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Presumptive treatment strategy for aspergillosis in allogeneic haematopoietic stem cell transplant recipients

Kumi Oshima[1,2], Yoshinobu Kanda[1,2], Yuki Asano-Mori[3], Nahoko Nishimoto[1], Shunya Arai[1], Sumimasa Nagai[1], Hiroyuki Sato[1], Takuro Watanabe[1], Noriko Hosoya[2], Koji Izutsu[1], Takashi Asai[1], Akira Hangaishi[1], Toru Motokura[1], Shigeru Chiba[2] and Mineo Kurokawa[1]*

[1]Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; [2]Department of Cell Therapy and Transplantation Medicine, University of Tokyo Hospital, Tokyo, Japan; [3]Department of Hematology, Japanese Red Cross Medical Center, Tokyo, Japan

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***Background*: The onset of invasive aspergillosis (IA) after allogeneic haematopoietic stem cell transplantation (HSCT) is bimodal. However, IA early after HSCT has become less frequent due to the shortened neutropenic period, and the clinical significance of empirical treatment for aspergillosis based on persistent febrile neutropenia (FN) became less clear. Therefore, we started a presumptive treatment strategy, in which anti-*Aspergillus* agents were started when patients developed positive serum test and/or infiltrates or nodules on X-ray or CT-scan associated with persistent FN, in 2002.**

***Methods*: We retrospectively reviewed the records of 114 adult patients who underwent allogeneic HSCT between September 2002 and December 2005 in high-efficiency particulate air-filtered clean rooms. Fluconazole was given as anti-*Candida* prophylaxis. The primary endpoint was the development of early IA, which was defined as probable or proven IA according to the EORTC/MSG criteria that developed between the day of HSCT and 7 days after engraftment.**

***Results*: Among 73 patients who experienced persistent FN for 7 days or longer, anti-*Aspergillus* agents were empirically started in 13 patients at the discretion of attending physicians, whereas 60 patients actually followed presumptive treatment strategy. Only 4 of 60 patients received anti-*Aspergillus* agents. Two patients in the presumptive group developed early IA, but were successfully treated with anti-*Aspergillus* agents started after the diagnosis of IA.**

***Conclusions*: These findings suggested the feasibility of a presumptive treatment strategy for aspergillosis in HSCT recipients. A randomized controlled trial is warranted to compare empirical and presumptive anti-*Aspergillus* strategy in allogeneic HSCT recipients.**

Keywords: empirical treatment, febrile neutropenia, invasive aspergillosis

Introduction

Invasive fungal infection (IFI) is one of the leading causes of transplant-related mortality and its incidence in allogeneic haematopoietic stem cell transplantation (HSCT) recipients ranges from 8 to 15%.[1–3] Invasive aspergillosis (IA) is the most common IFI after allogeneic HSCT.[1–4] The development of IA after allogeneic HSCT shows bimodal distribution, one in the neutropenic period early after HSCT and the other 2–3 months after HSCT when patients are taking glucocorticosteroid for acute graft-versus-host disease (GVHD).[1,3,5,6] IA early after HSCT, however, has become less frequent because of the shortened neutropenic period due to the use of peripheral blood stem cells (PBSC), granulocyte colony-stimulating factor (G-CSF) and high-efficiency particulate air (HEPA) filtration and/or laminar air flow.[5–12] Therefore, the clinical significance of empirical treatment for aspergillosis based on persistent febrile neutropenia (FN) has become less clear, although it is supported by old evidence and recent guidelines.[11–16]

Our transplantation unit moved to a new building in September 2002. At the same time, we changed the strategy against aspergillosis during the neutropenic period from

*Corresponding author. Tel: +81-3-5800-9092; Fax: +81-3-5840-8667; E-mail: kurokawa-tky@umin.ac.jp

empirical strategy to presumptive strategy, in which anti-*Aspergillus* agents were started based on positive serum test and/or infiltrates or nodules on X-ray or CT-scan associated with persistent FN.[17,18] In this report, we reviewed the outcomes of 114 patients who underwent allogeneic HSCT in the new transplant unit and evaluated the feasibility of the presumptive strategy during the early neutropenic period after allogeneic HSCT.

