

Fungal infections after hematopoietic stem cell transplantation

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Abstract Invasive fungal infections (IFIs) are associated with considerable morbidity and mortality after hematopoietic stem cell transplantation (HSCT). Despite that epidemiology of IFIs has changed notably by evolution in transplantation procedures as well as preventative strategies, the attributable mortality still remains high, mainly because of delayed initiation of treatment due to its diagnostic difficulty. Hence high-resolution computed tomography and non-culture based adjunctive diagnostic tests such as enzyme-linked immunosorbent assay for galactomannan and (1,3)- β -D-glucan have been incorporated into clinical practice, and global antifungal prophylaxis has been applied particularly to high-risk patients. Newer mold-active agents with higher efficacy and lower toxicity are currently being introduced as prophylaxis, and the combination of these agents are being evaluated as salvage therapy. This review summarizes recent advances in the diagnosis and management of IFIs in HSCT recipients. Further improvement of clinical outcome will be achieved by optimizing diagnostic, prophylactic and therapeutic approach based on individual patient's risk and situation.

Keywords Invasive fungal infections · Aspergillosis · Antifungal agents · Hematopoietic stem cell transplantation

1 Introduction

Invasive fungal infections (IFIs) are one of the major causes of morbidity and mortality after hematopoietic stem

cell transplantation (HSCT). The mortality rate associated with documented IFIs has been extremely high once developed, depending on the patients' group and the infection type [1–3]. This high mortality rate is mainly attributed to the difficulty of diagnosis retarding the initiation of treatment, because histopathological examinations require invasive procedures and fungal cultures have low sensitivity in detecting IFIs. Therefore, the prevention upfront may play an important role in reducing the incidence of IFIs and its attributable mortality, especially in high-risk patients.

To date, incessant efforts have been made to prevent the development of IFIs, with more effective and less toxic antifungal agents, and to diagnose early so that appropriate, timely treatment may be initiated. Despite increased risk of IFIs provided by an aggressive form immunosuppression and a growing population of high-risk patients, the advance in diagnostics, including computed tomography (CT) scan technology and laboratory adjunct markers, and the availability of newer mold-active agents have resulted in sequential improvement in the clinical outcome of IFIs [4, 5]. This study will review the progress in diagnostics and management of IFIs to outline the future directions.

2 Epidemiology and risk

The epidemiology of IFIs has substantially evolved, as transplant modalities and preventative strategies have changed over the last several decades [4, 5]. Although *Candida* infections mainly caused by *C. albicans* were recognized as common during early post-HSCT in the 1980s, the introduction of fluconazole (FLCZ) prophylaxis has reduced the overall attack rate of invasive

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candidiasis (IC) [6–8]. Mold infections, caused especially by *Aspergillus* spp., have been increasingly encountered during the 1990s. Successful use of prophylactic FLCZ has also caused a shift to FLCZ-resistant *C. glabrata* and *C. krusei*, and other molds including *Zygomycetes*, *Fusarium* spp. and *Scedosporium* spp. have emerged since the late 1990s.

Candida spp. are common inhabitants of the gastrointestinal (GI) tract and skin that mostly enter the body through a breach in the skin or mucosal membrane of the GI tract during severe neutropenia and/or mucositis. IC is described as either acute bloodstream infection or chronic hepatosplenic disease, which exclusively occurs during the pre-engraftment period (Table 1). Candidemia typically originates from either the GI tract or through intravenous catheters and mainly presents with non-specific symptoms of fever of unknown origin, a sepsis-like syndrome and multi-organ failure. It is a life-threatening infection and evolves into acute disseminated candidiasis including chorioretinitis and organ abscesses. In contrast, chronic disseminated candidiasis usually occurs after neutrophil engraftment, with increased inflammation causing fever, elevated liver enzymes and flank pain. Specific risk factors for IC include older age, prolonged neutropenia, high-dose total body irradiation, indwelling catheters, GI-tract colonization and severe GI-tract mucositis associated with conditioning or graft-versus-host disease (GVHD) [9].

Aspergillus spp. usually cause invasive sinonasal and/or pulmonary infection after inhalation or colonization into the sino-pulmonary tracts. The lung is the most common site where they produce nodular infiltrates with or without a “halo sign”, which may typically become larger and eventually cavitate to reveal “air crescent sign” with neutrophil recovery or remain as isolated nodules or dense lobular infiltrates [10]. The earliest symptoms are dry cough and low-grade fever, and pleuritic chest pain and hemoptysis can occasionally occur (Table 1). Invasive aspergillus sinusitis is aggressive and frequently complicated by skin, palate, and ocular and intracerebral extension. Pulmonary and sinus aspergillosis may occur concurrently, and a poorly controlled infection may progress to a disseminated disease, which is often suspected but rarely proved unless cerebral or cutaneous aspergillosis is diagnosed. The development of invasive aspergillosis (IA) shows bimodal distribution, one during the neutropenic period early after HSCT and the other during the receipt of corticosteroids for severe acute GVHD late after HSCT [1]. Late occurrence of IA has been increasingly observed, with a relatively decreased incidence of early-onset IA, because of a shortened neutropenic period due to the use of peripheral blood rather than bone marrow,

non-myeloablative rather than myeloablative conditioning regimens, granulocyte colony-stimulating factor and high-efficiency particulate air (HEPA) filtration [1–3]. An increased risk of IA is associated with older age, prior fungal exposure, prolonged neutropenia, CMV disease, HLA-mismatched or T cell-depleted graft, the development of GVHD and profound immune suppression due to the treatment of GVHD [1, 2].

Zygomycetes are angioinvasive and potentially disseminate systemically. The most common sites of infections are the paranasal sinuses, lung and skin, as the primary sites of infections with the potential for hematogenous dissemination in approximately one-fourth of the patients [11]. Zygomycosis has attracted particular attention, the clinical manifestation and risk in HSCT recipients of which also largely overlap with those of IA, but the outcome is poorer with an overall mortality rate of more than 50% [4, 11]. Breakthrough infections are increasingly reported during prophylaxis or treatment of IA with voriconazole (VRCZ) [12, 13].

3 Diagnosis

3.1 Revision of the definitions of IFIs

The first standard definitions for IFIs were introduced by a consensus group of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) in 2002 [14]. In the criteria, IFIs were classified into 3 levels according to the certainty of diagnosis: “proven”, “probable” and “possible”. Proven IFIs require a detection of fungus by histopathological documentation or culture of a specimen from a normally sterile site, whereas probable and possible IFIs depend on 3 elements: host factors, clinical manifestations and mycological evidence. Although the definitions are of particular value in allowing clinical researches to be compared by standardizing definitions, there remains the shortcoming that the original category “possible” potentially includes many ambiguous cases, totally different from highly suspicious cases despite the presence of neutropenia, non-specific pulmonary infiltrates and persistent fever refractory to broad-spectrum antibiotics. Therefore, the definitions were revised in 2008 [15], where the category “possible” was defined more strictly with sufficient clinical evidence to distinguish dubious cases from the more likely cases in the absence of mycological evidence. The definitions of “probable” were expanded as a reflection of advances in indirect assays that are highly specific for IFIs.

