JACOBS PUBLISHERS

OPEN ACCESS

Jacobs Journal of Hematology

Clinical Study

Proven Invasive Fungal Infection during the Pre-Engraftment Period in Pediatric Allogeneic Hematopoietic Stem Cell Transplantation for Non-Malignant Disorders

Abdalla Khalil*[1], Juma Natsheh[2], Polina Stepensky[3], Aharon Gefen[1], Ronit Leiba[4], Myriam Ben-Arush[1,5], Irina Zaidman[1]

[1]*Department of Pediatric Hematology Oncology, Ruth Rappaport Children's Hospital, Rambam Health Care Campus, Haifa, Israel*

[2]*Department of Pediatrics, Hadassah Hebrew University Medical Center, Jerusalem, Israel*

[3]*Department of Pediatric Hematology Oncology, Hadassah Hebrew University Medical Center, Jerusalem, Israel*

[4]*Epidemiology Unit, Rambam Health Care Campus, Haifa, Israel*

[5]*Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel*

**Corresponding author:* *Dr. Abdalla Khalil, Department of Pediatric Hematology Oncology, Ruth Rappaport Children's Hospital Rambam Health Care Campus, POB 9602, Haifa 31096, Israel, Tel: 972-4-7774718; Fax: 972-4-7774798; Email: h_abdalla@rambam.health.gov.il*

Received: *12-31-2015*

Accepted: *01-14-2016*

Published: *05-23-2016*

Abstract

Invasive fungal infection remains a significant cause of morbidity and mortality, particularly during the pre-engraftment neutropenic period, in pediatric allogeneic hematopoietic stem cell transplantation. We retrospectively analyzed epidemiology, incidence, and outcome of IFI in children with non-malignant diseases undergoing HSCT during the pre-engraftment period. Between 2004 and 2014, 236 patients underwent HSCT for non-malignant disorders at Rambam Health Care Campus in Haifa and Hadassah Hebrew University Medical Center in Jerusalem. Main diagnoses were thalassemia and severe immunodeficiency. Eighteen of 236 (7.6%) patients (9 males, 9 females) developed proven IFI. Median age was 5.47 years (0.44-18 years). Invasive candidiasis was seen in nine patients, invasive aspergillosis in five, both invasive candidiasis and invasive aspergillosis in two, Mucor and Fusarium in one patient each. In eight (44.4%) patients, IFI occurred secondary to engraftment failure; two received autologous back-up bone marrow stem cell infusion and survived, three died due to IFI complications, and three were treated with granulocyte transfusion, only two of whom survived. Three patients developed autologous recovery. IFI was the main cause of death in five (27.8%) patients. It was concluded that engraftment failure, thalassemic patients with high serum ferritin levels, and congenital or acquired immunodeficiency exhibited increased susceptibility to pre-engraftment IFI. Granulocyte transfusion and autologous stem cell infusion could be the therapeutic option for life-threatening pre-engraftment IFI. Our data may help guide the intensity of monitoring for prevention, early diagnosis, and treatment of IFI.

Keywords: Aspergillus; Candida; Hematopoietic Stem Cell Transplantation; Invasive Fungal Infection; Pre-Engraftment Period

Introduction

Invasive fungal infection (IFI) remains a significant cause of morbidity and mortality, particularly during the pre-engraftment neutropenic period in children undergoing allogenic hematopoietic stem cell transplantation (HSCT). In accordance with changes in HSCT, IFI complications during

HSCT change. The risks for IFI and the types of IFI that may occur are not continuous over the time course. The events of IFI vary if they occur during the pre-engraftment neutropenic period or during the post-engraftment period [1]. Allo-HSCT is characterized by a subsequent period of immunodeficiency due to multiple factors, such as graft manipulation and choice of graft type, intensity of conditioning, duration, prophylaxis and treatment. Invasive aspergillosis (IA), invasive candidiasis (IC) and zygomycosis are the most common types of IFI [1]. Early identification of the causative fungi is important for antifungal treatment, duration of therapy, surgical intervention and monitoring.

