Autoimmune disorder could also occur in combination with B-cell malignancy, including MM. Wada et al. [9] reported a case of MM complicated by autoimmune hemolytic anemia. Terpos et al. [10] also reported a case of MM developing SS. However, these case reports could not show a true association between disease activity of MM and subsequent autoimmune disorders.

Isshiki et al. [11] reported recurrence of preexisting autoimmune disorder in 3 patients with MM, following autologous PBSCT. It is unclear why transplant recipients are susceptible to autoimmune disorders, but the underlying mechanism has been postulated to be a depletion of CD25-positive regulatory T cells which maintain self-tolerance, resulting in escape of auto-reactive T cells [12]. The presence of conditioning-induced thymic damage might interfere with negative selection of autoreactive cells and, thereby, facilitate the development of cellular and humoral autoreactivity [13]. The use of G-CSF-mobilized grafts, containing predominantly Th2 cells, may also contribute to induction of the autoimmune phenomenon [14].

No case of MD following autologous PBSCT has yet been reported. In our patient, both MD and severe thrombocytopenia were observed around the same time, and both responded well to steroid treatment, while MM remained in complete remission during these episodes. This suggests that the autoimmune disorders occurred as a result of impaired immunity due to autologous transplantation rather than secondary to MM recurrence.

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Intracranial hemorrhage following allogeneic hematopoietic stem cell transplantation

Yuho Najima,[1] Kazuteru Ohashi,[1]* Maho Miyazawa,[2] Mikako Nakano,[2] Takeshi Kobayashi,[1] Takuya Yamashita,[1] Hideki Akiyama,[1] and Hisashi Sakamaki[1]

Charts and radiographs of 622 allogeneic hematopoietic stem cell transplant (HSCT) recipients, over a 20-year period, were retrospectively reviewed for intracranial hemorrhage (ICH) following transplant. A total of 21 cases of ICH were identified (3.4%) including 15 cases of intraparenchymal hemorrhage (IPH), two cases of subarachnoid hemorrhage (SAH), and four cases of subdural hematoma (SDH). The median time from transplantation to the onset of ICH was 63 days (range, 6–3,488 days). The clinical features of post-transplant ICH patients were similar and included hypertension, diabetes mellitus, chronic graft-versus-host disease (GVHD), systemic infection, and veno occlusive disease (VOD), recently referred to as sinusoidal obstruction syndrome, in addition to severe thrombocytopenia. Mortality rate was especially high (89%) after IPH with a median survival of 2 days (range, 0–148 days). In contrast, all patients with SAH or SDH following HSCT survived. The cause of post-transplant ICH appears to be multifactorial, including thrombocytopenia, hypertension, acute GVHD, VOD, and radiation therapy. Most patients in our series displayed severe thrombocytopenia at the onset of ICH, even though adequate prophylactic platelet transfusions were given. By univariate analysis, cord blood transplantation, acute GVHD, systemic infection, and VOD were related to the incidence of ICH, whereas prior CNS episodes and radiation therapy did not reach statistical significance. A multivariate analysis with logistic regression identified acute GVHD as the only factor that significantly influenced ICH occurrence. Am. J. Hematol. 84:298–301, 2009. © 2009 Wiley-Liss, Inc.

Introduction

HSCT recipients are at high risk for severe neurological complications [1]. These complications arise either from the primary disease for which the patient is undergoing HSCT, or as a consequence of immunosuppressive treatments, infection, or intracranial hemorrhage that may develop during HSCT [2,3]. Although the clinical course of subdural hematoma (SDH) or subarachnoid hemorrhage (SAH) can be relatively benign, intraparenchymal hemorrhage (IPH) has the worst outcome among these complications [4,5]. Pomeranz et al. [6], in a retrospective analysis of the clinical features of ICH, found that while SDH was usually due to a more specific factor such as thrombocytopenia and had a more benign course, IPH was rather sporadic and usually lethal.

This study describes the clinical courses of 21 cases of ICH among 622 allogeneic transplants performed over the last 20 years at a single institution and reviews their clinical outcomes.

Results

Clinical features of post-transplant ICH

Charts and brain CT of 622 allogeneic transplant recipients were retrospectively reviewed for ICH, and 21 patients (3.4%) eventually developed ICH at a median time of 63 days (range, 6–3488 days) after transplantation. Median age at the time of ICH was 42 years (range, 11–66 years) and 11 patients were men (52%). Eleven patients underwent unrelated hematopoietic stem cell transplant with a radiation-containing regimen.

