への G-CSF 投与と採取が開始された現在，ドナーの安全確保への細心の注意が望まれている。また，G-CSF と併用で末梢血への幹細胞動員を容易にする薬剤として，欧米，韓国等では可逆的に，chemokine stromal cell-derived factor-1α（SDF-1α）の CXC chemokine receptor 4（CXCR4）への結合を阻害する，plerixafor（Mozobil®）[64]が，多発性骨髄腫や非ホジキンリンパ腫[65]における自家（自己）末梢血幹細胞動員採取のため末梢血幹細胞の動員採取で承認されているが，わが国では使用できず，ドラッグ・ラグが生じている。早期のわが国での安全性の評価が望まれる。

一方，アフェレーシスに関連した治療の中で，わが国で行えないものに，extracorporeal photopheresis（ECP；体外フォトレーシス）がある[66]。ECP はヘムアフェレーシスで得られた白血球に 8-methoxypsoralen を添加し，その後 ultraviolet A（UVA）を照射したのち体内に戻し，抗腫瘍効果や，免疫調節効果を得ようとするものである。対象疾患によって，抗腫瘍効果（T 細胞性リンパ腫）や，免疫調節効果（GVHD，PSS〔progressive systemic sclerosis；強皮症〕など）が期待される。特に造血幹細胞移植では，難治性の GVHD に対する効果が期待される。わが国で，遠心分離によるアフェレーシスが十分行われていないことも導入が遅れている一因と考えられる。今後積極的な取り組みが必要である。

遠心式血液成分分離装置を用いたヘムアフェレーシスは，輸血，造血細胞移植，細胞治療のインフラとして，一般臨床やトランスレーショナルリサーチの場面で，その重要性はますます増大していくものと思われる。輸血部門，血液内科領域において，安全性に留意した上で新たな展開を模索することが重要と考えられる。

また現在，日本アフェレシス学会では，『アフェレシス』という呼称が統一して用いられ，日本輸血・細胞治療学会等では『アフェレーシス』が一般的に用いられている。今後用語の統一が望まれる。

（上田 恭典，大戸　斉）

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ORIGINAL ARTICLE

Role of autotransplantation in the treatment of acute promyelocytic leukemia patients in remission: Fukuoka BMT Group observations and a literature review

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We retrospectively analyzed the outcomes of 26 patients with acute promyelocytic leukemia (APL) in the first CR (CR1) or second CR (CR2), who underwent autologous PBSCT (auto-PBSCT) between 1992 and 2008. All patients received all-*trans* retinoic acid-based induction therapy. After two courses of consolidation chemotherapy, upfront auto-PBSCT was performed in 20 patients in the CR1. Five patients had a high WBC count of more than 10×10^9/L (high risk), while 15 patients had a count of less than 10×10^9/L (low risk) at initial presentation. In addition, six patients, who were considered as low-risk patients at presentation, had a relapse after three cycles of consolidation and 2 years of maintenance therapy, but gained the molecular remission after re-induction and consolidation, and underwent auto-PBSCT in the CR2. In 26 recipients, engraftment was rapid and no TRM was documented. All 20 patients autotransplanted in CR1 were still in CR at a median of 133 months (73–193 months), and six patients who underwent auto-PBSCT in CR2 were also still in CR at a median of 41 months (2–187 months) without maintenance therapy. PML/RARα chimeric mRNA was undetectable in PBSC or BM samples examined before auto-PBSCT. Despite a small number of cases studied, our retrospective observations suggest that auto-PBSCT may be an effective treatment option to continue durable CR in the treatment of high-risk APL. We review previous reports and discuss the role of autotransplantation in the treatment of APL patients in CR.

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Introduction

Combination therapy with upfront all-*trans* retinoic acid (ATRA) and anthracycline-based chemotherapy for induction and consolidation, as well as ATRA-based maintenance, has dramatically improved the outcomes of patients with acute promyelocytic leukemia (APL).[1,2] This treatment strategy is currently the standard of care for newly diagnosed APL patients. However, relapse occurs in about 20% of patients receiving ATRA and chemotherapy, and is still a major obstacle to cure for APL patients.[3–6] A recent multivariate analysis revealed that APL patients with a WBC count of more than 10×10^9/L at initial presentation have a significantly increased relapse risk and poorer survival.[1,3,7] New strategies include risk-adapted therapies to intensify the use of anthracyclines and/or cytarabine (CA) in either induction or consolidation of high-risk APL patients.[1,8,9]

Autologous hematopoietic SCT (HSCT) has been widely used to consolidate remission in patients with acute myelogenous leukemia.[10,11] As new risk-adapted treatment strategies combined with upfront ATRA have provided higher cure rates, HSCT might not always be necessary for APL patients who are in the first complete molecular remission at the end of consolidation.[12] Therefore, the role of HSCT in the front-line APL therapy has changed during recent years. In this paper, we use historical data to report the long-term safety and therapeutic efficacy of myeloablative therapy followed by autologous PBSCT (auto-PBSCT) in 20 APL patients in the first CR (CR1) and six patients in the second CR (CR2). We discuss the role of auto-HSCT for APL patients based on our observations and previously reported literature.

Materials and methods

Patient characteristics

Between April 1992 and November 2008, 26 APL patients, who were treated with myeloablative conditioning followed by auto-PBSCT in remission, were enrolled in this study at seven institutions of the Fukuoka Blood & Marrow Transplant Group (FBMTG). All patients were diagnosed as APL morphologically according to the FAB classification. The diagnosis was also confirmed by the presence of t(15; 17)(q22; q21) by karyotypic analysis and/or detection of the PML/RARα transcript by reverse transcription PCR (RT-PCR).

Clinical characteristics of these patients are listed in Table 1. The patients comprised 14 males and 12 females with a median age of 45 years (16–68 years). Of these 26 patients, 20 received upfront auto-PBSCT in CR1 between April 1992 and November 2002. Five of these 20 patients had a WBC count of more than 10×10^9/L and were considered as high-risk patinets,[1,3,7] and six patients showed additional karyotypic abnormalities with t(15;17) at presentation (Table 1). In contrast, after August 1993, according to the policy of each institution, APL patients in molecular CR1 after consolidation are no longer always indicated for upfront auto-PBSCT. Consequently, six patients received auto-PBSCT in CR2 during the period up to November 2008, although all of these six patients had a WBC count of less than 10×10^9/L at presentation, but had a relapse of APL after cessation of maintenance therapy for CR1. This study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki.