Materials and methods

Study patients

Medical records of 124 consecutive adult patients who underwent allogeneic HSCT at the University of Tokyo Hospital between September 2002 and December 2005 were reviewed. All patients received prophylactic antifungal agents. Of the 124 patients, 114 who received fluconazole at 200 mg/day as anti-*Candida* prophylaxis were included in this study.[19,20] The remaining 10 patients were excluded from this study, because they had recent IA and prophylactically received anti-*Aspergillus* agents including micafungin and itraconazole. Characteristics of the 114 patients are summarized in Table 1. The median age was 43 years (range, 20–66 years). Patients' underlying diseases included acute myeloblastic leukaemia (AML), acute lymphoblastic leukaemia (ALL), chronic myelogenous leukaemia (CML), myelodysplastic syndrome (MDS), non-Hodgkin lymphoma (NHL), aplastic anaemia (AA) and so on. Standard-risk diseases were defined as AML/ALL in first complete remission (CR1) or CR2, CML in first chronic phase (CP1) or CP2, chemosensitive NHL, MDS in refractory anaemia or refractory anaemia with ringed sideroblasts and non-malignant haematological disorders. All other diseases were classified as high-risk diseases. Eight patients had received previous autologous or allogeneic stem cell transplantation. Two patients had a previous history of probable IA prior to HSCT.

Table 1. Characteristics of the 114 patients who were included in this study

Characteristic	
Median recipient age, years (range)	43 (20–66)
Male/female	66/48
Underlying diagnosis, *n* (%)	
AML	30 (26.3)
ALL	20 (17.5)
AUL	1 (0.9)
CML	13 (11.4)
MDS	13 (11.4)
NHL	16 (14.0)
ATL	4 (3.5)
AA	7 (6.1)
Others	10 (8.8)
IA before HSCT, *n* (%)	2 (1.8)
Disease status, *n* (%)	
Standard-risk	65 (57.0)
High-risk	49 (43.0)
Donor, *n* (%)	
Related	67 (58.8)
Unrelated	47 (41.2)
Stem cell source, *n* (%)	
BM	55 (48.2)
PBSC	59 (51.8)
Number of transplantation, *n* (%)	
1	106 (93.0)
2	7 (6.1)
3	1 (0.9)
HLA mismatches at serological level, *n* (%)	30 (26.3)
HLA mismatches at genetic level, *n* (%)	36 (31.6)
Conditioning regimen, *n* (%)	
Myeloablative conditioning	74 (64.9)
Reduced-intensity conditioning	40 (35.1)
GVHD prophylaxis, *n* (%)	
Cyclosporine alone	4 (3.5)
Cyclosporine and short-term MTX	80 (70.2)
Tacrolimus and short-term MTX	3 (2.6)
In vivo T cell depleted	27 (23.7)
Engraftment, *n* (%)	112 (98.1)
Days of engraftment, median (range)	16.5 (9–43)
Antibacterial prophylaxis, *n* (%)	
Tosufloxacin	110 (96.5)
Ciprofloxacin	4 (3.5)
Use of G-CSF, *n* (%)	68 (59.6)

MTX, methotrexate; G-CSF, granulocyte colony-stimulating factor; AUL, acute unclassified leukaemia; ATL, adult T-cell leukaemia/lymphoma.

Transplantation procedure

The stem cell source was bone marrow (BM) from a related donor in 8, BM from an unrelated donor in 47 and PBSC from a related donor in 59. Myeloablative conditioning regimens were used in 74 patients, mainly with total body irradiation plus cyclophosphamide or busulfan plus cyclophosphamide. Fludarabine-based reduced-intensity conditioning regimens were conducted in 40 patients. In these regimens, fludarabine was combined with either busulfan at 8–16 mg/kg in total or melphalan at 140 mg/m^2 in total. In some patients, total body irradiation of 4 Gy in total was added. Therefore, the intensities of regimens were close to the myeloablative conventional regimens. Prophylaxis against GVHD was performed with calcineurin inhibitors (cyclosporine or tacrolimus) with or without short-term methotrexate in the majority of patients. *In vivo* T cell depletion using alemtuzumab or anti-thymocyte globulin was performed in 27 patients, concomitant with cyclosporine and short-term methotrexate.

Neutrophil engraftment was defined as an absolute neutrophil count >500 cells/mm^3 for 3 consecutive days. All patients were housed in double-door HEPA-filtered laminar air flow rooms and provided with low microbial diets until neutrophil engraftment. New quinolones were given prophylactically in all patients. Recombinant G-CSF was routinely administered for patients with non-malignant disease and those with lymphoid malignancies after HSCT. Chest X-ray and non-invasive screening serum tests for IA including galactomannan antigen test (Platelia Aspergillus, Bio-Rad Laboratories, Marnes-la-Coquette, France) and β-D-glucan (BDG) test (β-glucan Test Wako, Wako Pure Chemical Industries, Tokyo, Japan) were performed weekly. Initial empirical antibacterial treatment for FN was started with fourth-generation cephalosporins or carbapenems.[11] For patients with persistent or recurrent FN for 7 days or longer, we did not start anti-*Aspergillus* agents as an early presumptive treatment for aspergillosis until patients developed positive serum test and/or infiltrates or nodules on X-ray or CT-scan (presumptive group). Thirteen patients, however, received