The clinical characteristics of these 21 patients are summarized in Table I. ICH was symptomatic in 14 of the 21 patients and symptoms included loss of consciousness (seven patients), headache (three patients), hemiplegia (three patients), and seizure (one patient). Two asymptomatic patients had ICH which was incidentally found during systemic screening for infection. One patient developed ICH during deep sedation while on mechanical ventilation. The remaining four patients visited another emergency unit at the onset of ICH and, therefore, clinical information relating to their initial symptoms was unavailable. Among the 21 patients with ICH, 15 patients developed IPH, two patients developed SAH, and four patients developed SDH. Median onset of IPH occurred at 122 days (range, 16–3,488 days) after HSCT. In comparison, all patients with SAH or SDH developed their events earlier in the course of HSCT, with a median onset of 22 days (range, 6–38 days) for the SAH patients and 31 days (range, 17–41 days) for the SDH patients (Table I). A previous history of CNS events was present in nine patients including four with reversible encephalopathy syndromes induced by cyclosporine, three patients with leukemic CNS infiltration, one case of fungal infection, and one case of meningitis. Platelet counts at the onset of ICH were extremely low in 14 patients (82%) out of 17 patients whose clinical data was available. Acute GVHD was apparent in 11 patients and three patients displayed active chronic GVHD (two extensive and one limited) at the onset of ICH. Concomitant diseases included hypertension, hyperlipidemia, chronic kidney disease, dia-

[1]Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo, Japan; [2]Pharmacy Division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo, Japan

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*Correspondence to: Kazuteru Ohashi, MD, FACP, Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8677, Japan.
E-mail: k.ohashi@cick.jp

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TABLE I. Clinical Characteristics of 21 Patients Who Developed ICH

Case	Age/ Sex	Primary disease	Conditioning regimen	Type of HSCT	ICH								
					Type of ICH	Onset of ICH (days after HSCT)	Platelet counts at the onset ($\times 10^4/\mu l$)	Prior CNS events	Acute GVHD at the onset (grade)	Chronic GVHD at the onset (type)	Concomitant diseases	Survival (days after The onset of ICH)	Outcome/Cause of death
1	56/M	NHL	CA,CY,TBI	UCBSCT	IPH	16	0.5	Leukemic infiltration	Yes (3)	-	HTN	10	Dead/IPH
2	32/F	AML	BU,CY,TLI	UBMT	IPH	42	0.9	No	Yes (3)	-	No	148	Dead/aGVHD
3	47/M	AML	BU,CY,TLI	UBMT	IPH	43	1.6	No	Yes (1)	-	No	2	Dead/IPH
4	30/M	CML	CA,CY,TBI	UBMT	IPH	57	3.3	Fungal infection	Yes (4)	-	No	2	Dead/IPH
5	11/M	ALL	BU,VP,MEL	RBMT	IPH	63	0.1	Leukemic infiltration	Yes (4)	-	VOD,DIC	0	Dead/IPH
6	20/F	AML	BU,CY,VP	RBMT	IPH	88	1.7	No	Yes (1)	-	No	1	Dead/IPH
7	66/M	MDS	FLU,MEL,TBI	UCBSCT	IPH	121	0.5	CyA encephalopathy	No	No	No	10	Dead/infection
8	32/F	CML	BU,CY	RBMT	IPH	122	2.1	Meningitis	No	No	VOD	0	Dead/IPH
9	54/M	NHL	CY,TBI	UBMT	IPH	159	0.7	No	Yes (2)	No	No	5	Dead/IPH
10	31/M	AML	BU,CY,TLI	UBMT	IPH	216	0.7	No	Yes (4)	No	No	2	Dead/IPH
11	36/M	ALL	CA,CY,TBI	RBMT	IPH	235	2.0	CyA encephalopathy	No	Yes (ext.)	CKD	0	Dead/IPH
12	37/M	NHL	CA,CY,TBI	RBMT	IPH	374	1.0	No	No	Yes (lim.)	CKD	0	Dead/IPH
13	44/M	AML	BU,CY	RBMT	IPH	642	N/A	No	No	No	HL	0	Dead/IPH
14	42/F	AML	BU,CY,TLI	UBMT	IPH	1449	N/A	No	No	Yes (ext.)	VOD	8	Dead/IPH
15	39/F	ALL	CA,CY,TBI	RBMT	IPH	3488	N/A	No	Yes (1)	No	CKD	602+	Alive/–
16	53/F	MDS	BU,CY,TLI	RBMT	SAH	6	0.4	No	No	-	No	1199+	Alive/–
17	59/F	MDS	FLU,CY	RPBSCT	SAH	38	2.4	CyA encephalopathy	Yes (4)	-	No	52	Dead/aGVHD
18	23/M	CML	BU,CY	RBMT	SDH	17	N/A	No	No	-	No	5850+	Alive/–
19	43/F	MDS	BU,CY,TLI	UBMT	SDH	29	0.6	CyA encephalopathy	No	-	No	66	Dead/infection
20	50/F	ALL	CA,CY,TBI	UCBSCT	SDH	32	1.5	No	No	-	HTN,DM	1649+	Alive/–
21	45/F	ALL	CA,CY,TBI	UCBSCT	SDH	47	0.3	Leukemic infiltration	Yes (3)	-	VOD	63	Dead/relapse