Chemotherapy and collection of PBSCs

Remission induction therapy for 23 patients consisted of ATRA 45 mg/m^2 orally and anthracyclines including DNR or idarubicin intravenously (i.v.). The remaining three patients were treated with ATRA alone at the same dosage because their WBC count at presentation was less than 0.5×10^9/L. Consolidation chemotherapy consisted of cytosine arabinocide (CA) 500 mg/m^2 i.v. every 12 h for 6 days (intermediate-dose CA) combined with mitoxantrone 7 mg/m^2 i.v. for 3 days in the first course and etoposide 100 mg/m^2 i.v. for 5 days in the second course.[10,13]

In 20 patients who were assigned to receive upfront auto-PBSCT in CR1, auto-PBSCs were collected during the hematopoietic recovery phase after the second course of consolidation followed by G-CSF (filgrastim; Kyowa Hakko Kirin, Japan). Collected and unmanipulated PBSCs were cryopreserved until transplantation. The remaining six patients after the second consolidation chemotherapy were treated with an additional course of intermediate-dose CA combined with DNR 25 mg/m^2 for 4 days, followed by treatment with ATRA, and low-dose MTX and mercaptopurine as maintenance therapy as described by Sanz *et al.*[1] Thereafter, these six patients, who had a WBC count of less than 10×10^9/L at presentation and were considered as low-risk patients, had a relapse of leukemia at a median of 22 months (8–54 months) after diagnosis. Three patients showed molecular relapse, which was detected by RT-PCR, whereas the remaining three patients showed hematologic relapse. Three patients received re-induction cytotoxic chemotherapy without arsenic trioxide (ATO), and the remaining three patients received ATO 0.15 mg/m^2 with or without idarubicin. All six patients achieved molecular CR2 after one course of re-induction chemotherapy. Auto-PBSCs were collected after an additional two or three course of consolidation chemotherapy in these patients.

Auto-PBSCT

The pretransplant conditioning regimen for all patients consisted of BU 1 mg/kg every 6 h orally on days −8 to −5, etoposide 20 mg/kg i.v. on days −4 and −3 and CA 3 g/m^2 i.v. every 12 h on days −3 and −2 (BEA regimen).[10,13] Concurrently, G-CSF was administered to prime residual leukemic cells at a dose of 5 μg/kg on days −14 to −6, 10 μg/kg on days −5 to −4 in combination with continuous infusion of CA 100 mg/m^2 i.v. on days −12 to −6. Cryopreserved PBSCs were rapidly thawed in a 37 °C water bath and infused on day 0.

RT-PCR analysis

Nested RT-PCR analysis of PML/RARα chimeric mRNA was performed to detect minimal residual disease (MRD) with sensitivity threshold between 10^{-6} and 10^{-7} in PBSC samples in 15 of 20 patients who underwent auto-PBSCT in CR1, and BM samples in all six patients just before auto-PBSCT in CR2 as described previously.[14]

Results

Auto-PBSCT in CR1

Twenty patients received upfront high-dose chemotherapy (G-SCF-combined BEA regimen) with auto-PBSCT during CR1 after a median of 6 months (5–13 months) from the initial diagnosis. A median dose of 7.1×10^6 CD34$^+$ cells/kg was transplanted. No engraftment failure was observed. The median days to reach a granulocyte count above 0.5×10^9/L, a plt count above 20×10^9/L, and independence from plt transfusion was 15 days (range, 13–24 days), 11 days (range, 8–210 days) and 11 days (range, 6–191 days), respectively. No treatment-related deaths were observed after auto-PBSCT. Grades 1–2 stomatitis and diarrhea occurred in most recipients; however, significant adverse events, which were graded in accordance with National Cancer Institute Common Toxicity Criteria (version 2.0), above grade 3 were not observed.

At a median follow-up time of 133 months (73–193 months), all of 20 patients were continuing CR1 without maintenance therapy. Nested RT-PCR analysis was performed to test contamination of the infused PBSCs with APL cells. PML/RARα chimeric mRNA was undetectable in PBSCs samples obtained from all 15 patients. After auto-PBSCT (median follow-up time, 43 months), MRD could not be detected in BM samples from any of these patients (Table 1).

Auto-PBSCT in CR2

Six patients, who had relapse but achieved CR2 after re-induction chemotherapy, were assigned to receive

Table 1 Patient characteristics

	CR1 (n=20)	*CR2 (n=6)*
Age	45 (16–68)	45.5 (37–50)
Sex (male/female)	12/8	2/4
WBC at diagnosis		
$>10\times10^9$/L	5	0
$\leqslant10\times10^9$/L	15	6
Additional chromosomal abnormality		
Yes	6	1
No	14	5
Months from diagnosis to auto-PBSCT	6 (5–13)	25.5 (12–61)
Months from auto-PBSCT to present	133 (37–193)	41 (2–187)
Infused CD34+ cells ($\times10^6$cells/kg)	7.1 (1.03–20.2)	6.1 (0.5–11.2)
Minimal residual disease (PCR)		
Pretransplant BM samples	NE	Negative 100% (6 of 6)
Graft (PBSC) samples	Negative 100% (15 of 15)	NE

Abbreviations: auto-PBSCT = autologous PBSCT; CR1 = first CR; CR2 = second CR; NE = not examined.

auto-PBSCT. RT-PCR analyses of BM samples obtained from all six patients before auto-PBSCT were negative for MRD (Table 1). They received G-SCF-combined BEA regimen followed by auto-PBSCT after a median of 26 months (12–61 months) from the initial diagnosis. A median dose of 6.1×10^6 $CD34^+$ cells/kg was transplanted. Engraftment was documented in all six patients. The median days to reach a granulocyte count above 0.5×10^9/L, a plt count above 20×10^9/L and independence from plt transfusion was 11 days (range, 9–12 days), 14 days (range, 11–15 days) and 11 days (range, 8–15 days), respectively. No treatment-related deaths and no significant adverse events above grade 3 were documented. No significant difference was observed in hematopoietic recovery or adverse effect between 20 and 6 patients in CR1 and CR2, respectively, after auto-PBSCT. All six patients who received transplantation in CR2 remained in CR at a median follow-up time of 41 months (2–187 months) without maintenance therapy.

Discussion

The European Cooperative Group for Blood and Marrow Transplantation (EBMT) group conducted a survey on APL patients who underwent HSCT between 1993 and 2003 in Europe.[15] In their analysis of 625 APL patients, the number of auto-HSCT has decreased progressively for patients in CR1 since 1998, whereas that for patients in CR2 has remained stable. The emergence of a new risk-adapted treatment strategy combining ATRA and ATO has dramatically changed the role of auto-HSCT in the treatment of APL in CR1 and CR2. In contrast, allogeneic HSCT should be considered for patients with persistent MRD or without hematologic remission if an HLA-matched donor is available.[12]

