of Cyc	Thorax HRCT (halo signs)	GM+	Probable
18	IA	Neutropenia	Thorax HRCT (air cresent)	GM+	Probable
19	IA	Neutropenia	Sinus CT and black eschar	GM+/ Cytology, culture	Proven
20	IA	Prolonged use of Cs	Thorax HRCT (halo signs)	Sputum /Cytology, culture	Probable
21	IA	Prolonged use of Cs	Focal lesion on Cranial MRI	Cytology, culture/GM+	Proven
22	IA	Prolonged use of Cs	Thorax HRCT (halo signs)	GM+	Probable
23	IA	Prolonged use of Cs	Thorax HRCT (air cresent)	GM+	Probable

[1]ATG: Antithymocyt globulin, [2]cyc: Cyclosporin, [3]cs: Corticosteroid, [4]IA: Invasive Aspergillosis, [5]BAL: Bronchoalveolar lavage, [6]GM: Galactomannan

*nodul: dense, well circumscribed lesions without a halo sign.

Table 3. The Features of Children Cured from Invazive Fungal Disease

Patients	Suspected pathogen	Underlying Disease	Site of IFD	Definition	Treatment	Outcome	Survival time (months)
3	Candidiasis	AML	Blood	Proven	L-amp(5), caspo[4]	Cured from IFD	17
4	Candidiasis	ALL-RL	Blood-Liver/Spleen	Proven	L-amp(5)	Cured from IFD	19
6	Candidiasis	ALL	Blood	Proven	L-amp(5), caspo	Cured from IFD	21
7	IA	ALL	Brain	Proven	L-amp(3,5,8)	Cured from IFD	34
9	IA	AML	Lung	Probable	L-amp(3,5)	Cured from IFD	22
10	IA	AML	Lung	Probable	L-amp(5), VCZ	Cured from IFD	17
12	IA	ALL	Lung	Probable	L-amp(5)	Cured from IFD	33
13	IA	ALL-RL	Brain	Probable	L-amp(5), VCZ	Cured from IFD	20
15	IA	ALL	Lung	Probable	L-amp(5), VCZ, caspo	Cured from IFD	9
16	IA	A.A	Sinus	Proven	L-amp(5), VCZ	Cured from IFD	11
18	IA	ALL	Lung	Probable	L-amp(5), VCZ, caspo	Cured from IFD	13
20	IA	ALL	Lung	Probable	L-amp(5), VCZ	Cured from IFD	15
21	IA	ALL	Brain	Proven	L-amp(5), VCZ	Cured from IFD	22
22	IA	ALL	Lung	Probable	L-amp(5), VCZ	Cured from IFD	34

[1]AA: Aplastic anemia, [2]L-amp: liposomal amphotericine B, [3]RL: Relapse leukemia, [4]Caspo: caspofungin, [5]VCZ: Voricanazole

center was ongoing hospital renovation in the last 5 years. Several studies have suggested an association between IA and contamined ventilation systems, hospital construction or renovation [21-23]. In the current study, the respiratory tract was the most common site of the IA. Four children also had more than one site of infection where CNS was involved in three. The previous reports also suggested that respiratory tract followed by CNS in IA were the most frequent IFD in the leukemia and transplant settings [2,3,24,25]. Therefore, children with pulmonary IA should have a careful neurological examination.

The diagnosis of IFD in immunocompromised patients is difficult. In the current study, the episodes according to EORTC/MSG criteria were defined as proven in 10 (43%) children and probable in 13 (57%) cases. Histopathologic and/or

cytopathalogic evidence for proven infection is rather difficult, especially in children. This is in part because invasive diagnostic procedures are often contraindicated due to impaired clinical condition [26]. In ours study, we were able to do tissue biopsy only in 4 children. In recent years, early HRCT as a non-invasive method seems to be more valuable compared to chest X-ray in defining IFD [27]. The findings of thorax CT in IFD are given in details, mentioning that focal rather than diffuse infiltrates, macronodules with or without halo sign, wedge-shaped infiltrates, segmental and lobar consolidation can be found in pulmonary lesions. In addition radiologic data in pediatric population is limited and its presence is highly predictive, but not specific [28]. Although not totally specific, segmental, consolidation, multi-lobar consolidation, perihilar infiltrates and pleural based nodular masses can all be seen and raise suspicion about IFD in these immunosuppressive children [29,30]. In our setting, HRCT scans were obtained in the first week of febrile neutropenia in all children. Findings related to IFD were documented in 65% (n: 15/23) of these scans, although the concomitant chest X-rays had no signs related to infection.

Serum GM levels could lead to an early diagnosis and substantially improve the clinical outcome. The inclusion of GM in the EORTC/MSG criteria could at least partly resolve this problem [26]. We have determined GM positivity in 13 out of 17 patients with IA. In the previous reports, the sensitivity of the assay was found 64.5% for proven and 16.4% for IA [31-33]. However, the false positive rate was reported as 44% in children [34-36]. Therefore, the test should be used in conjunction with other diagnostic work-ups and serial sampling is required to maximize detection.

All children had clinical findings of infection (Table 1). Despite the heterogeneity of the combinations of criteria used for diagnosis, we found that the most frequently used diagnostic methods were clinical findings (100%) and radiogical methods (84%).