HSCT, hematopoietic stem cell transplantation; ICH, intracranial hemorrhage; CNS, central nervous system; GVHD, graft-versus-host disease; M, male, F, female; NHL, non-Hodgkin's lymphoma; AML, Acute myelogeneous leukemia; CML, chronic myelogeneous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; CA, cytarabine; CY, cyclophosphamide; TBI, total body irradiation; BU, busulfan; TLI, total lymphoid irradiation; VP, VP16; MEL, melphalan; FLU, fludarabine; UCBSCT, unrelated cord blood stem cell transplantation; UBMT, unrelated bone marrow transplantation; RBMT, related bone marrow transplantation; RPBSCT, related peripheral blood stem cell transplantation; IPH, intraparenchymal hemorrhage; SAH, subarachnoid hemorrhage; SDH, subdural hemorrhage; N/A, not available; CyA, cyclosporine; ext, extensive; lim, limited; HTN, hypertension; VOD, veno-occlusive disease; DIC, disseminated intravascular coagulation syndrome; CKD, chronic kidney disease; HL, hyperlipidemia; DM, diabetes mellitus.

betes mellitus, VOD, and disseminated intravascular coagulation in 10 patients (Table I).

Management, outcome, and risk factor of post-transplant ICH

Treatment options for post-transplant ICH were extremely limited and most cases were not eligible for neurosurgical intervention because of ICH severity, transfusion refractory thrombocytopenia, or poor general condition. Only two patients in our series underwent neurosurgery to avoid immediate death (Cases 2 and 18, Table I).

Post-transplant ICH has a dismal prognosis. According to Kaplan-Meier product-limit estimates, 5-year overall survival was 17.9%, which is much lower compared with patients without ICH (55.8%, $P < 0.00001$), as shown in Fig. 1. In the case of IPH, most patients died soon after the event, with the median survival after IPH of 2 days (range, 0–148 days). In contrast, patients with SAH or SDH had longer survival, and actually no deaths were attributed to SAH or SDH.

We sought to determine which variables were associated with the risk of developing ICH. Univariate and multivariate analyses of the risk factors for ICH are shown in Table II. By univariate analysis, cord blood transplantation, Grade III–IV acute GVHD, systemic infection, and VOD were related to the incidence of ICH, whereas prior CNS episodes and prior radiation did not reach statistical significance. A multivariate analysis with logistic regression identified Grade III–IV acute GVHD as the only factor that significantly influenced ICH occurrence.

Discussion

This study reviews the incidence of ICH among 622 recipients of allogeneic HSCT. Although some patients may have been overlooked due to lack of symptoms, the cumulative incidence of ICH in our series was 3.4%, which is equivalent to the incidence rates reported by others using similar definitions and methods [7]. The incidence of ICH in the setting of post-transplantation, however, was much higher than ICH not associated with HSCT in the general Japanese population, where IPH occurs in 1 per 1,000 patients and SAH occurs in 0.7 per 1,000 patients [8].