Table 1 Clinical features and treatments for candidemia/invasive candidiasis and invasive aspergillosis

Candidemia/invasive candidiasis		Invasive aspergillosis	
Common period of infections	Neutropenic period	Neutropenic period during immunosuppressive therapy for GVHD	
Major species	<i>C. albicans</i> , <i>C. krusei</i> , <i>C. glabrata</i> , <i>C. tropicalis</i> , <i>C. parapsilosis</i>	<i>A. fumigatus</i> , <i>A. flavus</i> , <i>A. niger</i> , <i>A. terreus</i> , <i>A. nidulans</i>	
Risk factors	Older age, prolonged neutropenia, high-dose TBI, indwelling catheters, GI-tract colonization and severe GI-tract mucositis	Older age, prior fungal exposure, prolonged neutropenia, CMV disease, HLA-mismatched or T cell-depleted transplantation, immunosuppressive therapy for GVHD	
Clinical manifestations	Fever, signs of sepsis, skin lesions, polyarthralgias, polymyalgias, chorioretinitis, elevated liver enzyme levels, organ abscesses (liver, kidneys)	Fever, respiratory signs or symptoms, sinus signs or symptoms, hemorrhagic stroke, cutaneous infections	
Diagnostic procedures	Ophthalmoscopy, CT, MRI, AUS, tissue biopsy, blood/puncture fluid culture, β -D-glucan	HRCT (halo sign, air-crescent sign), sinus CT, tissue biopsy, blood/sputum culture, BALF, β -D-glucan, GM ELISA	
Primary therapy	Echinocandins (preferred for <i>C. glabrata</i>) CSFG 70 mg and then 50 mg daily or MCFG 100 mg daily L-AMB 3–5 mg daily (preferred for <i>C. parapsilosis</i>)	Reference	Success rate
	Mora-Duarte [60]	Herbrecht [63]	52.8 vs 31.6%
	Kuse [59]		
	Pappas [61]		
	Pappas [61]		
	FLCZ 800 mg and then 400 mg daily (for less critically ill cases)		
Alternative therapy	VRCZ 600 mg and then 400 mg daily (for additional mold coverage) Posaconazole 800 mg and then 400 mg daily	Reference	Success rate
	Kullberg [62]	Comely [64]	1 vs 41%
Salvage therapy	No available data	Reference	Success rate
		Maertens [65]	50 vs 46%
		Walsh [67]	
		Caillot [66]	
Combination therapy	No available data	Reference	Success rate
		Kontoyannis [68]	45%
		Kontoyannis [70]	42%
		Marr [69]	52%
			Response rate
			0 vs 10%
			Overall survival
			0.42 (0.17–1.1)

3.2 Contributions of advanced diagnostic tools

Confirming the diagnosis of IFIs requires extreme caution, because it remains difficult even with various clinical manifestations, radiological findings, culture, histopathology and adjunctive tests. Improved CT technology and non-culture based diagnostic tools are expected to make great contributions to allow for prompt diagnosis.

Radiology plays an important role in the diagnosis and follow-up of IFIs, and especially high-resolution (HR) CT facilitates in detecting lung involvement at an early stage of IA. The early indicator for IA on CT scans is a “halo sign”, a macronodule surrounded by a perimeter of ground-glass opacity, which pathologically represents infarction and coagulative necrosis caused by angioinvasive aspergillosis and alveolar hemorrhage. Systemic CT screening for the “halo sign” in high-risk patients has been recommended to make a quicker diagnosis and initiate an effective antifungal treatment earlier in patients with suspected IA [10]. A large image study of IA has reported that initiation of antifungal treatment based on the identification of the “halo sign” results in a significantly better response to treatment (52 vs 29%, $P < 0.001$) and improved survival rates (71 vs 53%, $P < 0.01$) [16].

Enzyme-linked immunosorbent assays (ELISA) for galactomannan (GM) and (1,3)- β -D-glucan are useful adjunctive tests to identify IFIs and have been incorporated into the diagnostic criteria [15]. The GM ELISA test contributes to an earlier non-invasive establishment of diagnosis, which has a sensitivity of 76–100% and specificity of 90–99% (Table 2) [17–22]. The accuracy of this test is variable among different patient populations, depending on the choice of the cutoff value for defining a positive result [23]. The GM assay is more appropriate in

the case of a high pretest probability of IA. In spite of the recommended cutoff serum ratio of 1.5 by the manufacturer, lower thresholds have been investigated and a cutoff of 0.5 has been adopted for approval by the US Food and Drug Administration (FDA). False-positive reactions should be considered in patients receiving fungus-derived antibiotics such as piperacillin-tazobactam and amoxicillin-clavulanate and in patients having GI-tract mucositis due to chronic GVHD [24–26].

(1,3)- β -D-Glucan is another potential marker for detection of numerous yeasts and molds except *Cryptococcus* spp. and *Zygomycetes* [27]. (1,3)- β -D-Glucan assay has been pioneered in Japan in 1995, and three commercial kits have been widely used for the diagnosis of IFIs over a decade (Table 3) [27, 28]. Clinical usefulness of the (1,3)- β -D-glucan assay was also recognized outside of Japan, and Fungitell (Associates of Cape Cod) was approved by the FDA in 2004 [29–32]. Unfortunately, the cutoff values differ according to each manufacturer’s instruction and the measured values are totally incommensurable because they use different β -D-glucan standards and different species of horseshoe crab as a source of reagent, resulting in different affinity and reactivity to β -D-glucan. According to the manufacturer’s instructions, the cutoff values are defined as 20 pg/mL for the Fungitec G-Test MK (Seikagaku Cooperation), 11 pg/mL for the β -Glucan Test WAKO (Wako Pure Chemical) and β -G Star (Maruha Cooperation), and 80 pg/mL for Fungitell. However, the optimal cutoff values are still under clinical evaluation. Several studies have recently proposed a serum β -D-glucan level of ≥ 60 pg/mL as an appropriate cutoff value for Fungitell [32].

The detection of fungi DNA by PCR has been under investigation, the technical standardization and validation of which has not yet been attained for clinical use.