Auto-HSCT in CR1

Large multicenter studies have shown that treatment regimens combining upfront ATRA and chemotherapy have provided high cure rates.[2–7] In addition, multivariate analyses have revealed that the most important factor predicting relapse is a WBC count of more than 10×10^9/L at initial presentation.[1,3,7] Therefore, many investigators have tried to classify the risk for each patient and modulate the regimen to intensify the use of anthracyclines and/or CA in either induction or consolidation therapy for high-risk patients.[1,9] A joint analysis by the Spanish cooperative group 'Programa para el Estudio de la Terapéutica en Hemopatía Maligna' (PETHEMA) and the European 'French-Belgian-Swiss APL' group compared the outcomes of PETHEMA LPA99 and European APL 2000 trials, and demonstrated that addition of high-dose of CA in consolidation has benefited high-risk patients.[5] Thereafter, through the combined analysis of APL93 and APL2000 trials, the European APL group confirmed a dramatically improved outcome of APL patients with high WBC counts when treated with escalating dose of CA in APL2000 trial: between these two trials, CR rate increased from 89 to 93%, and the 5-year cumulative incidence of relapse decreased from 40 to 9.5% in patients with high WBC counts (between 10 to 50×10^9/L). In patients with very high WBC counts of more than 50×10^9/L, the CR rate increased from 82 to 91%, and 5-year cumulative incidence of relapse decreased from 59 to 24%.[9] These results showed that upfront ATRA combined with intensified consolidation with high-dose CA has potential benefits for high-risk APL patients. Therefore, in terms of the intensification of chemotherapy, theoretically auto-PBSCT would be the best therapy to maximize the antileukemia effect and to minimize TRM by rapid engraftment, especially for high-risk patients, if TRM can be reduced as much as possible. In our study, upfront auto-PBSCT was performed in 20 APL patients in CR1 after consolidation, which included 5 high-risk patients. Engraftment was rapid, and TRM was not documented. All of these patients continued in CR for more than 10 years without maintenance therapy, indicating that leukemia-free survival (LFS) at 10 years was 100%. All infused PBSCs were negative for MRD in good-risk and high-risk patients who were examined (Table 1). Thus, high-dose chemotherapy followed by auto-PBSCT and deep CR status, reflecting negative MRD in PBSCs, would have benefited our patients, as several reports indicated that molecular CR would be an important factor for patients autotransplanted in CR2. In contrast, according to the multicenter retrospective survey from the EBMT group, LFS at 5 years was ~70% in 149 APL patients in CR1 receiving auto-HSCT, indicating that the results in APL patients autotransplanted in CR1 were not better than those in patients given ATRA-combining risk-adapted chemotherapy.[5] In addition, a long-term follow-up study with a median of 10 years of follow-up (APL 93 trial) recently revealed the benefits of prolonged maintenance with ATRA plus chemotherapy for high-risk patients with initial WBC counts of more than 5×10^9/L.[16] In these high-risk patients, cumulative incidence of relapse declined from 68.4% with no maintenance to 20.6% with combined ATRA and chemotherapy maintenance. On the basis of

these results, auto-HSCT is not routinely recommended for APL patients in CR1,[12,17] although there has been no randomized trial to compare outcomes among APL patients in CR1 receiving upfront auto-HSCT vs ATRA-combining risk-adapted chemotherapy for induction, consolidation and maintenance.[15,18–21]

Auto-HSCT in CR2

For APL patients who had relapse after ATRA-containing regimens, ATO is presently considered as the best treatment option; 2-year OS was reported to be approximately between 50 and 60% after repeated cycles of ATO combined with chemotherapy in relapsed APL patients achieving CR2.[22,23] The consolidation strategy after ATO-induced CR2 generally consists of HSCT, and the choice of transplant modality is mainly based on PCR status. Allogeneic HSCT is restrictively chosen for patients failing to achieve molecular CR2, as allogeneic HSCT offers greater antileukemic activity because of its GVL effect, but obviously involves a greater risk of TRM.[23] Auto-HSCT was recently considered to be one of the most useful options for consolidation in relapsed APL patients.[12,15,17,23]

In a report of the EBMT group, 195 APL patients in CR2 autotransplanted between 1993 and 2003 were enrolled in the survey; the 5-year estimate of LFS was 51%.[15] Although no data were available on re-induction and consolidation chemotherapy, or on PCR status of the graft and/or recipients before transplant, the results demonstrated that a large population of patients in CR2 achieved long-term OS after auto-HSCT. De Botton *et al.*[24] also discussed the benefit of auto-HSCT in 50 APL patients who relapsed after ATRA-containing treatment in 2004. The relapse-free survival at 7 years was 79.4% with a TRM of 6% after auto-HSCT. They analyzed MRD status in 30 available cases. PCR before auto-HSCT was positive in PBSCs in two cases and negative in 28 cases. One out of two cases autografted with a positive PCR assessment relapsed, and only three out of 28 cases (11%) autografted with negative PCR also relapsed.[24] In the patients autografted with negative PCR, relapse-free survival at 7 years increased to 87.3%, indicating that auto-HSCT would be

Table 2 Comparison of outocomes from the studies in the autotransplanation for APL

Author (publication)	N	*Age (median)*	*Disease status at transplant*	*Source of Auto-transplant*	*Pretransplant BM % PCR negative*	*Pretransplant graft % PCR negative*	*% of TRM*	*Outocome %DFS/%EFS/ %RFS/%LFS (interval) median survival median duration of CR*
Mandelli *et al.*[6]	187	30	CR1: 129	BM	NA	NA	CR1: 18	CR1: 42 ± 4 (7-year LFS)
			CR2: 58				CR2: 23	CR2: 22 ± 8 (4-year LFS)
Meloni *et al.*[26]	15	38	CR2: 15	BM	53.3 (8 of 15)	NA	0	Median duration of CR PCR+*: 5 months PCR−**: 28.5 months
Ferrant *et al.*[19]	36	NA	CR1: 36	BM	NA	NA	NA	70% (3 years LFS) 83% (3 years OS)
Roman *et al.*[21]	10	47	CR1: 8 CR2: 1 PR: 1	BM 4 PB 6	100 (2 of 2)	NA	0	Median survival: 41 months
Lo Coco *et al.*[25]	8	40	CR2: 8	BM	100 (8 of 8)	NA	0	Median duration of CR: 11 months
Ottaviani *et al.*[20]	16	30	CR1: 13 PR: 1 CR2: 1 CR3: 1	BM	81.3 (13 of 16)	NA	0	Median survival CR1: 55 months CR2: 16 months
Thomas *et al.*[28]	22	NA	CR2: 22	BM 5 PB 17	88.9 (8 of 9)	33.3 (2 of 6)	9	77 (3 years DFS)
Ferrara *et al.*[27]	6	38	CR2: 6	BM or PB	100 (6 of 6)	100 (6 of 6)	0	Median duration of CR: 36 months
de Botton *et al.*[24]	50	45	CR2: 50	BM 43 PB 7	93.3 (28 of 30)	90.9 (20 of 22)	6	79 (7 years RFS) 61 (7 years EFS)
Sanz *et al.*[15]	344	50 (CR1)	CR1: 149	CR1: BM 92, PB 57	NA	NA	CR1: 10	CR1: 70 (5 years LFS)
		38 (CR2)	CR2: 195	CR2: BM 91, PB 104			CR2: 16	CR2: 51 (5 years LFS)
Thirugnanam *et al.*[29]	14	33	CR2: 12 CR3: 2	PB	100 (14 of 14)	NA	0	83 ± 15 (5 years EFS)
Kamimura *et al.* (present study)	26	45	CR1: 20	PB		CR1: 100 (15 of 15)	0	CR1: 100 (11 years LFS)
			CR2: 6		CR2: 100 (6 of 6)			CR2: 100 (3 years LFS)

Abbreviations: CR1 = first CR; CR2 = second CR; DFS = disease-free survival; LFS = leukemia-free survival; NA = not available; *PCR+ = pretransplant BM PCR positive; **PCR− = pretransplant BM PCR negative.

effective for the treatment of relapsed APL if performed in molecular CR2; this was consistent with previous reports[25–27] (Table 2).