Dvorak et al [2] found predictors of IFI to include age older than 10 years at HSCT, diagnosis such as severe aplastic anemia (SAA), acute myeloid leukemia (AML), relapse of original disease, graft versus host disease (GVHD), viral infection and use of high-dose steroids, and possibly related to the occurrence of IFI post-HSCT. The major risk factors for late IFI are GVHD, the use of corticosteroids and cytomegalovirus (CMV) disease [3,4].

The relationship between risk factors for prolonged neutropenia, engraftment failure, the underlying disease and IFI during the pre-engraftment period of HSCT in non-malignant diseases has not been clearly defined. However, studies that focus on pediatric patients are scarce and use relatively small cohorts [4]. Our study analyzed IFI during the pre-engraftment period of HSCT, including epidemiology, incidence, outcome and identification of high-risk groups of pediatric patients with non-malignant diseases, and risk factors for prolonged neutropenia and engraftment failure.

Patients and Methods

Patients: The medical record of all pediatric patients who underwent allo-HSCT for non-malignant disorders at Rambam Health Care Campus in Haifa and Hadassah Hebrew University Medical Center in Jerusalem from January 2004 to September 2014 were reviewed. The study was approved by the local institutional ethical committees and performed according to the declaration of Helsinki. Information about prior fungal infections was also recorded, although only fungal infections occurring pre-engraftment were included in the analysis.

Transplant Protocols: The conditioning regimen was dependent on diagnosis, donor type and medical center choice. The conditioning regimen for thalassemia patients was according to "Pesaro classification", type of donor available, and whether the HSCT was first or second. Patients with class III thalassemia received protocol P26 [5], including hydroxyurea azathioprine from day -45 pretransplant and busulfan, followed by cyclophosphamide (Cy). Protocol 26.1 [6], based on protocol P26 with the addition of thiotepa and ATG (antithymocyte globulin), was used for the second transplant. Children with severe aplastic anemia (SAA) were transplanted using a generic protocol Cy/ATG. Most patients with severe combined immunodeficiency (SCID) were transplanted, often using a generic conditioning regimen with Flu/Melph/ATG-modifications.

Most patients received a short course of methotrexate together with cyclosporine A for standard prophylaxis for GVHD. Corticosteroids were not used prophylactically.

Supportive Care: All patients were nursed in protective isolation in single rooms with high-efficiency particulate air (HEPA) filters. All patients had indwelling central venous catheters. For fungal prophylaxis, all patients were treated with a standard antifungal prophylaxis that consisted of amphotericin B (until 2009) or intravenous fluconazole at a dose of 5-6 mg/kg/day from the start of conditioning.

Data collected: Baseline information recorded for each patient included age, gender, underlying disease, conditioning regimen, transplant type, donor type, and stem cell source. Time to engraftment was defined as time from transplant until the neutrophil count exceeded 0.5x10^9/l. For patients who failed to engraft, secondary neutropenia was counted after day 30 post-HSCT.

Statistical Analysis: All patients were included in the analysis. Categorical values were compared using the Pearson chi-square test. Continuous variables were compared using the unpaired two-tailed t-test. A p value less than 0.05 was considered as statistically significant.

Definitions: The date of confirmation was defined as the day on which a positive culture was obtained. Diagnosis of proven fungal infection was based on a positive culture, obtained from the infected tissue or peripheral blood, or pathological or cytological evidence for fungal infection in the presence of typical clinical and radiological findings [7].

Fungal colonization, as evidenced by isolation of fungi from a single superficial site (urine, sputum, throat, oropharynx, skin, or wound), was not classified as IFI. A diagnosis of disseminated infection required histological evidence of tissue invasion and/or positive cultures from two or more normally sterile sites.