Table 2 Evaluation of cutoff values for the serum galactomannan ELISA assay

Reference	Study patients	Positive cutoff	IA	Sensitivity	Specificity	PPV	NPV
Herbrecht [17]	Hematological malignancy	1.5×1	Overall	29.4	94.8	57.7	84.9
Maertens [18]	Hematological malignancy	1.0×1	Proven	100	94.6	68.1	100
			Proven/probable	92.3	94.6	72	98.8
		1.0×2	Proven	100	98.1	85.7	100
Maertens [19]	HSCT recipients		Proven/probable	89.7	98.1	87.5	98.4
		1.0×1	Proven	94.4	85.4	58.6	98.6
		1.0×2	Proven	94.4	98.8	94.4	98.8
Kawazu [20]	Hematological malignancy	0.6×2	Overall	100	93	55	100
Maertens [21]	Hematological malignancy	0.8×1	Proven/probable	96.5	97.3	93.3	98.6
		0.5×2	Proven/probable	96.5	98.6	98.6	98.4
Maerten [22]	Hematological malignancy	1.5×1	Proven/probable	76.3	97.5	85.3	95.6
		0.5×1	Proven/probable	97.4	90.5	66.1	99.4
		0.5×2	Proven/probable	92.1	97.5	87.5	98.5

Table 3 Comparison of the 4 commercial kits for the serum (1,3)- β -D-glucan assay

Cutoff by manufacturer (pg)	Assay method	Standard β -glucan	Origin of lysate	Reference	Study patients	Positive cutoff	Sensitivity	Specificity	PPV	NPV
Fungitec G-Test MK	Kinetic chromogenic	Pachyman	<i>Tachypleus tridentatus</i>	Obayashi [27] Obayashi [28]	Proven IFIs Autopsy cases with IFIs	20 pg \times 1 30 pg \times 1 60 pg \times 1 80 pg \times 1	90 95.1 85.4 78.0	100 85.7 95.2 98.4	59 47.1 70.4 86.7	97 99.2 98.0 97.1
β -Glucan test WAKO	Kinetic turbidimetry	Carboxymethyl-curdhan	<i>Limulus polyhemus</i>	No available data						
β -G Star	End point chromogenic	Lentinan	<i>Tachypleus tridentatus</i>	No available data						
Fungitell	Kinetic chromogenic	Pachyman	<i>Limulus polyhemus</i>	Pazos [29] Pickering [30] Ostrosky-Zeichner [31] Odabashi [32]	Proven/probable IA Proven IFIs Proven/probable IFIs Proven/probable IFIs	120 pg \times 1 80 pg \times 1 60 pg \times 1 80 pg \times 1 60 pg \times 1 60 pg \times 2	87.5 93.3 69.9 64.4 100 65	89.6 77.2 87.1 92.4 90 96	70 51.9 83.8 89 43 57	96.3 97.8 75.1 73 100 97

Combining non-culture based diagnostics is an important research direction that may improve the overall predictive value of these systems.

4 Management

There are four major strategies to manage IFIs, consisting of antifungal prophylaxis, empirical therapy, presumptive therapy and treatment.

4.1 Prophylaxis

Effective prevention is made possible by protected environment and prophylactic use of antifungal agents.

4.1.1 Protected environment

Installation of HEPA filters has been recommended by the Centers for Disease Control (CDC) for high-risk patients [33], which appears to provide significant benefits to neutropenic patients. However, the effectiveness of a protected environment in preventing IFIs is still controversial. A multicenter study comparing HEPA filtration and single-room isolation with any combination of hand washing, and wearing gloves, mask and gown in 5065 HSCT recipients supported the benefits of a protected environment [34], whereas a recent meta-analysis of 16 trials did not show the significant benefits for reducing mortality rates among severely neutropenic patients with hematological malignancies and HSCT recipients [35]. There are some limitations, in that HEPA filtration cannot be applied to all patients at risk for longer periods even if it is highly effective for a limited period, and environmental fungal exposure is essentially inevitable in patients treated later or followed up as outpatients.

4.1.2 Prophylactic antifungal agents

Global prophylaxis with antifungal agents has been an exclusively common practice in transplant settings (Table 4). Prophylactic FLCZ offers a great benefit to high-risk patients, especially in preventing *Candida* infections during neutropenia. Two randomized studies performed during the early 1990s established the efficacy of FLCZ at 400 mg/day in decreasing the incidence of IFIs mainly caused by *Candida albicans* [6, 7]. An extended survival benefit beyond the period of prophylaxis was seen when FLCZ was continued up to 75 days after HSCT ($P = 0.0001$), compared to stopping prophylaxis at the time of engraftment [8]. However, the most serious limitation is its narrow spectrum of activity, which has led to the investigation of antifungal prophylaxis using mold-

Table 4 Prophylaxis and empirical therapy for invasive fungal infections

Strategy	Reference	Study drug	Comparator	Study patients	n	Incidence of IFIs	IFI-related death	Overall death	Success rate
Primary prophylaxis	Goodmann [6]	FLCZ 400 mg	Placebo	HSCT recipients	356	2.8 vs 15.8%	–	30.7 vs 26%	
	Slavin [7]	FLCZ 400 mg	Placebo	HSCT recipients	300	7 vs 18%	–	20 vs 35%	
	Marr [8]	FLCZ 400 mg	Placebo	Long-term follow-up of the study by Slavin et al. [7]	300	3 vs 20% (candidiasis only)	1 vs 14% (candidiasis only)	44 vs 57%	
Secondary prophylaxis	Winston [36]	ITCZ 400 mg	FLCZ 400 mg	HSCT recipients	138	9 vs 25%	9 vs 18%	45 vs 42%	
	Marr [37]	ITCZ 7.5 mg/kg	FLCZ 400 mg	HSCT recipients	296	7 vs 15% (on treatment)	75 vs 67% (IFI-free survival)	61 vs 69% (overall survival)	
	Glasmacher [38]	ITCZ	Placebo, no treatment, oral polyene, FLCZ	Neutropenic patients with hematological malignancy	3597	3.3 vs 5.3%	2.2 vs 3.3%	11.4 vs 11.5%	
	Cornely [41]	Posaconazole 600 mg	FLCZ 400 mg/ITCZ 400 mg	AML/MDS neutropenia	602	2 vs 8%	2 vs 5%	16 vs 22%	
	Ullmann [42]	Posaconazole 600 mg	FLCZ 400 mg	HSCT recipients with GVHD	600	5.3 vs 9.0%	1 vs 4%	25 vs 28%	
	Penack [46]	L-AMB 50 mg	No systemic prophylaxis	Neutropenic patients with hematological malignancy	132	6.7 vs 35%	3 vs 12%	5 vs 14%	
	van Burik [44]	MCFG 50 mg	FLCZ 400 mg	HSCT recipients	882	Overall success rate	80.0 vs 73.5%		
	Maittuzzi [45]	CSFG 50 mg	ITCZ 200 mg iv	AML/high-risk MDS	192	Overall success rate	52 vs 51%		
	Wingard [43]	VRCZ 400 mg	FLCZ 400 mg	HSCT recipients	600	IFI-free survival rate	78 vs 76% at day 180		
	de Fabritius [47]	CSFG 50 mg		HSCT recipients	18	Outcome: stable 4, improved 12, progressed 2 at day 30			
Empirical therapy	Cordonnier [48]	VRCZ 400 mg		Hematological malignancy	11	Outcome: no relapse, and 1 treatment delay			
	Cordonnier [49]	VRCZ 400 mg po or 8 mg/kg iv		HSCT recipients	45	Outcome: incidence of IFIs 7%			
	Walsh [51]	L-AMB	AMB	Patients with persistent fever and neutropenia	687	3.2 vs 7.8%			50 vs 49%
	Walsh [52]	VRCZ	AMB		837	1.9 vs 5.0%			26.0 vs 30.6%
	Boogaerts [53]	ITCZ	AMB		384	2.7 vs 2.7%			47 vs 38%
	Walsh [54]	CSFG	L-AMB		1095	5.2 vs 4.5%			33.9 vs 33.7%