Thomas *et al.*[28] reported their experience using ATO as re-induction therapy for 28 relapsed APL patients. Nine of 24 patients achieving molecular CR underwent auto-HSCT, and all of them continued in CR2 (2-year LFS and OS rates of 100%). In our study, six patients had a relapse of APL after cessation of maintenance therapy, although all six patients were considered as low-risk patients; three patients were treated with chemotherapy as re-induction for relapsed APL before the era of ATO, whereas the remaining three underwent ATO-containing chemotherapy. All six patients gained molecular CR2 after one course of re-induction chemotherapy, and PBSCs were collected after the subsequent chemotherapy (Table 1). Six patients received auto-HSCT in CR2, and were continued in CR without maintenance at a median of 41 months (2–187 months). Furthermore, Thirugnanam *et al.*[29] recently showed that, following remission induction with an ATO-based regimen in relapsed APL patients, consolidation with auto-HSCT was associated with a significantly superior clinical outcome compared with ATO-based maintenance; EFS at 5 years was 83.3% in patients who underwent auto-HSCT vs 34.4% in those who did not. In addition, as recent evidence has revealed that ATO treatments comprising at least two cycles can result in molecular CR in nearly 80% of relapsed APL patients,[23] auto-HSCT may become more beneficial for APL patients in molecular CR2 receiving ATO treatment.

Intensification of pretransplant conditioning by G-CSF priming may have improved LFS in our study, especially patients with high risk and in CR2. G-CSF can stimulate the proliferation of myeloid leukemic cells because most express receptors for G-CSF, and increase susceptibility of leukemic cells to cell cycle-specific agent CA. Moreover, recent evidences have shown that G-CSF can mobilize hematopoietic stem/progenitor cells from BM niche by disruption of adhesion molecules such as CXCR4/SDF1 and VCAM1/VLA4.[30] In this context, G-CSF can also mobilize leukemic stem cells into circulation from BM niche, resulting in the increased susceptibility to chemotherapeutic agents. Clinical studies support a potential role to reduce leukemic relapse after HSCT or chemotherapy.[10,31–34] Large study will be required to assess further the efficacy of G-CSF priming for pretransplant conditioning.

Future perspective

Recently, Lee *et al.*[35] reported the importance of serial measurement of MRD by PCR analysis during therapy in 70 newly diagnosed APL patients. According to their prospective study, MRD after upfront ATRA and anthracycline-based induction chemotherapy was detectable in half of the patients and was undetectable in the remaining half. All patients negative for PCR after induction had a favorable clinical course thereafter, without relapse. In contrast, after the first consolidation, MRD was still detectable exclusively in about 30% of the patients positive for PCR after induction, who were highly susceptible to subsequent hematologic relapses, despite additional consolidation.[35] Therefore, patients who remain PCR positive after the first consolidation may be candidates requiring further ATO-containing treatment followed by HSCT: auto-HSCT has a strong anti-APL activity and potent roles in the treatment of APL, particularly in molecular CR, whereas allogeneic HSCT would be recommended for patients who fail to gain molecular CR. Large prospective studies and careful follow-ups with serial quantification of MRD would be needed to assess the value of an individualized, response-oriented treatment strategy and the role of HSCT in the treatment of APL patients.

Conflict of interest

The authors declare no conflict of interest.

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Different Risk Factors Related to Adenovirus- or BK Virus-Associated Hemorrhagic Cystitis following Allogeneic Stem Cell Transplantation

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Virus-associated hemorrhagic cystitis (HC) is a major cause of morbidity and mortality following allogeneic hematopoietic stem cell transplantation (HSCT). Although numerous studies have attempted to identify factors that predispose patients to viral HC, its causes remain controversial. We analyzed retrospectively the results of 266 allogeneic HSCTs to identify factors associated with HC. Of this group, 42 patients (15.8%) were diagnosed with viral HC, because of either adenovirus (ADV; n = 26; 9.8%) or BK virus (BKV; n = 16; 6.0%). ADV-HC was frequently associated with T cell purging, and was less common in patients with acute graft-versus-host-disease (GVHD). Conversely, BKV-HC was more frequently observed in patients with excessive immune reactions such as GVHD, preengraftment immune reaction, and hemophagocytic syndrome. These observations indicate that ADV- and BKV-HC may differ significantly in their risk factors and pathogenesis. Profound immune deficiency is more likely to be associated with ADV-HC, whereas immune hyperactivity might play a key role in BKV-HC.

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KEY WORDS: Hemorrhagic cystitis, Adenovirus, BK virus, Stem cell transplantation, Immune reaction

INTRODUCTION

Hemorrhagic cystitis (HC) is one of the most common complications following hematopoietic stem cell transplantation (HSCT), which remarkably decreases patients' quality of life, and potentially causes therapy-related mortality [1-3]. Clinical manifestations of HC vary from painless microscopic hematuria to gross hematuria, clot formation within the urinary tract, and obstructive renal failure [4]. Early-onset HC that occurs during or shortly after high-dose chemotherapy as part of the conditioning regimen is generally related to cyclophosphamide (CY) toxicity, whereas late-onset HC is mainly attributed to viral infection. BK virus (BKV) is most frequently associated with late-onset HC [5-10], although adenovirus (ADV)- and JC virus (JCV)-associated HC also occur: ADV type 11 is the prominent pathogen for HC, especially in Japan [11-17]. In general, primary ADV and BKV infections typically occur during childhood and remain latent in the genitourinary tract, but these viral infections are prevalent in allo-HSCT recipients and can cause viral-induced HC [1,2].

A number of retrospective studies have proposed a variety of risk factors for HC following allogeneic HSCT (allo-HSCT), including busulfan (BU)-containing myeloablative conditions, unrelated donors, and the occurrence of graft-versus-host disease (GVHD); however, these risk factors were not observed consistently. The analysis of risk factors is likely to be complicated by many variables, including the clinical definitions of HC, the HSCT protocols, or the number and age of patients analyzed. We performed a retrospective analysis of 42 Japanese adult allo-HSCT recipients with either ADV-HC (n = 26) or BKV-HC (n = 16), confirmed by polymerase chain reaction (PCR) examination, to identify risk factors for viral HC.