The success rate of treatment for candidiasis and IA were found 60%, 71% respectively. Two children with hepatosplenic candidiasis, L-AmB were changed to caspofungin, due to stable response. Comparative trials with adults have demonstrated that caspofungin was effective as a first line drug for invasive candidiasis and as a second line agent for IA [37]. However, there is limited data on the use of caspofungin in pediatrics cases [37,38]. Groll et al. [39] have recently displayed favorable safety and tolerance in with caspofungin 64 immunocompromised children. In our study, three out of 6 children with candidiasis was lost. All of them had positive blood cultures; in patient 1 both C.crusei and K. pneumonia, in patient 2 and 5, C. albicans were grown. Patient 5 also had hepatosplenic candidiasis. Of these 3 deaths, only two (33%) (patients 1 and 5) were related to candidiasis. The underlying host factor for patient 1 was severe AA treated with ATG, cyclosporine and steroids, and for patient 2 and 5 was relapse/resistant AML (Table 4).

In our study, the success rate of VCZ monotherapy in IA was found 75%. Patterson et al. [40] reported a good response in the primary treatment of IA with VCZ compared to amphotericin B (52.8% vs. 31.6%) and increased survival rates (70.8% vs. 57.9%). Therefore, VCZ was approved as a first line treatment in IA [41]. In our study, four children with IA required combination therapy with caspofungin due to stable response to VCZ. All of these children had impaired bone marrow function either due to relapse or bone marrow failure. Caspofungin has been shown effective as a salvage therapy for IA in adults [41]. Prospective controlled studies in analyzing the efficacy of combination therapy is a few in children. However, the recent reports on this issue addressed that combination therapy in children with hematological diseases increased the survival rates [38,41-43].

In current study, six out of 17 (35%) children with IA was lost. Among those 6 cases, underlying diseases were ALL in 4 and AA in 2. Only five deaths (30%) were related to IFD. The causes of death in these fatal cases were massive haemoptysis (patients 8, 11, 23), respiratory failure (patients 14, 17). Two of them had also positive blood cultures for K. pneumonia and S. maltophilia (patients 14, 17) in addition to fungal disease. One death (patient 19) was related to relapse/resistant leukemia. Three out of 4 patients with leukemia were in remission but, all of them were on prolonged use of steroid and had neutropenia.

In our series, the total mortality of IFD was found 30% (n: 7/23) excluding patients with relapse leukemia. In a study

Table 4. The Features of Lost Children

Patients	Suspected pathogen	Underlying Disease	Treatment phase	Site of IFD	Definition	Treatment	Outcome	Survival time (months)
1	Candidiasis	A.A[1]	ATG[1],cyc[2],cs[3]	Blood	Proven	L-amp(5)[2]	Died of IFD and sepsis	2,1
2	Candidiasis	AML-RL	Resistant leukemia	Blood-Liver/Spleen	Proven	L-amp(5)	Died of RL[3]	4
5	Candidiasis	AML-RL	Consolidation	Blood	Proven	L-amp(5)	Died of IFD	1,2
8	IA	ALL	Induction	Lung	Probable	L-amp(5)	Died of IFD	1.8
11	IA	ALL	Induction	Lung	Probable	L-amp(5)	Died of IFD	1.8
14	IA	A.A	ATG,cyc,cs	Lung	Probable	L-amp(5)	Died of IFD and sepsis	2
17	IA	A.A	Cyc	Lung	Probable	L-amp(5), VCZ, caspo	Died of IFD and sepsis	1,4
19	IA	ALL-RL	Resistant leukemia	Sinus	Proven	L-amp(5), VCZ, caspo	Died of RL	2
23	IA	ALL	Induction	Lung	Probable	L-amp(5),	Died of IFD	1.8

[1]AA: Aplastic anemia, [2]L-amp: liposomal amphotericine B, [3]RL: Relapse leukemia, [4]Caspo: caspofungin, [5]VCZ: Voricanazole

from Japan, IFD related mortality in a total of 334 pediatric patients with hematologic malignancies, aplastic anemia and solid tumors was determined as 48.2% [44]. In the same study, when the children with hematologic malignancies and aplastic anemia were separately evaluated, IFD related death rate increased to 58.8% (n: 10/17). ALL-BFM study group including 2021 children, reported infection related death rate as 2.1% (n:43). The cause of death was IFD in 9 of these 43 fatal cases (21%) [45]. One of the major AML study group, MRC, has reported total death rate as 13.8% in 341 children. The main cause of death was infection (65.9%). Of them, fungal diseases were significant problem, causing 23% of all infective deaths [46]. Invasive fungal infections have been also identified as a growing threat in patients with AA and were reported as the major cause of death in this population [16, 17]. Considering all of these data, the incidence of fungal infections is considerably increasing in immunocompromised children causing high mortality and morbidity.

It is concluded that fungal infections are an important problem in children with hematologic malignancies and SAA. Both the underlying disease and prolonged neutropenia are important risk factors. It is rather difficult in children to prove the fungal disease. Therefore, any sign in children under risk of developing IFD should be carefully evaluated for an urgent treatment to decrease the morbidity and mortality. The availability of new antifungal treatments and early diagnostic tests will also help to improve the survival rates in this patient group.

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