active agents with a broader spectrum of antifungal activity including *non-albicans Candida* spp. and molds.

Itraconazole (ITCZ) is a synthetic triazole, which is available as oral and parenteral dosage forms. Two prophylactic trials evaluated ITCZ solution compared with FLCZ in HSCT recipients [36, 37]. In a randomized trial comparing ITCZ at 400 mg/day versus FLCZ at 400 mg/day for 100 days after HSCT among 138 allo-HSCT recipients, ITCZ reduced IFIs more effectively ($P = 0.01$), but failed to improve an attributable mortality [36]. Another trial compared intravenous ITCZ at 200 mg/day or oral suspension at 7.5 mg/kg/day with FLCZ at 400 mg/day in 296 allo-HSCT recipients. A statistically significant reduction of invasive mold infections was achieved only in the subset of patients who tolerated the drug ($P = 0.03$), because prophylaxis was associated with a higher rate of toxicity and gastrointestinal intolerance leading to 36% withdrawal rate [37]. This study also documented higher toxic death rate under concomitant use of cyclophosphamide. Although a meta-analysis of 13 ITCZ prophylaxis trials in neutropenic patients with hematological malignancies showed both successful reductions in the rate of IFIs ($P = 0.002$) and IFI-related death ($P = 0.04$), a significant effect was seen only in patients who received oral solution and not in patients receiving capsules [38]. The bioavailability of ITCZ depends on the formulation and reliable serum concentrations are constantly provided by intravenous (iv) formulation or oral suspension than capsules. A correlation between the ITCZ levels greater than 250 or 500 ng/mL and increased efficacy is suggested by limited evidence [39, 40]. ITCZ has important interactions with several drugs, especially cytochrome P450-metabolized drugs including calcineurin inhibitors such as cyclosporine A and tacrolimus.

Posaconazole is suitable for prophylaxis especially in high-risk patients, despite being available only as an oral formulation. The efficacy and safety of posaconazole have been evaluated in two large studies comparing it to FLCZ, leading to FDA approval of the drug for prophylaxis in febrile neutropenia patients with hematological malignancies and allo-HSCT recipients with GVHD [41, 42]. One study evaluated posaconazole 600 mg/day by comparing with FLCZ or ITCZ at 400 mg/day each in 602 patients with acute myeloid leukemia or myelodysplastic syndrome during febrile neutropenia [41]. It reported a reduced incidence of IFIs ($P < 0.001$) and trends toward improved survival ($P = 0.04$) in the posaconazole arm. The other study randomized 600 allo-HSCT recipients with GVHD to receive either posaconazole at 600 mg/day or FLCZ at 400 mg/day, which resulted in fewer overall IFIs ($P = 0.07$) and IA ($P = 0.006$), and significantly lower IFI-related mortality ($P = 0.046$) in the posaconazole arm [42].

Voriconazole (VRCZ) is also attractive, although little data are available from sufficiently powered randomized trials. In a large randomized trial only presented in the abstract form comparing prophylactic VRCZ to FLCZ in 600 HSCT recipients, VRCZ tended to decrease the incidence of IFIs ($P = 0.11$), especially IA ($P = 0.05$), despite failing to produce fungal-free survival ($P = 0.72$) [43]. Adverse reactions, such as visual disturbance and liver toxicity, and drug interactions with multiple drugs including calcineurin inhibitors require consideration. Another major concern about widespread use of VRCZ prophylaxis is the emergence of zygomycosis [12, 13], although it is still controversial whether a causal relationship exists. It is preferable to monitor plasma VRCZ levels, because serum concentrations are highly variable between patients and the levels of some patients are low during oral dosing of 200 mg twice daily [13].

Echinocandins and polyenes are also considered candidates, despite their potential utilities being limited by the administration route. Micafungin (MCFG) at 50 mg/day has been compared to FLCZ at 400 mg/day in a large double-blind trial on 882 HSCT recipients. MCFG prophylaxis during neutropenia was associated with greater treatment success, compared to FLCZ prophylaxis ($P = 0.03$), leading to FDA approval for the use as prophylaxis in this setting [44]. A comparative study of caspofungin (CSFG) at 50 mg/day with intravenous ITCZ at 200 mg/day showed similar efficacy and safety among patients with hematological malignancies ($P = 0.92$) [45].

The utility of amphotericin B deoxychoate (AMB) as prophylaxis has been historically limited by excessive nephrotoxicities and infusion-related reactions, and the insufficient power of available studies hampers the use of lipid formulation of amphotericin B as part of a prophylactic strategy. However, a recent study evaluating intermittent application of low-dose liposomal AMB (L-AMB) at 50 mg every other day in 132 neutropenic patients reported a significantly decreased incidence of IFIs ($P = 0.001$) in the L-AMB arm, without grade 3–4 toxicities [46].

4.1.3 Recommended prophylactic strategy

Recommended prophylactic strategies vary according to patient's risk and situation, and post-transplant period. Choice of a particular antifungal agent for prophylaxis also depends on its availability and toxicity, fungal pathogens that are a great threat, local fungal flora and susceptibility patterns.

4.1.3.1 During neutropenia, early period after HSCT
Among patients who stay in the HEPA-filtered rooms and frequently monitored with radiography, serological

examinations and culture tests, FLCZ may be used as prophylaxis for the time from conditioning until development of severe GVHD, because of its lower toxicity. In contrast, anti-mold azoles and echinocandins appear to be preferred for patients without HEPA filtrations, high-risk patients against mold infections or if an institution's incidence of mold infections is high. ITCZ and VRCZ have important side effects and drug–drug interactions, and therefore may be recommended only in patients who can tolerate the drug and are not at increased risk for significant drug interactions.