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Table 1. Pretransplantation Characteristics of the 266 Patients

Characteristics	Total	ADV-HC (n = 26)	BKV-HC (n = 16)	No-HC (n = 224)	*P* Value	
					ADV versus No	BKV versus No
Age, median (range)	48 (16-69)	48.5 (17-69)	52 (29-63)	46.5 (16-68)	.34	.21
Sex, male/female	152/114	17/9	11/5	124/100	.33	.3
Underlying disease					***.004***	.27
MDS/AML	106	5	10	91		
CML	11	1	1	9		
ALL	33	1	1	31		
ML	80	11	2	67		
AA	14	4	0	10		
Others	22	4	2	16		
Disease status at transplantation					.9	***.011***
Standard risk	123	13	1	109		
High risk	143	13	15	115		
Conditioning regimen					.93	.58
Conventional	134	13	7	114		
Reduced intensity	132	13	9	110		
Stem cell source					.66	.55
Related PB	69	6	6	57		
Related BM	14	3	0	11		
Unrelated BM	105	8	5	92		
Unrelated CB	64	7	5	52		
Haploidentical PB/BM	14	2	0	12		
Cycles of prior therapies, median (range)	5 (0-23)	4 (0-23)	4.5 (0-12)	5 (0-19)	.68	.79
Times of HSCT					.14	.5
1st	190	22	10	158		
≥2nd	76	4	6	66		
HLA matching					.6	.17
Full-matched	143	13	6	124		
Mismatched	123	13	10	100		
GVHD prophylaxis*					.61	.5
CsA-based	126	11	9	106		
FK-based	139	15	7	117		
In vivo T cell purging					***.025***	.8
Yes	19	5	0	14		
No	247	21	16	210		
IgG-antibody for ADV (titer)					.84	.26
≤ ×4	159	15	11	133		
×8	13	1	0	12		
×16	32	3	1	28		
Unknown	62	7	4	51		

MDS/AML indicates myelodysplastic syndrome/acute myelogenous leukemia; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; ML, malignant lymphoma; AA, aplastic anemia; PB, peripheral blood; BM, bone marrow; CB, cord blood; HSCT, hematopoietic stem cell transplantation; CsA, cyclosporine; FK, tacrolimus; GVHD, graft-versus-host disease; ADV, adenovirus; BKV, BK virus.
*One case that used only mPSL (methylprednisolone) was excluded.

PATIENTS AND METHODS

Patients

The medical records of 266 patients (152 men and 114 women; median age = 48 years), who underwent allo-HSCT at Kyushu University Hospital between January 2002 and June 2010, were reviewed; a subset of these patients has been described earlier [11]. Patient characteristics are listed in Table 1. Primary diseases included myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML; n = 106), chronic myelogenous leukemia (n = 11), acute lymphoblastic leukemia (ALL; n = 33), malignant lymphoma (n = 80), aplastic anemia (n = 14), and others (n = 22). Patients with any of the following conditions were classified as standard risk: acute leukemia (AML or ALL) in remission; chronic myelogenous leukemia in chronic phase; MDS classified as refractory anemia orrefractory anemia with ringed sideroblasts. All others (n = 143) were categorized as high risk. This study was approved by the institutional review board of Kyushu University Hospital.

Transplantation Procedures

A total of 134 patients received conventional preparative regimens, either 12 Gy total body irradiation/CY (n = 94) or BU/CY (n = 40). The remaining 132 cases received purine analog-based reduced-intensity conditioning consisting of either fludarabine (Flu)/CY (n = 25), Flu/BU (n = 69), or Flu/melphalan (n = 38). Low-dose total body irradiation (2-4 Gy), antithymocyte globulin (ATG), and alemtuzumab were administered in 73, 14, and 4 cases, respectively (Table 1). The sources of stem cells included related granulocyte colony-stimulating factor-mobilized peripheral blood (n = 82), related bone marrow (n = 15), unrelated

bone marrow (n = 105), or unrelated cord blood (n = 64). Human leukocyte antigen (HLA)-matching varied from haploidentical (3 of 6) to identical (6 of 6). Of 266 patients, 126 and 139 received cyclosporine- or tacrolimus-based GVHD prophylaxis, respectively; the remaining 1 patient received methylprednisolone alone. A total of 76 patients had received at least 1 prior autologous (n = 28) or allogeneic (n = 48) HSCT, and the reason of second or more transplantations was either relapse (n = 65) or graft failure (n = 11) (Table 1). Acyclovir was given as prophylaxis against herpes simplex virus reactivation, 1000 mg/day orally from days −7 to 35 after HSCT.

Diagnosis and Treatment of Viral HC

Urinalysis was routinely performed at least once a week beginning with the initiation of preparative regimens until discharge or when clinical signs of cystitis appeared after that. If microscopic or macroscopic hematuria and/or bladder irritation existed, urine was further analyzed by rapid immunochromatography and PCR method to detect ADV antigen [11] or ADV, as well as BKV and JCV, viral DNA. Only patients with viruria confirmed by PCR were diagnosed with viral HC and included in our analysis.

All patients with viral HC were treated by supportive modalities including hyperhydration, forced diuresis, and/or blood transfusions. In addition, continuous bladder irrigation and/or administration of antiviral agents were performed based on each physician's decision. According to previous reports with a minor modification [9,10], the response criteria were defined as follows: complete response (CR), the complete resolution of HC symptoms accompanied by eradication of ADV or at least a 2-log reduction of BKV viral load; partial response (PR), a significant improvement of HC symptoms accompanied by persisting microhematuria or continued detection of ADV or BKV in the urine samples; and no change, no improvement or worsening of HC.

Statistical Analysis

The aim of this study was to identify factors correlating with the development of viral HC. Chi-square tests were used for univariate comparisons to examine categoric variables, including sex, underlying diseases, disease status, conditioning regimen, stem cell source, HLA matching, GVHD prophylaxis, and prior HSCT. A numerical variable (age) was compared using the Mann-Whitney test. Odds ratios (ORs) were calculated using a logistic regression analysis, and variables were analyzed using a multivariate stepwise logistic regression model. Survival following allo-HSCT was measured from the date of stem cell infusion until the date of death. The survival period was calculated using the Kaplan-Meier method. P values $<.05$ were considered statistically significant. All statistical analyses were performed using SPSS 17.0 software (SPSS Japan Inc., Tokyo, Japan).

RESULTS

Incidence of Viral HC

In our series, a total of 42 of 266 allo-HSCT recipients (15.8%) developed viral HC. Of these, 26 (9.8%) were diagnosed with ADV-HC, including coinfection with BKV (n = 3) or JCV (n = 1), and 16 (6.0%) with BKV-HC alone. The immunochromatography assay for ADV antigen was positive in 20 of 24 tested urine samples of ADV-HC patients, although false-positive results were obtained in 4 of 13 BKV-HC patients, confirming the reliability of this assay for diagnosing ADV-HC [11].

ADV-HC has predominantly been reported from Japanese transplantation centers [11-17], whereas BKV-HC is frequently seen worldwide [5-10]. Because the role of BKV in HC pathogenesis remains unclear, because it is commonly found in the urine of unaffected patients, we analyzed ADV-HC and BKV-HC separately and compared it to patients without HC (n = 224).

Pretransplantation Characteristics of Patients with ADV-HC and BKV-HC

Six of 128 (4.7%) patients who underwent HSCT for acute leukemia (MDS/AML and ALL) developed ADV-HC, which was significantly less frequent than in the 20 of 122 (16.4%) patients suffering from other disorders (P = .004). A high incidence of ADV-HC was found in patients who received T cell purging using ATG or alemtuzumab (26.3%; 5 of 19) compared with those who did not (9.1%; 21 of 231; P = .025). Some studies have reported a close association between positive results of anti-ADV antibody and the development of ADV-HC [12,16], whereas another group [14] and our study could not detect such a relationship between them (Table 1). In contrast, BKV-HC was closely related to the status of underlying diseases at HSCT: high-risk patients developed BKV-HC more frequently than standard-risk patients (11.5%, n = 130 versus 0.9%, n = 110; P = .011). There was no association among viral HC with sex, stem cell source, or HLA matching. Moreover, the incidence of viral-HC was not affected by the usage of BU (BU-containing, 12.8%, n = 109 versus non-BU, 17.8%, n = 157; P = 0.27), usage of CY (CY-containing, 16.4%, n = 159 versus non-CY, 15.0%, n = 107; P = 0.76), or prior history of treatment (number of cycles of pretransplantation therapy) (Table 1).