Results

Of the 236 patients identified as undergoing allo-HSCT for non-malignant disorders between January 2004 and September 2014, 18 (7.6%) patients developed proven pre-engraftment IFI, despite having no evidence for fungal infection on admission to HSCT, with a ratio of male:female of 9:9, aged 0.44-18 years (median age 5.47 years).

Table 1. Pre-Engraftment IFI in allo-HSCT for Non-Malignant Disorders.

Pt no.	Gender	Age (years)	Year of HSCT	Diagnosis	Day of ANC >500	Day of IFI	Fungus	Location	Diagnostic procedure	Associated infection	IFI cause of death	SC source	Donor relation
1	F	17.6	2004	Dyskeratosis congenita	+12	+9	C. albicans	CVC	MB	E. coli	N	PBSC	UR
2	M	0.5	2004	Wolmans disease	NE	+17	C. albicans	CVC	MB	Acinetobac.	Y	BM	RS
3	F	12.2	2004	BTM	NE	+13	C. tropicalis C. albicans	Blood + CVC	MB		Y	PBSC	RS
4	M	9.8	2005	SAA	+12	+3	C. tropicalis	CVC + blood	MB	Acinetobac.	N	BM	RS
5	F	4.3	2006	BTM	+21	+1	C. tropicalis	CVC	MB		N	BM	RS
6	M	1.3	2006	X-linked hyper IgM syndrome	+19	0	C. albicans	CVC	MB		N	BM	RS
7	F	0.73	2007	SCID	NE	-2	C. fumigatus C. tropicalis	CVC + lung	MB + CT	Para-infection	N	PBSC	RS
8	F	0.5	2008	SCID	+12	+4	C. tropicalis	CVC	MB		Y	BM	RS
9	M	18	2008	SAA	+22	+1	C. dubliniensis	CVC	MB	E. coli	N	PBSC	RS
10	F	0.44	2009	SCID	NE	+69	C. albicans	CVC	MB	Stenotro-phomonas	Y	BM	UR
11	F	1.36	2010	LAD	NE	+32	A. galactoman.	CVC + blood	MB		N	UCB	UR
12	M	1.18	2011	SCID	+10	-5	A. galactoman.	Blood	MB		N	PBSC	UR
13	F	6.35	2011	SAA	+11	-5	A. galactoman.	Blood	MB		Y	PBSC	UR
14	M	0.9	2011	Hypereosinophilic syndrome	+14	+6	A. galactoman.	CVC	MB		N	BM	RS
15	M	5.4	2013	Fanconi	NE	-1	A. galactoman. C. albicans	Blood	MB		N	PBSC	UR
16	F	8	2013	BTM	NE	+13	Trichosporon asahii fusarium	Blood + soft tissue	CT + MB		N	PBSC	RS
17	F	9	2012	BTM	NE	+40	Mucor	Mandible	MB		N	PBSC	UR

18	M	0.9	2014	Osteope-trosis	+17	+13	A. galacto-man.	Blood	MB	RSV	N	BM	RS

Notes
A=Aspergillus; BM=bone marrow; BTM=beta thalassemia major; C=candida; CVC=central venous catheter; MB=microbiology; LAD=leukocyte adhesion deficiency; NE=non-engraftment; PBSC=peripheral blood stem cells; SAA=severe aplastic anemia; RS=related sibling; RSV=respiratory syncytial virus; UCB=unrelated cord blood; UR=unrelated

Eleven (61%) patients had HLA-matched related donors and seven (39%) had matched unrelated donors. Nine (50%) patients received peripheral blood stem cells (PBSC), eight (44.4%) received bone marrow (BM), and one (5.6%) cord blood (CB).

Among these 18 patients with non-malignant diseases, B thalassemia major and SCID were the most common underlying diseases, found in four patients (22%) each, followed by SAA in three (16.7%), and leukocyte adhesion deficiency (LAD), X-linked hyper-IgM syndrome, hypereosinophylic syndrome, Fanconi syndrome, dyskeratosis congenita, osteopetrosis, and Wolmans disease in one patient (5.5%) each.