4.1.3.2 During immunosuppressive therapy for GVHD, late after HSCT In patients with acute GVHD treated with high-dose corticosteroids or those with severe chronic GVHD necessitating treatment with multiple immunosuppressive agents, it remains inconclusive whether anti-mold prophylaxis achieves a better outcome, compared to a prompt initiation of treatment based on early detection of IFIs. But at least in outpatients, the prophylaxis may be indicated because of the difficulty in early detection. Oral anti-mold azoles are favored candidates for antifungal prophylaxis in outpatients, while the need for parenteral administration is a disadvantage for echinocandin used as prolonged prophylaxis. Oral posaconazole seems to be most suitable for long-term use, because data advocating ITCZ prophylaxis are less conclusive and the data quality of VRCZ prophylaxis is currently inadequate.

4.1.3.3 Secondary prophylaxis For patients with a history of IFIs, prophylactic antifungal agents should be considered to prevent relapse of previously treated IFIs, because recurrence of infections is extremely high and associated with a very poor prognosis. Secondary prophylaxis for IA appears to be successful when anti-mold azoles, echinocandins or L-AMB are given [47–49]. The latest study in abstract form evaluating the efficacy of VRCZ as secondary prophylaxis in 45 HSCT recipients with previous proven or probable IFI reported a lower incidence than the expected rate (incidence of IFIs 7%) [49].

4.2 Empirical therapy

Empirical antifungal therapy is considered standard practice in neutropenic patients with persistent or recurrent fever while on broad-spectrum antibiotics and codified in the Infectious Disease Society of America (IDSA) guideline [50]. However, this strategy is based on two, old randomized controlled trials published in the 1980s, before the era of FLCZ prophylaxis. Careful attention is required while applying the empirical therapy in a transplant setting, because most of the recipients stay in a HEPA-filtered

room and receive prophylactic FLCZ during the neutropenic period. Non-albicans *Candida* or molds including *Aspergillus* spp. should be considered as pathogens of IFIs while selecting antifungal agents. While AMB has been used as the golden standard agent for empirical antifungal therapy until recently, L-AMB, ITCZ and echinocandins have become applicable with almost equivalent efficacy and with less toxicity [51–54]. In a randomized multicenter trial comparing L-AMB with AMB in patients with neutropenia and persistent fever, toxicities were significantly less frequent among patients treated with L-AMB than among those treated with AMB ($P = 0.009$), with an equivalent success rate between both groups [51]. Although the randomized comparison of VRCZ with L-AMB failed to meet the predefined margin for non-inferiority, the rate of breakthrough infections was significantly lower in the VRCZ arm ($P = 0.02$), suggesting the potential use of VRCZ for empirical therapy [52]. CSFG was shown to be equivalent to L-AMB in preventing breakthrough infections [54].

However, the clinical significance of empirical therapy for IFIs based on persistent febrile neutropenia (FN) has become less clear in transplant settings where a relatively rapid recovery of neutrophils can be expected. Based on specific symptoms and examination outcomes, non-albicans *Candida* infections should be suspected in patients with severe mucositis and/or prolonged diarrhea, whereas mold-active agents should be used in patients with respiratory and/or sinonasal symptoms and abnormal radiological findings suspicious of IA.

4.3 Presumptive therapy

Although empirical therapy has been the standard practice for persistent FN, overtreatment has emerged as a major shortcoming resulting in increased toxicity and treatment-related cost. This might be overcome by a presumptive approach where treatment is only initiated when non-culture based microbiological tests are positive and/or radiological findings suggestive of IFIs are detected. Maertens et al. [55] assessed the feasibility of a presumptive therapy with a combined serum GM assay and HRCT against IA in neutropenic patients with hematological malignancy. They started L-AMB for patients with two consecutive positive galactomannan tests or with CT findings suggestive of IFIs, regardless of the presence or absence of FN. This presumptive therapy successfully reduced the use of anti-*Aspergillus* agents compared to empirical therapy from 35 to 7.7% (a reduction rate 78%) and did not fail to detect IA. In a recent study by Oshima et al. [56], suspected cases of IA were successfully treated with a presumptive therapy using serum GM assay and/or β -D-glucan and CT and/or X-ray in HSCT recipients receiving FLCZ prophylaxis in

HEPA-filtered rooms. These results may propose a necessity to reevaluate the merits of routine initiation of empirical therapy in neutropenic patients with persistent fever.

4.4 Treatment

Although AMB is highly effective and has been the gold standard historically for the treatment of IFIs, newer antifungal agents offer advantages in efficacy and toxicity. Hence, the IDSA released an update in its treatment guideline for *Candida* and *Aspergillus* infections [57, 58]. The IDSA guideline for candidiasis, updated in 2009, lists FLCZ, L-AMB, echinocandins and VRCZ as the preferred treatment before specific identification (Table 1) [59–62]. Echinocandins or L-AMB are preferred as primary therapy; echinocandins have especially found their greatest use, because a series of randomized, blinded, controlled trials comparing echinocandins with comparator agents have shown similar rate of success and minimal toxicity associated with the echinocandins [59, 60]. The only trial that compared the efficacy between two different echinocandins reported equivalent response rates and the two different dosages of MCFG showed similar response rates, leading to the conclusion that 100 mg daily of MCFG was appropriate for candidemia [61]. FLCZ is recommended for patients without recent azole exposure and who are not critically ill, while VRCZ for patients who need additional coverage of molds. After specific identification, an echinocandin or L-AMB is preferred for infections due to *C. glabrata* or *C. parapsilosis*, respectively, while all but FLCZ are applicable for infections due to *C. krusei*.

The updated guideline for aspergillosis recommends VRCZ as a first-line therapy for IA and L-AMB as an alternative (Table 1) [63, 64], based on a randomized study of VRCZ with AMB for primary treatment of IA suggesting the superiority of VRCZ over AMB with higher response rates and an improved survival rate [63]. Organ function should be considered in choosing an appropriate first-line agent, because the presence of liver dysfunction complicates VRCZ therapy and renal dysfunction complicates L-AMB therapy. Although there have been no randomized, controlled trials of any echinocandin as primary therapy, CSFG has received FDA approval for treatment of IA and was granted for second-line therapy [65]. CSFG, ITCZ and posaconazole have been shown to achieve 40–50% complete or partial responses when given to patients who have failed or are intolerant to VRCZ or AMB [65–67].