Table 2. Clinical and Laboratory Manifestations of Viral-Associated HC

Case	Diagnosis	Graft	Conditioning	Preengraftment Allo-reaction	Maximum Grade of GVHD	Immunosuppressive Agents at HC Onset	Onset (Day)	Hematuria	Bladder Irritation	ADV-IC	Viruria (PCR)	Antiviral Agents	Response	CMV Reactivation	VZV Reactivation	Outcome
1	LPL	UBM	Conv	(−)	(−)	FK	2	macro	No	(+)	ADV	CDV	CR	(−)	(−)	survive
2	AA	RBM	RIC (ATG)	NA	NA	FK	4	macro	Yes	(+)	ADV	CDV	CR	NA	(−)	dead by infection
3	AA	RBM	RIC (Campath)	(−)	(−)	CsA	7	OB	No	(+)	ADV	CDV	CR	antigenemia	(+)	survive
4	ATL	UCB	Conv	NA	NA	CsA	7	macro	Yes	(−)	ADV	CDV + FCV	PR (viruria+)	NA	(−)	ATL relapse
5	ATL	UCB	RIC	HPS	(−)	FK	11	macro	No	(+)	ADV + BKV	CDV	PR (viruria+)	(−)	(−)	dead by bleeding
6	HPS	UCB	RIC (ATG)	(−)	(−)	FK/PSL	13	macro	Yes	(+)	ADV + BKV	CDV	PR (viruria+)	antigenemia	(−)	survive
7	ATL	RPB	Conv	PIR	acute(**IV**)	CsA/mPSL	19	OB	Yes	(+)	ADV	CDV	PR (OB+)	antigenemia	(−)	dead by infection
8	NK leukemia	UCB	Conv	(−)	(−)	CsA/PSL	22	macro	Yes	(+)	ADV	CDV + ribavirin	NC	antigenemia	(−)	survive
9	AA	haplo-BM	Conv	(−)	acute(**II**)	FK/PSL	25	macro	No	(+)	ADV	CDV	CR	antigenemia	(−)	survive
10	PTCL-u	UCB	RIC	(−)	(−)	FK	29	OB	Yes	(+)	ADV	GCV	CR	gastritis	(+)	survive
11	AA	UBM	RIC (ATG)	PIR	(−)	CsA	30	macro	Yes	(+)	ADV	CDV	PR (OB+)	antigenemia	(−)	dead by bleeding
12	AML	UBM	Conv	HPS	acute(**II**)	FK/PSL	31	macro	No	(+)	ADV	None	CR	antigenemia	(+)	dead by PD
13	NK lymphoma	RPB	RIC	PIR	acute(**IV**)	FK/mPSL	47	macro	Yes	(+)	ADV	CDV	CR	colitis	(−)	dead by TMA
14	MM	RPB	RIC	(−)	chronic(extensive)	CsA/PSL	79	OB	Yes	(+)	ADV	CDV	CR	antigenemia	(−)	survive
15	ATL	haplo-PB	RIC (ATG)	(−)	(−)	FK/PSL	120	macro	Yes	(+)	ADV	CDV	CR	antigenemia	(−)	survive
16	AML	UBM	Conv	(−)	(−)	FK	144	macro	Yes	(−)	ADV	CDV	CR	antigenemia	(−)	survive
17	AITL	UCB	RIC	PIR	acute(**III**)	CsA/PSL/MMF/ basiliximab	149	macro	No	NA	ADV	CDV	CR	antigenemia	(−)	dead by infection
18	MDS/AML	RPB	RIC	PIR	acute(**II**)	CsA/PSL	183	macro	Yes	(+)	ADV	CDV	PR (OB+)	antigenemia	(−)	dead by PD
19	MF	RPB	Conv	(−)	chronic (extensive)	CsA/PSL	184	macro	Yes	(+)	ADV	CDV	CR	(−)	(+)	survive
20	HCL	RPB	Conv	PIR	acute(**II**), chronic(limited)	CsA/PSL	265	OB	Yes	(+)	ADV	CDV	CR	(−)	(−)	survive
21	AML	RBM	Conv	(−)	acute(**I**), chronic (extensive)	CsA/PSL	266	macro	Yes	NA	ADV	None	CR	(−)	(−)	survive
22	CML	RBM	Conv	(−)	chronic(extensive)	PSL	281	macro	Yes	(+)	ADV	CDV	CR	antigenemia	(−)	survive
23	MF	UBM	RIC	(−)	(−)	FK/PSL	368	macro	Yes	(+)	ADV	CDV	CR	antigenemia	(−)	survive
24	AML	UBM	Conv	(−)	(−)	(−)	455	macro	Yes	(−)	ADV + JCV	None	PR (viruria+)	gastritis/colitis	(−)	dead by PD
25	ALL	UCB	RIC	(−)	(−)	(−)	484	macro	No	(+)	ADV	CDV	CR	(−)	(−)	survive
26	DLBCL	UBM	Conv	PIR	chronic(limited)	FK	875	macro	Yes	(−)	ADV + BKV	CDV	CR	antigenemia	(+)	survive
27	AML	UCB	RIC	HPS	(−)	CsA	6	OB	Yes	(+)	BKV	None	CR	antigenemia	(−)	dead by PD
28	ALL	UBM	Conv	PIR	acute(**II**)	FK	7	OB	Yes	(−)	BKV	None	PR (OB+)	antigenemia	(−)	dead by PD
29	Gastric Ca	RPB	RIC	HPS	NA	CsA/PSL	11	macro	No	(−)	BKV	None	NC	NA	(−)	dead by infection
30	DLBCL	UCB	RIC	PIR	acute(**III**)	CsA/PSL	29	macro	Yes	NA	BKV	None	NC	(−)	(−)	dead by PD
31	MDS/AML	UBM	Conv	(−)	acute(**III**)	FK/mPSL/MMF/ basiliximab	40	macro	No	NA	BKV	None	NC	antigenemia	(−)	dead by GVHD
32	ATL	UCB	RIC	PIR	acute(**II**)	CsA	42	macro	Yes	(−)	BKV	None	CR	antigenemia	(−)	dead by PD
33	MDS/AML	RPB	Conv	PIR	acute(**II**)	CsA/mPSL	44	OB	Yes	(−)	BKV	None	CR	antigenemia	(+)	dead by PD
34	MDS/AML	RPB	RIC	(−)	acute(**II**)	CsA/mPSL	49	macro	Yes	(+)	BKV	None	CR	antigenemia	(−)	dead by PD
35	MDS/AML	UBM	RIC	PIR	acute(**II**)	FK/mPSL	50	OB	Yes	(−)	BKV	None	CR	antigenemia	(−)	dead by PD