All the four thalassemic patients were class III, and mean serum ferritin level was 2873 ng/ml (1068-5155). One patient was splenectomized for symptomatic hypersplenism and two underwent a second HSCT one year after the first transplant rejection. Table 1 summarizes pre-engraftment IFI in allo-HSCT for non-malignant disorders.

Among 10/18 (55.6%) patients who had donor engraftment, the median time to proven IFI from pre-engraftment was 11.9 days (range, 3-21 days). Median engraftment day was 15 days (range, 10-22 days). Three of the 10 engrafted patients (30%) died, two (#8, #13) due to IFI complications, and one (#1) due to other complications.

Among the 18 patients, there were 8 (44.4%) non-engrafted cases, two (#16, #17) of whom received their own back-up bone marrow reinfusion and engrafted. Three patients (#7, #11, #15) did not recover with donor cells and thus experienced a prolonged period of neutropenia that accompanied autologous recovery and survived; they proceeded to a second allogeneic HSCT using the same donor. However, three of eight non-engrafted patients (#2, #3, #10) died due to IFI complications.

Among the six (33.3%) patients who died, IFI was the main cause of death for five (27.8%), and one (5.5%) patient died of other complications. Twelve (66.7%) survived.

Only two (#16, #17) of three patients who were treated with granulocyte transfusion (GTX) on a daily basis and autologous stem cell infusion in addition to appropriate antifungal agents for non-engraftment or early graft rejection and active fungal infection survived.
Invasive candidiasis (IC) was the most frequent infection (9/18 patients, 50%), followed by invasive aspergillosis (IA) in five (27.8%). Two patients (11%) developed both Candida and Aspergillus. Mucor and Fusarium were isolated in one patient each (5.5%). Four (44.4%) of the nine IC patients died, while only one (20%) of the five IA cases died. Pre-engraftment IFI-related mortality was 22.2% in 2004–2009 and 5.5% in 2010–2014.

Only one case (#7) of pre-engraftment IFI caused by Aspergillus was diagnosed before 2009 and only one case (#15) of Candida after 2009.

Of the IC species, distribution was four by C. albicans, three by C. tropicalis, one by both C. albicans and C. tropicalis, one by C. dubliniensis, one by both C. tropicalis and Aspergillus and one by C. albicans and Aspergillus.

Sites of involvement included central venous catheter (CVC) (n=8), blood (n=4), both blood and CVC (n=3), and both CVC and lungs, both blood and soft tissue infection or mandible, one patient each. Five patients had more than one site of involvement.

There were no statistically significant differences between stem cell source or donor type.

Discussion

In this study, we focused on the recent trends and clinical outcomes of proven pre-engraftment IFI, according to published consensus criteria [7]. IFIs vary with the events that occur during the pre-engraftment neutropenic period, in the early post-engraftment period until approximately day 100 post-HSCT, and those in the late post-engraftment period after day 100. However, since a prolonged neutropenic period is a known risk factor for fungal infection [8], shortening its duration may have an advantage.

Important related recipient factors include age and the underlying disease for HSCT [4]. Factors related to HSCT procedure include conditioning, HLA-relatedness (related/ unrelated, matched/ mismatched), stem cell source (PBSC, BM,

CB), and stem cell dosing. Factors related to HSCT complications include duration of pre-engraftment neutropenic period, graft failure or rejection, and the use of corticosteroids for the prevention of GVHD.

In our pediatric cohort, we found an incidence of pre-engraftment IFI of 7.6% with a mortality rate of 27.8%, more than the 5% reported by Van Burik et al [9]. The higher IFI rate observed in this group of patients most probably reflects the active prolonged diseases, such as thalassemia and congenital or acquired immunodeficiency. Based on prior studies, IFI in pediatric allo-HSCT recipients is estimated to occur in 8-17% of children with malignant and non-malignant diseases, with a mortality rate of 35-50% [10].