The combination of two agents with a different spectrum of activity may lead to synergistic activity and decreased toxicity. Triazoles and polyenes target the lipomembrane of the fungus, whereas the echinocandins target the (1–3)- β -D-glucan synthesis in the fungal cell wall. Concurrent

administration of echinocandins with either VRCZ or polyenes showed potential utility, especially for IA. A retrospective review has reported benefit with a combination of CSFG and L-AMB [68], and another later study comparing VRCZ and CSFG with VRCZ alone for salvage therapy after AMB failure in IA has shown significantly lower mortality rate ($P = 0.048$) and fewer IA-related deaths ($P = 0.024$) in patients who were receiving the combination therapy compared to VRCZ alone [69]. However, the other study concluded that the addition of ITCZ to L-AMB could not improve the outcome of IA [70] and therefore the efficacy of this strategy is still uncertain.

5 Conclusion

Despite these remarkable progresses, IFIs are an ongoing threat, and therefore further investigations are imperative to reduce IFI-related mortality rates in the transplant setting. Optimization of preventative strategies by choosing more effective and less toxic antifungal agents as the situation demands, combination therapy with these agents, establishment of optimal cutoff of existing diagnostic tests and development of novel methods for early detection might show the direction that is best for improving the overall outcome of HSCT. Therapeutic drug monitoring also represents an important component, especially for the use of triazoles, because of the marked inter- and inpatient variability.

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Brief report

Successful engraftment after reduced-intensity umbilical cord blood transplantation for myelofibrosis

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Although allogeneic hematopoietic stem cell transplantation has recently been applied to patients with myelofibrosis with reproducible engraftment and resolution of marrow fibrosis, no data describe the outcomes of umbilical cord blood transplantation. We describe 14 patients with primary (n = 1) and secondary myelofibrosis (n = 13) who underwent reduced-

intensity umbilical cord blood transplantation. Conditioning regimens included fludarabine and graft-versus-host disease prophylaxis composed cyclosporine/tacrolimus alone (n = 6) or a combination of tacrolimus and mycophenolate mofetil (n = 8). Thirteen patients achieved neutrophil engraftment at a median of 23 days. The cumulative incidence of neutrophil

and platelet engraftment was 92.9% at day 60 and 42.9% at day 100, respectively. Posttransplantation chimerism analysis showed full donor type in all patients at a median of 14 days. The use of umbilical cord blood could be feasible even for patients with severe marrow fibrosis, from the viewpoint of donor cell engraftment. (*Blood*. 2010;116(4):649-652)

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is considered the only curative therapy for primary myelofibrosis (MF) and MF secondary to hematologic malignancies.¹ Myeloablative conditioning regimens are associated with high rates of transplantation-related mortality (TRM), especially among elderly patients.²⁻⁴ Recent reports indicate that reduced-intensity conditioning (RIC) regimens can improve outcomes in such patients.⁵⁻⁸ These reports also confirm the safety and effectiveness of bone marrow (BM) and mobilized peripheral blood stem cells (PBSCs) from matched related or unrelated donors as stem cell sources. In contrast, the feasibility of umbilical cord blood transplantation (CBT) for MF is unknown.

CBT is a valuable alternative to allo-HSCT for treating patients with hematologic diseases who do not have matched related or unrelated donors and who need urgent transplantation.⁹⁻¹² On the other hand, engraftment delay or failure is one of the most critical issues that can arise after CBT. The limited doses of total nucleated cells and CD34⁺ cells in umbilical cord blood and a human leukocyte antigen (HLA) disparity influence the kinetics of hematopoietic recovery.¹³⁻¹⁵ Considering these disadvantages of CBT, delayed engraftment or engraftment failure is a great concern for MF patients who undergo CBT.¹⁶ The goal of this study is to evaluate the feasibility of reduced-intensity CBT (RI-CBT) for MF.

Methods

The records of all patients who underwent RI-CBT at Toranomon Hospital from August 2003 and December 2008 were reviewed to identify patients who had histologically confirmed MF before starting the conditioning

regimen. Marrow fibrosis was assessed on silver-stained BM trephine biopsies and classified into 4 grades according to the World Health Organization classification.¹⁷ All the patients were incurable using conventional approaches and lacked an HLA-identical sibling or a suitable unrelated donor from the Japan Marrow Donor Program. Cord blood units serologically matching more than or equal to 4 of 6 HLA antigens and containing at least 1.8×10^7 nucleated cells/kg of recipient body weight before freezing were obtained from the Japan Cord Blood Bank Network. Conditioning regimens were determined at the discretion of each physician according to the patients' disease, disease status, and history of prior therapy. Information about baseline demographics, clinical characteristics, transplantation, and its outcome were collected from medical records. Assessment of engraftment, chimerism (one or more times a week), pre-engraftment immune reactions, graft-versus-host disease (GVHD), and supportive care during transplantation were performed as previously reported.¹⁸⁻²⁰ Cumulative incidences were estimated for neutrophil and platelet engraftment. Overall survival was estimated using the Kaplan-Meier method, taking the interval from date of transplantation to death or last contact.²¹ The Institutional Review Board of Toranomon Hospital approved the study, and written informed consent was provided by all patients to use their records in accordance with the Declaration of Helsinki.

Results and discussion

Fourteen MF patients (median age, 57.5 years; range, 46-72 years) were extracted. Table 1 shows the clinical characteristics of the patients. They had primary MF (n = 1), leukemic transformation from MF secondary to polycythemia vera or essential thrombocytosis (n = 2), or MF secondary to acute myeloid leukemia (AML; n = 11; AML with multilineage dysplasia in all patients except for one with de novo AML). All but one patient had the highest-grade

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Table 1. Patient characteristics

Patient no.	Age, y/sex	Diagnosis	Disease status	Time from diagnosis to transplantation, d	Pretransplantation MF grade	Splenomegaly	Cytogenetics
1	55/M	AML/MF/ET	PIF	1732	3	Yes	Normal
2	53/M	PMF	Untreated	307	3	Yes	NA
3	61/M	AML/MDS	PIF	116	3	Yes	Complex*
4	51/F	AML/MDS	PIF	740	3	Yes	Normal
5	61/F	AML/MDS	PIF	227	3	Yes	NA
6	55/M	AML/MDS	Untreated	299	3	Yes	Complex
7	46/M	AML/MDS	Untreated	600	3	Yes	NA
8	58/M	AML/MDS	Untreated	544	3	Yes	Complex
9	67/F	AML/MF/PV	Untreated	150	3	Yes	t(3;3)(q21;q26), -7
10	53/M	De novo AML	PIF	111	3	No	Complex
11	57/F	AML/MDS	Untreated	352	3	Yes	Complex with t(9;22)(q34;q11)
12	62/M	AML/MDS	Untreated	147	3	Yes	add(1)(p32), -7
13	72/F	AML/MDS	PIF	329	2	No	Complex with t(9;22)(q34;q11)
14	66/M	AML/MDS	Untreated	92	3	No	Normal

AML indicate acute myeloid leukemia; MF, myelofibrosis; ET, essential thrombocythemia; PIF, primary induction failure; PMF, primary myelofibrosis; AML/MDS, acute myeloid leukemia with multilineage dysplasia; NA, not available; and PV, polycythemia vera.