36	AML	UBM	Conv	PIR	acute(IV)	FK/PSL/MMF/etanercept	52	macro	No	(+)	BKV	CDV	PR (viruria+)	(−)	(−)	dead by GVHD
37	AML	UCB	RIC	PIR	acute(III)	CsA/PSL	61	macro	Yes	(−)	BKV	CDV	PR (OB+)	antigenemia	(−)	AML relapse
38	AML	UCB	Conv	PIR	(−)	CsA	66	macro	Yes	(−)	BKV	None	PR (OB+)	antigenemia	(−)	dead by infection
39	MDS/AML	RPB	RIC	PIR	acute(II)	CsA/PSL	86	macro	No	(−)	BKV	CDV	PR (viruria+)	antigenemia	(−)	survive
40	AML	RPB	Conv	HPS	chronic(limited)	CsA/PSL	122	macro	No	NA	BKV	CDV + Ara-A	PR (viruria+)	antigenemia	(−)	dead by PD
41	Thymic Ca	RPB	RIC	(−)	acute(II), chronic(extensive)	CsA/PSL	134	macro	No	(−)	BKV	None	NC	(−)	(−)	dead by PD
42	CML	UBM	Conv	PIR	chronic(extensive)	FK/PSL	443	macro	No	(+)	BKV	None	NC	antigenemia	(−)	dead by renal failure

LPL indicates lymphoplasmacytic lymphoma; AA, aplastic anemia; ATL, adult T cell lymphoma; HPS, hemophagocytic syndrome; PTCL-u, peripheral T cell lymphoma unclassified; AML, acute myelogenous leukemia; MM, multiple myeloma; AITL, angioimmunoblastic T cell lymphoma; MDS, myelodysplastic syndrome; MF, myelofibrosis; HCL, hairy cell leukemia; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; DLBCL, diffuse large B cell lymphoma; UBM, unrelated bone marrow; RBM, related bone marrow; UCB, unrelated cord blood; RPB, related peripheral blood; Conv, conventional conditioning; RIC, reduced-intensity conditioning; ATG, antithymocyte globulin; NA, not assessed; PIR, preengraftment immune reaction; FK, tacrolimus; CsA, cyclosporin A; PSL, prednisolone; mPSL, methylprednisolone; MMF, mycophenolate mofetil; OB, occult blood; CDV, cidofovir; FCV, foscarnet; GCV, ganciclovir; CR, complete remission; PR, partial remission; NC, no change; VZV, varicella zoster virus; PD, progressive disease; TMA, thrombotic microangiitis; GVHD, graft-versus-host disease.

Clinical Findings of ADV-HC and BKV-HC

The clinical features of patients developing viral HC are shown in Table 2. The median onset of clinical symptoms was 49.5 days (range: 2-875 days), consistent with previous reports [13-15]. Most of the patients with ADV-HC (53.8%) and BKV-HC (75%) presented their symptoms more than 1 month after transplantation, indicating that viral HC was caused by the reactivation of latent infection rather than acute infection during the transplantation course. The frequency of macrohematuria or bladder irritation was similar between patients with ADV-HC and those with BKV-HC; however, the symptoms were more severe in ADV-HC.

At the onset of viral HC, almost all the patients were receiving immunosuppressive agents as either prophylaxis or treatment of GVHD or hemophagocytic syndrome (HPS). Interestingly, the incidence of allogeneic immune reactions was significantly different between patients with ADV-HC and BKV-HC. Acute GVHD (aGVHD) (maximum; grade II to IV) throughout the clinical course was marginally less frequent in ADV-HC patients than in patients without viral HC (P = .054; Table 3), possibly because of T cell purging. In contrast, patients with BKV-HC were more likely to have developed either noninfectious fever before engraftments known as a preengraftment immune reaction (PIR) [18] or HPS (80%) or postengraftment aGVHD (grade II-IV; 73.3%) than patients without viral HC (43.8%, P = .013 and 50.5%, P = .099, respectively; Table 3).

Finally, we found a significant or marginal increase of proven cytomegalovirus diseases (e.g. gastritis, colitis) or varicella zoster virus reactivation in ADV-HC patients compared with non-HC individuals (12.5% versus 3.6%, P = .05, and 20.8% versus 12.9%, P = .29, respectively).

Treatment and Outcome of Viral HC

In addition to supportive treatments, 8 of 46 patients with HC required continuous bladder irrigation. Twenty-two of 26 patients with ADV-HC and 4 of 16 patients with BKV-HC were treated with low-dose cidofovir (CDV; 1 mg/kg/day, 3 times a week), as previously reported [11]. In addition to CDV treatment, foscarnet was subsequently administered for 1 patient, ribavirin for 1, and Ara-A for the other because of their inadequate responses. Clinical features were relieved in all except 1 ADV-HC patient; 18 patients obtained CR, 7 obtained PR with persistent viruria (n = 4) or microhematuria (n = 3) without bladder irritation, and in the remaining case, autologous stem cell rescue for the primary graft failure improved his symptoms (case 8). Of the 16 patients with BKV-HC, symptoms persisted in 6 patients, although 5 of them did not receive antiviral agents because of either relapse of their

Table 3. Posttransplantation Characteristics of the 233 Engrafted Recipients

Characteristics	Total	ADV-HC (n = 24)	BKV-HC (n = 15)	No-HC (n = 194)	*P* Value	
					ADV versus No	BKV versus No
PIR and/or HPS					.56	***.013***
Yes	106	9	12	85		
No	127	15	3	109		
aGVHD*					***.054***	.099
No	89	16	4	69		
Grade I	28	1	0	27		
Grade II	79	4	7	68		
Grade III	25	1	3	21		
Grade IV	12	2	1	9		
CMV reactivation					.69	.47
Yes (antigenemia/diseases)	158/10	15/3	12/0	131/7		
No	65	6	3	56		
VZV reactivation					.29	.49
Yes	31	5	1	25		
No	202	19	14	169		

PIR indicates preengraftment immune reaction; HPS, hemophagocytic syndrome; aGVHD, acute graft-versus-host disease; CMV, cytomegalovirus; VZV, varicella zoster virus.
*Maximum grade throughout the observation period.
Statistically significant differences were indicated by italics.

underlying diseases, low performance status, or insufficient organ function (Table 2).

Nine of 26 patients with ADV-HC died because of infection (n = 4), disease progression (n = 3), and bleeding (n = 2). Of the surviving patients, 1 relapsed and 16 remained disease-free. In contrast, 14 of 16 patients with BKV-HC died because of disease progression (n = 9), other infection (n = 2), GVHD (n = 2), or renal failure (n = 1), and 1 patient relapsed, leaving a single disease-free survivor from the patients with this complication. In our study, the 1-year overall survival after HSCT was only 16.1% ± 10.2% for patients with BKV-HC, significantly lower than that of patients without HC (52.5% ± 3.4%) or with ADV-HC (63.7% ± 10.4%).