The incidence of proven or probable IFI in pediatric patients during the first year post-HSCT was 13%, comparable to the rate reported in adult patients; however, unlike IFI in adult patients, the majority of IFI in children occurred within the first month after transplantation [2]. Hovi et al [10] reported a higher frequency of IFI in the post-engraftment periods. However, recent reports showed that the highest frequency of IFI was observed in the pre-engraftment period, even if some episodes were reported many months post-HSCT [2].

IC was the most common cause of death, occurring in 4/5 dead patients. It seems that IC has a higher mortality incidence than IA (p=.08). Compared with the years 2004–2009, pre-engraftment IFI-related mortality decreased and overall survival increased in recent years, due to the control of underlying diseases, certainty of IFI diagnosis, younger age, the use of novel antifungal agents, and improved supportive care.

Pappas et al [11] noted that breaks in the integrity of skin and gastrointestinal (GI) tract mucosal barriers, such as occurs during mucositis, may lead to IC, particularly in the context of immunosuppression. Recent studies report incidence rates in the 5% range, with risk factors including GI-tract colonization, CMV disease, and prior episodes of bacteremia [12]. In our group, 27.8% (5/18) patients had an episode of bacteremia either prior to or during IFI. None had CMV infection, but 11% (2/18) patients had parainfluenza infection.

IA was reported in five (27.8%) of our patients but was the cause of death in only one. Aspergillus galactomannan was the most frequent. The most common affected sites were blood and CVC. Upton et al [13] reported a higher suspicion of IA longer post-HSCT, especially in patients who had long-term immunosuppression associated with GVHD.

Increased susceptibility to IA is observed among acquired or congenital immunodeficiencies, such as SCID, LAD and SAA.

In our study, seven patients who developed pre-engraftment IA had congenital immunodeficiencies (3 SCID, with Fanconi syndrome, LAD, SAA and hypereosinophilic syndrome in one patient each).

The role of prophylactic antifungal medication was retrospectively assessed regarding the incidence of pre-engraftment IFI. We found a statistically significant association of pre-engraftment IC or IA before and after 2009 (p=.003). Only one case of pre-engraftment IA was diagnosed before 2009 and only one case of IC after 2009. IFI caused by candida may be better prevented by antifungal prophylaxis with fluconazole. Pre-engraftment IFI due to fungi other than *C*andida spp, such as Aspergillus, Fusarium and Mucor, are becoming a major problem among HSCT patients receiving systemic antifungal prophylaxis with fluconazole.

Mucor spp. are ubiquitous in the soil. Inhalation of spores or penetration through the skin become angio-invasive with the potential to disseminate systemically [14].

Fusarium spp. are plant pathogens found in the soil and are waterborne. Anaissie et al [15] documented the transmission to HSCT recipients through water sources.

The 1-year overall survival rates were equally poor (~20%) in patients after diagnosis of aspergillosis, zygomycosis, and fusariosis in patients undergoing allo-HSCT [16].

Pre-transplant iron overload (IO) is significantly associated with a lower overall survival rate and a higher incidence of fungal infections. The toxicity of IO is related in part to intracellular generation of free radicals, resulting in oxidative damage and organ dysfunction, and in part to increased susceptibility to infection resulting from suppression of host immune responses, and from iron's role as an essential cofactor in pathogen growth [17]. Iron is important for the proliferation of fungal organism, and iron availability is critical for the growth of Mucorales [18]. Altes et al [19] found that severe IO at autopsy represented by a high LIC of 150micromol/g or greater was significantly associated with IA in patients who died post-HSCT.

Ozyilmaz et al [20] found that pre-transplant serum ferritin levels of over 1000 ng/ml were associated with a 3.4-fold high risk of fungal pulmonary infections. All our thalassemic patients (#3, #5, #16, #17) had serum ferritin levels over 1000ng/mL, but none had pulmonary fungal infections.