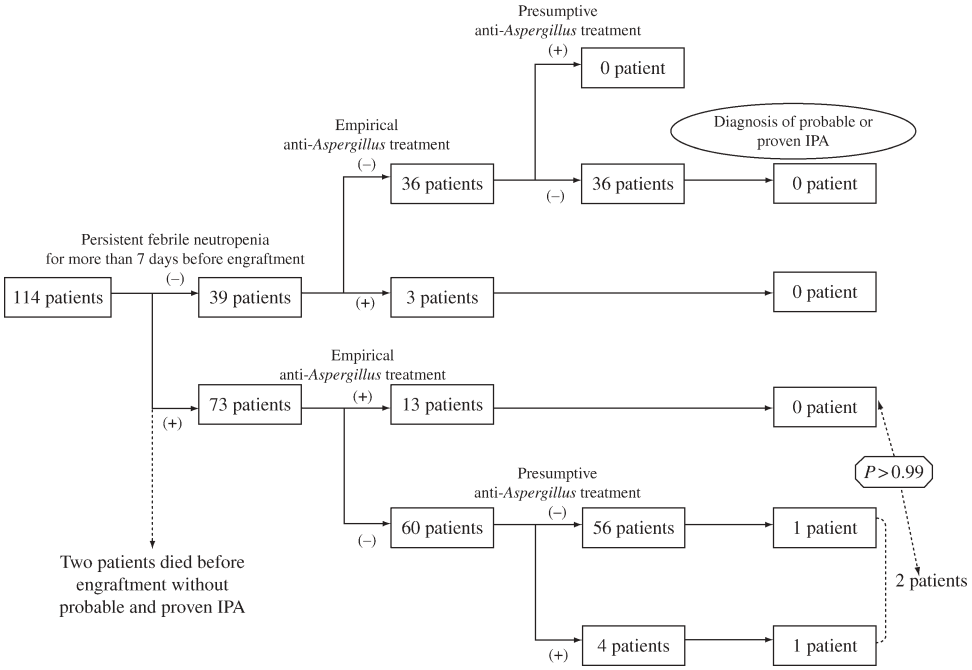

Figure 1. Treatment and outcome of patients included in the study. One hundred and fourteen patients were included in the study. One hundred and nine patients experienced FN. Seventy-three patients experienced persistent or recurrent FN for 7 days or longer. Thirteen patients in the empirical group received anti-*Aspergillus* treatment and the remaining 60 patients were included in the presumptive group. Four patients actually received anti-*Aspergillus* treatment presumptively. In total, early IA was observed in two patients in the presumptive group and none in the empirical group (3.3% versus 0%, $P > 0.99$). IPA, invasive pulmonary aspergillosis.

anti-*Aspergillus* agents empirically at the discretion of attending physicians (empirical group, Figure 1).

Definition of IA

The primary endpoint of this study was the development of probable or proven IA, that was diagnosed according to the criteria 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).[21] Microbiological criteria included two consecutive positive galactomannan tests using a reduced cutoff of 0.6 optical density index (O.D.I.).[22] IA that occurred between the day of HSCT and 7 days after engraftment was defined as early IA. The cumulative incidences of IA were calculated using Gray's method considering death without IA as a competing risk.[23]

Results

Clinical outcomes after allogeneic HSCT

Engraftment was observed in all patients at a median of 16.5 days (9–43 days) after HSCT, except for two who experienced early death before engraftment. Forty-two patients, 37.5% of those who achieved engraftment, developed grade II–IV acute GVHD at a median of 21 days after HSCT. Fifty-eight patients, 58.6% of those who survived more than 100 days after HSCT, developed chronic GVHD. Thirty-six patients relapsed at a median of 123.5 days after HSCT. Two-year overall survival of all subjects was 52.4% with a median follow-up duration of surviving patients of 822 (range 107–1603) days after HSCT.

Incidence of IA

Sixteen patients developed probable or proven IFI with a cumulative incidence of 15.1%, including 13 IA, 2 mucormycosis and 1 candidiasis (Table 2). The cumulative incidence of IA was 11.6% (Figure 2) and the median onset was 169.5 days (range, 12–531 days) after HSCT. Twelve out of 13 IA patients suffered from invasive pulmonary aspergillosis (IPA), whereas one developed gastrointestinal aspergillosis. No statistically significant risk factor was identified for the incidence of IA except for male sex (17.8% for male patients versus 2.1% for female patients, $P = 0.018$).