Risk Factors for ADV-HC and BKV-HC

Univariate analysis using logistic regression identified strong associations between ADV-HC and (1) the underlying disease (acute leukemia versus others; $P = .004$), (2) T cell purging ($P = .025$), and (3) less occurrence of severe aGVHD (grade II-IV versus grade 0-I; $P = .054$). Multivariate logistic regression analysis confirmed that all 3 factors had significant or marginal association with developing ADV-HC; ORs were 4.488 (95% confidence interval (CI) = 1.625-12.40; $P = .004$), 4.176 (95% CI = 0.942-18.50; $P = .06$), and 0.390 (95% CI = 0.148-1.025; $P = .054$), respectively (Table 4).

Similar analysis of BKV-HC patients identified a significant relationship with disease status at HSCT (high risk versus standard risk; $P = .011$), PIR and/or HPS ($P = .013$), and marginal to aGVHD (grade II-IV versus grade 0-I; $P = .099$). Multivariate analysis showed that high-risk disease status (OR = 14.34; 95% CI = 1.81-113.4; $P = .012$) and presence of PIR and/or HPS (OR = 4.13; 95% CI = 1.06-16.14; $P = .041$) were the risk factors for BKV-HC (Table 4).

DISCUSSION

BKV is frequently isolated from asymptomatic patients before or following HSCT [5-8], and even from healthy individuals [19-21], indicating that the presence of BKV in urine samples is not always associated with HC. In contrast, ADV is almost exclusively detected in patients with HC, indicating a likely causative role [11-17,22]. BKV is more

Table 4. Results of Multivariate Logistic Regression Analysis

Characteristics		Odds Ratio (95% CI)	*P* Value
ADV-HC			
Underlying disease	Others versus acute leukemia	4.488 (1.625-12.40)	***.004***
In vivo T cell purging	Yes versus no	4.176 (0.942-18.50)	.06
aGVHD	grade II to IV versus 0 to I	0.390 (0.148-1.025)	.054
BKV-HC			
Disease status at transplantation	High risk versus standard risk	14.34 (1.812-113.4)	***.012***
PIR and/or HPS	Yes versus no	4.132 (1.058-16.14)	***.041***

Odds ratio were calculated by the backward or the forward stepwise selection methods.
Statistically significant differences were indicated by italics.

frequently detected in the urine of the posttransplantation patients compared with ADV, increasing from 7% to 47% for BKV and by 4% for ADV [6]. Despite a high BKV reactivation rate, HC occurs in only a fraction of patients with sustained BK viruria, whereas the majority of HSCT recipients with ADV viruria progress to HC [6,14,23]. In our study, we retrospectively analyzed 266 patients to identify the typical clinical features of Japanese adult allogeneic HSCT recipients who develop viral HC. The cumulative incidence of viral HC was 15.8% overall in our study group, 9.8% because of ADV and 6.0% because of BKV. We found that the factors associated with ADV- or BKV-HC were significantly different.

In the present study, approximately one-half of the ADV-HC cases were early onset (<1 month post-HSCT) and were closely associated with the underlying diseases including lymphoid malignancies and usage of ATG or alemtuzumab as a part of conditioning for AA patients, consistent with previous reports [22,24-26]. In contrast, late-onset ADV-HC (>1 month post-HSCT) was associated with chronic GVHD (6 of 14 patients) and the administration of prolonged immunosuppressants (12 of 14 patients; Table 2). In addition, 15 of 24 patients with ADV-HC were positive for the cytomegalovirus antigen test throughout HSCT, possibly indicating a general impairment of immune protection against viral reactivation. This suggests that profound immune suppression, such as T cell depletion or persistent GVHD and the resultant prolonged administration of immunosuppressants, may be a critical factor in the etiology of ADV-HC.

Only a subset of HSCT recipients with BK viruria progress to clinical BKV-HC, suggesting that other factors may be involved in this complication. Previous reports have shown that BKV-HC is extremely rare in autologous HSCT recipients [14,27,28], although their intensity of myeloablative preparative regimens, as well as the level and incidence of BK viruria, were similar among patients with autologous and allogeneic HSCT [27]. Here we have identified a significant association between occurrence of BKV-HC and aGVHD or non-T cell purging, consistent with previous reports from others [6,7,9,22,29]. Ten of 16 (62.5%) cases with BKV-HC developed their symptoms between engraftment and 100 days post-HSCT, in which aGVHD were frequently occurred, suggesting that immune reactions mediated by donor T cells may be an important contributing factor for developing BKV-HC. In addition, PIR and/or HPS, which are also indicative of excessive allogeneic immune reactions, were more frequently observed in patients with BKV-HC (80%, 12 of 15 patients) than among those without viral HC (43%, 85 of 194 patients). The lower frequency of severe aGVHD among Japanese HSCT recipients than in Western countries [30,31] may, in part, account for the lower incidence of BKV-HC in Japan.

Our findings indicate that ADV- and BKV-HC may develop because of different mechanisms in allo-HSCT recipients. Although under normal circumstances, BKV and ADV remain latent in the urinary tracts following primary infection, analysis of urine samples using PCR indicates that BKV is able to replicate in healthy adults [19-21], although it does not typically lead to HC. BKV-HC was frequently found in patients with excessive allogeneic immune reactions such as GVHD, PIR, and HPS. Because BKV is usually not sufficient to cause HC, BKV might cooperate with excessive immune reactions to cause HC, although it remains unclear whether this immune attack can target the uroepithelium or not. In contrast, ADV is usually undetectable in the urine of healthy adults, indicating that ADV does not replicate under the normal immune status [32]. In the allo-HSCT recipients, ADV-HC was associated with T cell purging and the underlying disease. It is conceivable that severe immune suppression allows ADV replication to occur in the urinary tract, leading to local inflammation and subsequent development of HC. Because BKV viruria may be asymptomatic, it is likely that ADV is more virulent than BKV for developing HC.

The influence of HC on the outcome of HSCT remains controversial. In our study, 22 of 26 patients developing ADV-HC were promptly initiated low-dose CDV, as previously reported [11], resulting in CR in 15 patients and PR in 6 others, and the 1-year overall survival in patients with ADV-HC was similar to those without ADV-HC (63.7% versus 52.5%). In contrast, patients with BKV-HC had a very low probability of survival (<20%), although others have reported that the clinical course of BKV-HC was less severe than ADV-HC [6]. In these patients, the main cause of death was not the BKV-HC but the progression of the underlying diseases; 15 of 16 cases with BKV-HC underwent allo-HSCT against the uncontrolled diseases. Irrelevant immune reactions and the resultant administration of immunosuppressants might contribute to the reduction of the graft-versus-leukemia effect.

In conclusion, we have identified different related factors in HSCT recipients to develop either ADV-HC or BKV-HC, although there are the limitations to a retrospective, single-center analysis. Severe immunosuppression might play a pivotal role for ADV reactivation and subsequent development of ADV-HC, whereas an excessive immune reaction might be critical for the development of BKV-HC. Earlier diagnosis and intervention for ADV-HC with low-dose CDV therapy may provide a survival benefit. It will be interesting to see if these associations are found in other adult populations.

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