Goussetis et al [21] reported that the use of an ATG-containing preparative regimen in pediatric patients with hemoglobinopathies had no effect on post-HSCT infections.

The progression of IFI was successfully controlled in two of three patients who were treated with GTX in addition to autologous stem cell infusion and antifungal therapy. GTX

seemed to be effective in controlling life-threatening pre-engraftment IFI, although the experience is too small to draw any conclusions. Prospective randomized trials are needed to support the routine usage of GTX or autologous stem cell infusion in patients who develop life threatening IFI, poorly controlled, and therefore at risk for mortality.

An important consideration is whether GTX during the pre-engraftment period may cause a delay in engraftment. Adkins et al [22] reported that adverse reactions were not associated with human leukocyte antigen (HLA) and, also, leukocyte compatibility did affect the peak count of polymorphonuclear cells and delayed neutrophil engraftment. In our study, patients who received GTX did not achieve engraftment, but it is difficult to attribute the engraftment failure to leukocyte incompatibility alone. However, since this cohort of patients is small, caution is necessary in definitively describing risk group stratification for developing pre-engraftment IFI in children, and a multi-center study needs to be carried out.

IFI frequently occurs in patients with both congenital and acquired phagocytic and cellular immune defects, such as severe combined immunodeficiency, X-linked hyper-IgM syndrome, hyper-IgE syndrome, and SAA. Eleven (61%) of our patients were diagnosed with congenital or acquired immunodeficiency. Increased susceptibility to fungal infections was observed among these patients. Candida and Aspergillus were the most commonly implicated, five (27.8%) by Candida, four (22.2%) by Aspergillus, and two (11%) patients by both. Dvorak et al [2] reported age older than 10 years at HSCT was a predictor of IFI, and we found a statistically significant association of age on pre-engraftment IFI (15/18 patients). This different outcome may be attributed to the different patient ages at HSCT due to early diagnosis after birth.

Conclusions

Pediatric patients with non-malignant diseases and a high risk for prolonged neutropenia or engraftment failure, such as thalassemic patients with high serum ferritin levels, congenital or acquired immunodeficiency, or previous prolonged neutropenia, exhibit increased susceptibility to pre-engraftment IFI. GTX as well as autologous stem cell infusion could be therapeutic options for life threatening pre-engraftment IFI and may improve survival. Compared with 2004–2009, pre-engraftment IFI-related mortality decreased and overall survival increased in recent years. Our data may help guide the intensity of monitoring for the prevention, early diagnosis, and treatment of IFI to improve outcome. Risk group stratification should guide intensity of monitoring for prevention and early diagnosis. However, since this cohort of patients is small, caution is necessary in definitively describing risk group stratification for developing pre-engraftment IFI in children, and a multi-center study needs to be conducted.

Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Castagnola E, Faraci M, Moroni C, Bandettini R, Granata C et al. Invasive mycoses in children receiving hemopoietic SCT. Bone Marrow Transplant. 2008, 41: 107-111.

2. Dvorak CC, Steinbach WJ, Brown JMY, Agarwal R. Risks and outcome of invasive fungal infections in pediatric patients undergoing allogeneic hematopoietic cell transplantation. Bone Marrow Transplant. 2005, 36(7): 621-629.

3. Grow WB, Moreb JS, Roque D, Manion K, Leather H et al. Late onset of invasive aspergillus infection in bone marrow transplant patients at an university hospital. Bone Marrow Transplant. 2002, 29(1): 15-19.

4. Hol JA, Wolfs TFW, Bierings MB, Lindemans CA, Versluys ABJ et al. Predictors of invasive fungal infection in pediatric allogeneic hematopoietic SCT recipients. Bone Marrow Transplant. 2014, 49(1): 95-101.

5. Sodani P, Gaziev D, Polchi P, Erer B, Giardini C et al. New approach for bone marrow transplantation in patients with class 3 thalassemia aged younger than 17 years. Blood. 2004, 104(4): 1201-1203.