FN and early IA

One hundred and nine patients experienced FN with median duration of 12 days (range, 1–39 days). A median of three (range 1–5) antibiotics per patient were used for empirical antibacterial treatment during FN. Seventy-three patients experienced persistent or recurrent FN for 7 days or longer. The median duration of neutropenia was 21 and 20 days in the empirical group and presumptive group, respectively ($P = 0.91$). Thirteen patients in the empirical group received anti-*Aspergillus* treatment at a median of 9 days (range, 3–21 days) after the onset of FN (Figure 1). Amphotericin B was administered empirically in three patients, which was terminated within 2 days because of renal dysfunction. Micafungin was given to the other 10 patients for a median of 16.5 days (range, 3–76 days). Sixty patients followed the presumptive treatment strategy. Of the 60 patients in the presumptive group, 4 patients actually received anti-*Aspergillus* treatment presumptively, triggered by an elevation of BDG in 1 and infiltrates or nodules on chest

Table 2. Incidence of probable or proven IFI after allogeneic HSCT

	No.
Diagnosis of IFI after HSCT	
Proven diagnosis	4
Probable diagnosis	12
Onset of IFI after HSCT	
Early IFI	2
Late IFI	14
Incidence of late IFI	
Patients without FN	6
Empirical group	0
Presumptive group	8
Organisms that caused IFI	
Aspergillus spp.	13
Candida glabrata	1
Mucor spp.	2
Treatment for IFI	
Amphotericin B	5
Itraconazole	2
Micafungin	4
Voriconazole	3
None	2
Outcome	
Improved	8
No change or progression	6

X-ray or CT-scan in 3. One of them was subsequently diagnosed to have probable IA within a week, because galactomannan test became positive. We changed the anti-*Aspergillus* agent from micafungin to voriconazole and IPA was successfully treated (patient no. 4 in Table 3). Another patient in the presumptive group, who did not receive empirical or presumptive anti-*Aspergillus* agents, developed positive galactomannan test and nodules on CT-scan simultaneously, and was diagnosed to have probable IA (patient no. 5 in Table 3). This patient was also successfully treated with micafungin.

In total, early IA was observed in two patients in the presumptive group and none in the empirical group (3.3% versus 0%, $P > 0.99$). There was no significant difference in the duration of FN between the two groups (15.6 days versus 17.7 days, $P = 0.26$). There was no death that was directly associated with early IA in the whole population.

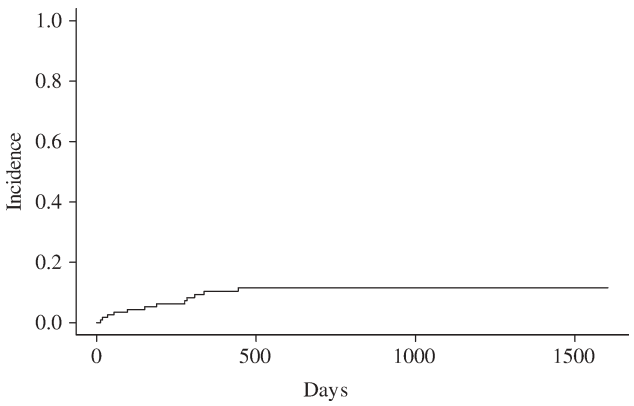

Figure 2. Cumulative incidence of IA after allogeneic HSCT. The cumulative incidence of IA was 11.6% in this study.

Discussion

In this study, the incidences of probable or proven IFI and IA were 15.1% and 11.6%, respectively, which were compatible with other recent studies.[1–3,5,7,8] Only three patients developed IFI other than IA, probably due to the prophylactic use of fluconazole.[5,7,8] Among the 13 patients with IA, only 2 developed IA early after HSCT. Both patients were successfully treated with anti-*Aspergillus* agents and therefore there was no death that was directly related to early IA.

Empirical anti-*Aspergillus* treatment has been recommended for patients with persistent FN.[11,24] However, this strategy is based on two old randomized controlled trials published in the 1980s, before the era of fluconazole prophylaxis.[12,25] Until recently, the standard antifungal agent in this setting has been amphotericin B deoxycholate.[12,25] This approach is limited by the substantial infusion-related toxicity and nephrotoxicity caused by this agent. Recently, lipid formulations of amphotericin B and intravenous itraconazole appeared to have equivalent efficacy compared with conventional amphotericin B with less toxicity.[13,16] Voriconazole and caspofungin were also reported to have similar efficacy.[14,15] However, these alternative agents are very expensive and still more toxic than fluconazole.