*Complex karyotype was defined as 3 or more abnormalities at pretransplantation evaluation.

MF. The median time from diagnosis to transplantation was 303 days (range, 92-1732 days). Table 2 shows the transplantation characteristics. All received purine analog-based conditioning regimens composing fludarabine phosphate (125-180 mg/m²), melphalan (80-140 mg/m²), or intravenous busulfan (12.8 mg/kg) and 0 to 8 Gy of total body irradiation. GVHD prophylaxis included tacrolimus and mycophenolate mofetil for 8 patients, tacrolimus, or cyclosporine A alone in 6. Neutrophil and platelet engraftment was achieved in 13 and 6 patients, respectively, of the 14 patients. The median time to engraftment was 23 days (range, 14-43 days) and 53 days (range, 44-102 days) for neutrophils and platelets, respectively. The cumulative incidence of neutrophil engraftment at day 60 and platelet engraftment at day 100 was 92.9% and 42.9%, respectively (Figure 1A-B). Chimerism analysis of the peripheral blood of 8 patients and the BM of 6 showed that donor chimerism was complete (donor > 90%) in all of them. The median length of time required to achieve complete donor chimerism was 14 days (range, 7-33 days; Figure 1A). Of the 14 patients, 9 (64%) developed pre-engraftment immune reactions. Five (36%) developed acute GVHD grades 2 to 4. No extensive chronic GVHD was observed in 6 evaluable patients (Table 3). Five patients remained alive at last contact, representing an estimated probability of

overall survival of 28.6% at 4 years (Figure 1C). All the patients who could not achieve platelet engraftment died, whereas 4 of 7 patients (57%) who achieved platelet engraftment survived. In 9 patients who died after RI-CBT, 5 patients died of relapsed leukemia. Non-relapse-related causes of death composed infection (n = 2), GVHD (n = 1), and multiple organ failure (n = 1). Marrow fibrosis disappeared in 2 evaluated patients who survived beyond 100 days.

This study demonstrated that umbilical cord blood results in successful engraftment, even for patients with severe marrow fibrosis in the setting of the RIC regimen, which was similar to that of other stem cell sources, such as BM and PBSCs.^{2-8,22} Although marrow fibrosis has historically been considered as a relative contraindication to transplantation because of concerns over an insufficient and/or dysfunctional niche in which allogeneic hematopoietic stem cell engraftment may proceed, recent outcomes of allo-HSCT for MF support the concept that marrow fibrosis is not an absolute barrier to allogeneic hematopoietic stem cell engraftment.¹ However, data from these reports are limited to transplantations with BM and PBSCs, and no information is available about umbilical cord blood. Delayed hematopoietic recovery and low engraftment rate, perhaps because of limited infused cell doses and

Table 2. Transplantation characteristics

Patient no.	TNC, ×10 ⁷ /kg	CD34 ⁺ , ×10 ⁵ /kg	Sex match	HLA match	Blood type match	Conditioning regimen	GVHD prophylaxis
1	2.52	0.823	MM	4/6	MM	F125/M80/TBI4	CsA
2	2.62	0.678	MM	4/6	MM	F125/M80/TBI4 + SRT	TAC
3	3.17	1.60	Match	4/6	Match	F125/M80/TBI4	TAC
4	2.43	NA	MM	4/6	Match	F125/M80/TBI4	TAC
5	3.94	2.26	MM	5/6	Match	F180/M140	TAC/MMF
6	2.31	0.887	MM	4/6	MM	F125/M80/TBI4	TAC
7	2.72	1.03	Match	4/6	MM	F125/Mel140/TBI4	TAC/MMF
8	2.46	0.773	MM	4/6	Match	F180/M140	TAC
9	1.99	1.24	MM	4/6	MM	F125/M80/TBI4 + SRT	TAC/MMF
10	3.25	0.547	MM	4/6	Match	F125/M140/TBI4	TAC/MMF
11	3.31	1.31	MM	4/6	Match	F125/M80/TBI8	TAC/MMF
12	2.37	0.873	MM	4/6	MM	F125/M80/TBI8	TAC/MMF
13	2.51	0.993	MM	4/6	Match	Flu180/B12.8/TBI2	TAC/MMF
14	2.50	0.554	MM	5/6	Match	F125/M120	TAC/MMF

TNC indicates total nucleated cell count; MM, mismatch; F, fludarabine (mg/m²); M, melphalan (mg/m²); TBI, total body irradiation; CsA, cyclosporine; SRT, splenic radiation; TAC, tacrolimus; MMF, mycophenolate mofetil; and B, intravenous busulfan (mg/kg).

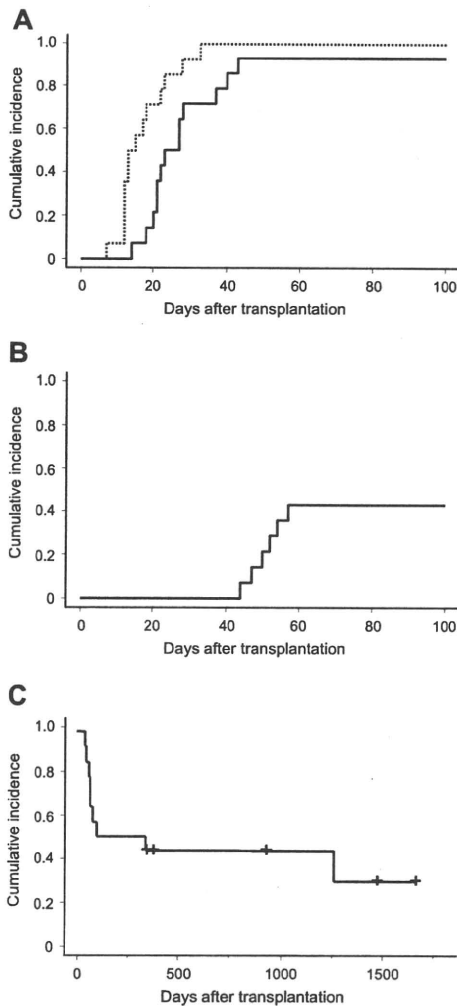


Figure 1. Cumulative incidence of engraftment. (A) Solid and broken lines indicate cumulative incidence of neutrophil engraftment and complete donor chimerism, respectively. (B) Cumulative incidence of platelet engraftment. (C) Overall survival.