The etiology of post-transplant ICH appears to be multifactorial and includes thrombocytopenia, hypertension, acute GVHD, VOD, prior CNS episodes, and radiation therapy. Of particular interest is the finding that most patients in our series displayed severe thrombocytopenia at the onset of ICH, despite adequate prophylactic platelet transfusions which was triggered when a patient's platelet level dropped below 20,000/μl without acute bleeding. Although the precise mechanism of severe thrombocytopenia with refractoriness to platelet transfusion was unknown, cerebro-microvascular endothelial injury could be a possible underlying problem. Our patients with acute GVHD were also more likely to experience ICH, especially those receiving steroids or calcineurin-inhibitors, medications that are known to harm the CNS [9]. In a recent case report, Campbell et al. [10] described several cases of GVHD with cerebral vasculitis resulting in parenchymal hemorrhage. Uckan et al. [7] have also recently reported that life-threatening neurological complications including ICH was more frequently observed in patients carrying severe acute GVHD. In their case series, all patients who developed ICH were complicated with Grade III–IV acute GVHD. In our series, patients with severe acute GVHD (>Grade III) might have an increased risk for ICH on both univariate and multivariate analysis. Although further clarification is warranted, these suggest a possible causal relationship between severe GVHD and ICH. Radiation therapy is also thought

TABLE II. Univariate and multivariate analysis factors for ICH

	Univariate *P**	Multivariate *P***	Hazard ratio (95%CI)
Donor (unrelated/related)	0.120	0.741	0.83 (0.28–2.48)
Radiation containing regimen	0.134	0.443	1.53 (0.51–4.59)
Prior CNS events (yes/no)	0.061	0.312	1.78 (0.58–5.40)
CBSCT (yes/no)	0.006	0.213	1.51 (0.79–2.93)
Acute GVHD grade III-IV (yes/no)	0.006	0.046	1.41 (1.01–1.97)
Systemic infection (yes/no)	0.0069	0.399	1.52 (0.57–4.03)
VOD (yes/no)	0.009	0.125	2.63 (0.77–9.00)

*Univariate analysis with the χ^2 test for categoric variables and the nonparametric Mann-Whitney U test for continuous variables.

**Multivariate analysis with the multiple logistic regression analysis for appropriate variables to evaluate the risk of ICH. Statistical significance was determined at the .05 level. All *P* values were two sided. The statistical data were obtained using the SPSS software package (SPSS 11.0 inc., Chicago, IL).

to contribute to the development of ICH [11], and the majority of our ICH patients received some form of radiation therapy. Laboratory-proven coagulopathies, however, were not evident in our series except for one patient (Case 5) who developed IPH.

Consistent with previous reports, the clinical course of patients with ICH in our series was dismal with a 5-year overall survival rate of only 17.9%. In contrast, among the 601 patients without ICH, the 5-year overall survival was 55.8% (see Fig. 1). In our series, SAH and SDH were observed by Day 50 after allogeneic HSCT and conservative therapy resolved the clinical symptoms in all but one patient, with no death attributed to SAH or SDH. Colosimo et al. [4] reported that 16 of 17 cases of SDH occurred within 60 days after allogeneic HSCT and none were fatal. Pomeranz et al. [6] also reported that all SDH events (13 cases per 471 HSCT cases) were observed within 42 days after HSCT. In contrast, IPH events occurred later, with a wide distribution of onset with a median of 122 days after transplant and a range of 16–3,488 days. Most patients died from their IPH event, especially those whose event occurred early in the course of the HSCT. The cause of this clinical discrepancy remains unclear, but may be due to predisposing factors unique to each disease entity.

Although not statistically significant, patients with VOD also appeared predisposed to developing ICH. This may not be solely due to a side effect of the tissue plasminogen activator administered with antithrombin-III as part of the VOD therapy. On the basis of a recent report, this combination appears to reverse the course of VOD without increased risk of bleeding [12].

This study is limited because of its retrospective nature, and we may have overlooked some patients with ICH, despite detailed database analysis and extensive chart review. Nevertheless, our aim was to review the clinical outcomes of 622 allogeneic transplant patients after ICH over a 20-year period and to provide useful insights into this phenomenon.

Methods

Patient demographics. We retrospectively reviewed 622 patients (370 men, 252 women; median age, 37 years; range, 0–67 years) with various diseases who underwent allogeneic transplantation at our institution. Between September 1986 and December 2006, 499 patients received bone marrow transplantations (264 related, 231 unrelated, four syngenic), 79 received related peripheral blood stem cell transplantations and 44 patients received unrelated cord blood stem cell transplantations. Their underlying diseases included chronic myeloid leukemia (n = 139), acute nonlymphoid leukemia (n = 175), acute lymphoid leukemia (n = 133), myelodysplastic syndrome (n = 83), non-Hodgkin's lymphoma (n = 31), severe aplastic anemia (n = 41), myelofibrosis (n = 5), multiple myeloma (n = 13), and adult T-cell leukemia/lymphoma (n = 2).