6. Gaziev J, Sodani P, Lucarelli G, Polchi P, Marktel S et al. Second hematopoietic SCT in patients with thalassemia recurrence following rejection of the first graft. Bone Marrow Transplant. 2008, 42(6): 397-404.

7. Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J et al. 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. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an International Consensus. Clin Infect Dis. 2002, 34(1): 7–14.

8. Johnson E, Gilmore M, Newman J, Stephens M. Preventing fungal infections in immunocompromised patients. Br J Nurs. 2000, 9(17): 1154-1156, 1158-1164.

9. Van Burik J, Weisdorf D, Mandell GL, BennettJE, Doolin R. Infections in Recipients of Hematopoietic Stem Cell Transplantation. Principles and Practice of Infectious Diseases. Churchill Livingstone, Philadelphia. 2005, 3486-3501.

10. Hovi L, Saarinen-Pihkala UM, Vettenranta K, Saxen H. Invasive fungal infections in pediatric bone marrow transplant recipients: single center experience of 10 years. Bone Marrow

Transplant. 2000, 26(9): 999-1004.

11. Pappas PG. Invasive candidiasis. Infect Dis Clin North Am. 2006, 20(3): 485-506.

12. Marr KA, Seidel K, White TC, Bowden RA. Candidemia in allogeneic blood and marrow transplant recipients: evolution of risk factors after the adoption of prophylactic fluconazole. J Infect Dis. 2000, 181(1): 309-316.

13. Upton A, Kirby KA, Carpenter P, Boeckh M, Marr KA. Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality. Clin Infect Dis. 2007, 44(4): 531-540.

14. Petrikkos G, Skiada A, Lortholary O, Roilides E, Walsh TJ et al. Epidemiology and clinical manifestations of mucormycosis. Clin Infect Dis. 2012, 54: S23-S34.

15. Anaissie EJ, Kuchar RT, Rex JH, Francesconi A, Kasai M et al. Fusariosis associated with pathogenic Fusarium species colonization of a hospital water system: a new paradigm for the epidemiology of opportunistic mold infection. Clin Infect Dis. 2001, 33(11): 1871-1878.

16. Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin Infect Dis. 2002, 34(7): 909-917.

17. Rahav G, Volach V, Shapiro M, Rund D, Rachmilewitz EA et al. Severe infections in thalassemic patients: prevalence and predisposing factors. Br J Haematol. 2006, 133(6): 667-674.

18. Spellberg B, Edwards J Jr, Ibrahim A. Novel perspectives on mucormycosis: pathophysiology, presentation, and management. Clin Microbial Rev. 2005, 18(3): 556-569.

19. Altes A, Remacha AF, Sorda P, Sancho FJ, Sureda A et al. Frequent severe liver iron overload after stem-cell transplantation and its possible association with invasive aspergillosis. Bone Marrow Transplant. 2004, 34(6): 505-509.

20. Ozyilmaz E, Aydogdu M, Sucak G, Aki SZ, Ozkurt ZN et al. Risk factors for fungal pulmonary infections in hematopoietic stem cell transplantation recipients: the role of iron overload. Bone Marrow Transplant. 2010, 45(10): 1528-1533.

21. Goussetis E, Efstathiou E, Paisiou A, Avgerinou G, Zisaki K et al. Infectious complications following allogeneic stem cell transplantation by using anti-thymocyte globulin-based myeloablative conditioning regimens in children with hemoglobinopathies. Transpl Infect Dis. 2015, 17(2): 201-207.

22. Adkins DR, Goodnough LT, Shenoy S, Brown R, Moellering J et al. Effect of leukocyte compatibility on neutrophils increment after transfusion of granulocyte colony-mobilized prophylactic granulocyte transfusions and on clinical outcomes after stem cell transplantation. Blood. 2000, 95(11): 3605-3612.