HLA disparities, might limit the use of umbilical cord blood in these cases.^{13-15,19} However, the present study demonstrated an equivalent or superior engraftment rate after CBT compared with allo-HSCT using other stem cell sources.¹⁻⁸ We also confirmed an early chimerism switching in the present study. All 14 patients achieved complete donor chimerism at a median of 14 days, which was much earlier than that with neutrophil engraftment. Moreover, we histologically confirmed that RI-CBT had the potential to cure marrow fibrosis in 2 evaluated patients. These data suggest that RI-CBT is an encouraging strategy for treating MF.

Despite successful engraftment, overall survival was poor in the present study compared with previous reports. However, this result does not eliminate the feasibility of RI-CBT for MF patients. Our patient series included only one primary MF. In 13 of 14 patients, MF coexisted with AML simultaneously. High prevalence of concurrent AML with MF in the present study probably made overall survival poorer. However, MF with AML is also challenging issues in real clinical settings. Physicians occasionally face rapidly growing AML cases with concurrent marrow fibrosis, especially in the elderly, for whom urgent allo-HSCT is the only curative therapy. For those patients, CBT is attractive because of its accessibility. In this viewpoint, we think that the feasibility of RI-CBT suggested in the present study is encouraging.

In conclusion, our data suggest that RI-CBT is feasible, even for patients with severe marrow fibrosis, from the viewpoint of donor cell engraftment. Especially for MF with AML, further improvements are required in the next place to overcome poor survival resulting from relapse.

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Table 3. Outcome of RI-CBT

Patient no.	Neutrophil engraftment, d	Platelet engraftment, d	Pre-engraftment immune reactions*	aGVHD 2-4	aGVHD 3-4	cGVHD	Survival	Survival from transplantation, d	Cause of death
1	27	52	No	Yes	No	No	Dead	1264	Relapse
2	22	54	Yes	No	No	NE	Alive	1672	NA
3	23	Not engrafted	Yes	Yes	Yes	NE	Dead	68	Infection
4	40	102	Yes	Yes	No	Limited	Alive	1481	NA
5	18	44	Yes	No	No	No	Dead	344	Relapse
6	14	Not engrafted	Yes	No	No	NE	Dead	78	Relapse
7	21	57	Yes	Yes	Yes	Limited	Alive	937	NA
8	Not engrafted	Not engrafted	No	No	No	NE	Dead	42	Infection
9	37	Not engrafted	Yes	No	No	NE	Dead	45	MOF
10	28	Not engrafted	Yes	Yes	Yes	NE	Dead	64	GVHD
11	27	Not engrafted	Yes	No	No	NE	Dead	61	Relapse
12	43	NA	No	No	No	Limited	Alive	392	NA
13	21	47	No	No	No	Limited	Alive	355	NA
14	20	50	No	No	No	NE	Dead	100	Relapse

aGVHD indicates acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; NE, not evaluable; NA, not applicable; and MOF, multiple organ failure.

*Pre-engraftment immune reactions were diagnosed when febrile patients developed skin eruption, diarrhea, jaundice, or body weight gain of more than 10% of baseline, with no direct evidence of infection or adverse effects of medication, developing more than 6 days before engraftment.¹⁸

Authorship

Contribution: S. Takagi performed transplantation, analyzed extracted data, and contributed to writing the paper; Y.O. analyzed histologic sections; N.U., K.T., K.I., M.T., H.Y., Y.A.-M., K.M., A.W., and S.M. performed transplantation and contributed to writing the paper; N.M. performed transplantation and supported

statistical analysis; K.O. reviewed histologic sections and contributed to writing the paper; and S. Taniguchi reviewed the study method and organized this study.

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Title: Successful sustained engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with severe aplastic anemia

Running title: RI-UCBT for adults with SAA

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Abstract

We retrospectively analyzed 12 consecutive adult severe aplastic anemia (SAA) patients who received unrelated umbilical cord blood transplantation after a reduced-intensity conditioning regimen (RI-UCBT). The conditioning regimen consisted of fludarabine 125 mg/m², melphalan 80 mg/m², and 4 Gy of total body irradiation. The median infused total nucleated cell number and CD34⁺ cell number were 2.50 x 10⁷/kg and 0.76 x 10⁵/kg, respectively. Eleven of the twelve patients achieved primary neutrophil and platelet engraftment. All patients who achieved engraftment had complete hematological recovery with complete donor chimerism, except for one patient who developed late graft failure 3 years after RI-UCBT. Two of the twelve patients died from idiopathic pneumonia syndrome, and the remaining 10 patients are alive, having survived for a median of 36 months. Our encouraging results indicate that RI-UCBT may become a viable therapeutic option for adult SAA patients who lack suitable HLA-matched donors and fail immunosuppressive therapy.

Key words:

severe aplastic anemia; adult patients; umbilical cord blood transplantation; reduced intensity conditioning regimen;

Introduction

Bone marrow transplantation from an HLA-matched sibling is recommended as first-line therapy for younger patients with severe aplastic anemia (SAA) ^{1,2}. However, many patients lack HLA-matched sibling donors. Bone marrow transplantation from an HLA-matched unrelated donor has been an alternative therapeutic option for patients who fail one or more courses of immunosuppressive therapy (IST), but high rates of graft failure (GF), graft-versus-host disease (GVHD), and infection still remains to be solved ³. The number of unrelated umbilical cord blood transplantation (UCBT) has been increasing ⁴. However, little information has been available on whether UCBT is feasible for SAA patients. We reported successful urgent UCBT using reduced-intensity (RI) conditioning for a 70-year-old SAA patient in 2003 ⁵. Here we present successful sustained engraftment of 11 consecutive patients with SAA who received RI-UCBT with the same RI conditioning regimen following the first report.

Methods

This study included 12 consecutive adult patients with acquired SAA who underwent RI-UCBT at our institute from September 2002 through January 2009. The patients' characteristics and umbilical cord blood (UCB) units are summarized in Table 1A. Their median age was 49 (range, 20-70) years. Four cases of severe, 6 of very severe, and 2 of fulminant type were included according to criteria as previously reported^{2,6}. Fulminant type was defined as no neutrophils in the peripheral blood at diagnosis despite administration of granulocyte-colony stimulating factor. Ten patients, except for the two patients with fulminant type, had failed at least one course of IST. All patients gave their written informed consent in accordance with the Declaration of Helsinki, and the study was approved by the Toramori Hospital institutional review board. UCB units were obtained from the Japanese Cord Blood Bank Network, and single UCB unit was infused in all the studied patients. All UCB units were serologically typed for HLA-A, -B, and -DR antigen before selection, and were tested by high-resolution DNA typing before transplant. The degree of mismatch is expressed by using antigen level at HLA-A and -B, and allele level at DRB1. ABO incompatibility was not incorporated as one of the factors used in CB unit selection. The median total nucleated cell (TNC) number and CD34⁺ cell number at