A presumptive strategy has been expected to decrease the use of these anti-*Aspergillus* agents by postponing anti-*Aspergillus* treatment until more specific findings are detected in patients with persistent FN. Several findings have been considered specific for IA, such as halo sign on CT-scan in neutropenic patients.[21] In addition, blood tests to detect *Aspergillus* constituents have been investigated, including galactomannan antigen test, BDG test and PCR to detect *Aspergillus* DNA.[22,26,27] Their clinical roles, however, have not been clarified.[26] Previously, we prospectively compared the sensitivity and specificity of these tests and found that the galactomannan test was the most suitable test for the diagnosis of IA with the best cutoff of 0.6 O.D.I.[22] In this study, we included not only blood galactomannan test with this cutoff index and halo sign on CT-scan but also blood BDG test and infiltrates or nodules on X-ray or CT-scan as triggers to start anti-*Aspergillus* treatment to increase sensitivity rather than specificity. By this presumptive strategy, only 2 of the 60 patients with persistent FN developed early IA, both of whom were successfully treated with anti-*Aspergillus* agents after the diagnosis of probable IA. This enabled us to decrease the use of anti-*Aspergillus* agents that are expensive and potentially toxic (4 of 60 in the presumptive group versus 13 of 13 in the empirical group). Considering the low incidence of early IA in the presumptive group, most patients in the empirical group might have been unnecessarily exposed to anti-*Aspergillus* agents. This is a retrospective study and therefore there are several limitations. Especially, we could not exclude the possibility of selection bias that high-risk patients tended to be treated empirically at the discretion of the attending physicians. However, there was no difference in the duration of neutropenia between the two groups. Both patients with a previous history of IA were included in the presumptive group.

Maertens *et al*.[28] recently showed the feasibility of preemptive therapy against IA. They started liposomal amphotericin B

Table 3. Characteristics of patients who received anti-*Aspergillus* agents presumptively (nos. 1–4) and patients who developed early IA (nos. 4 and 5)

No.	Age	Sex	Diagnosis	Prior IA	Triggers to start anti-*Aspergillus* agents	Anti-*Aspergillus* agents	Diagnosis of early IA	Outcome
1	57	Male	AML	—	Elevation of BDG	MCFG	No	Death due to AML progression
2	56	Male	AML	—	XP findings (consolidation)	MCFG	No	Alive
3	35	Male	CAEBV	—	CT findings (small multiple nodules with halo)	MCFG → ITC	No	Alive
4	56	Female	ALL	—	CT findings (nodules with halo) (positive galactomannan test after a week)	MCFG → VRC	Yes	Alive
5	54	Male	MDS	Probable IPA	CT findings (nodules with halo) and positive galactomannan test	MCFG → ITC	Yes	Alive

CAEBV, chronic active Epstein–Barr virus infection; IPA, invasive pulmonary aspergillosis; MCFG, micafungin; ITC, itraconazole; VRC, voriconazole; XP, X-ray photograph.

for patients with two consecutive positive galactomannan tests or with CT findings suggestive of IFI, regardless of the presence or absence of FN. They successfully reduced the use of anti-*Aspergillus* agents and no undetected cases of IA were identified. This approach may be more sensitive than our presumptive strategy to add anti-*Aspergillus* agents only for patients with persistent FN associated with positive serum test and/or radiological evidence. However, frequent galactomannan testing (thrice weekly) is required for this preemptive approach and thus it can be performed in only a limited number of centres.

Recently, prophylactic use of itraconazole, an anti-*Aspergillus* agent, has been evaluated in allogeneic HSCT recipients in two randomized controlled trials.[29,30] The incidence of IA was lower in the itraconazole group than the fluconazole group in both trials. The difference in the incidence of IA appeared 2 or 3 months after HSCT, not in the neutropenic period early after HSCT. Therefore, the prophylactic use of anti-*Aspergillus* agents should be considered for patients at higher-risk for IA, including patients receiving steroid for GVHD or neutropenic patients with a recent history of IA. However, for patients who are receiving anti-*Aspergillus* prophylaxis, another approach other than empirical or presumptive therapy, may be required.

In conclusion, these findings suggested the feasibility of a presumptive strategy for IA in HSCT recipients, provided that they were treated in a HEPA-filtered laminar air flow room. A randomized controlled trial is warranted to compare the efficacy and safety of presumptive and empirical strategy early after HSCT.

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Transparency declarations

None to declare.

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