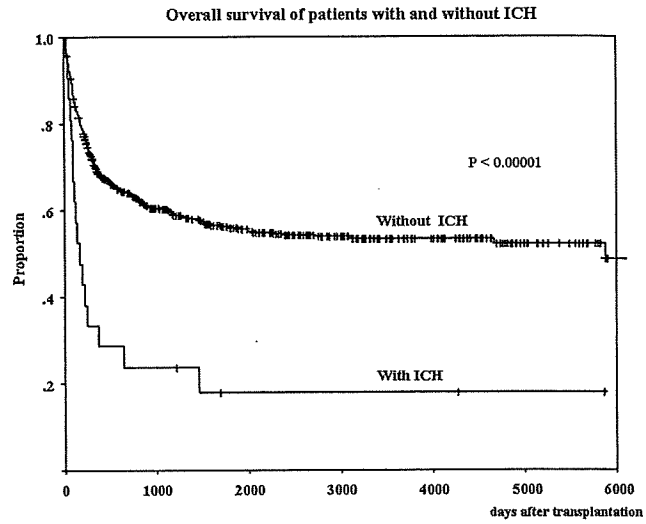

Figure 1. Calculation of overall patient survival with and without ICH, using the Kaplan-Meier method.

Preparative regimen, GVHD prophylaxis, and transfusion policy. Preparative therapy was performed according to the primary disease and type of transplant. Generally, patients with lymphoid malignancy were conditioned using a combination of total body irradiation (TBI) of 12 Gy and chemotherapy, including cytarabine at 8 g/m^2 and cyclophosphamide (CY) at 120 mg/kg. TBI was performed with partial transmission to the anterior–posterior eye with 33% shielding. Conversely, patients with myeloid malignancy were conditioned using a non-TBI containing regimen that included busulfan (BU) at 16 mg/kg and CY at 120 mg/kg. Plasma concentrations of busulfan were not monitored. Total lymphoid irradiation (TLI, 7 Gy) was included in BU/CY regimens in cases with mismatch or unrelated transplantation. Patients with severe aplastic anemia were also conditioned using a TLI-containing regimen. Cyclosporine (CyA) or tacrolimus (FK) plus short-term methotrexate were used for GVHD prophylaxis. FK was used in cases involving either unrelated or mismatched transplantation. Acute and chronic GVHD were diagnosed and graded according to previously established criteria. Prophylactic platelet transfusion was triggered when a patient's platelet level dropped below 20,000/μl without acute bleeding [13].

Definition of ICH and statistical analysis. On the basis of brain computed tomography (CT) findings, ICH was classified as IPH, SAH, or SDH. Ischemic events such as cerebral infarction or transient ischemic attack were not included. Post-traumatic hematoma, meningoencephalitis, or abscess was also excluded. Overall survival and relapse-free survival rate were estimated by Kaplan-Meier product-limit estimates. The log-rank test was used to assess differences between groups of patients with or without ICH. A multivariate analysis was done to determine the risk factors for causing ICH by the logistic regression model.

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ORIGINAL ARTICLE

A comparative assessment of the RIFLE, AKIN and conventional criteria for acute kidney injury after hematopoietic SCT

M Ando[1,3], J Mori[2], K Ohashi[2], H Akiyama[2], T Morito[1,3], K Tsuchiya[3], K Nitta[3] and H Sakamaki[2]

[1]*Department of Nephrology, Tokyo Metropolitan Cancer Center Komagome Hospital, Tokyo, Japan;* [2]*Department of Hematology, Tokyo Metropolitan Cancer Center Komagome Hospital, Tokyo, Japan and* [3]*Department IV of Medicine, Tokyo Women's Medical University, Tokyo, Japan*

An observational cohort study was conducted to compare the performance of the RIFLE (risk, injury, failure, loss and end-stage kidney disease), AKIN (acute kidney injury network) and conventional graded criteria to identify acute kidney injury (AKI) following SCT and to predict long-term mortality in 141 myeloablative allogeneic SCT (m-allo), 60 non-myeloablative allogeneic SCT (nm-allo) and 48 autologous SCT (auto) cases. The AKIN criteria had less ability to identify patients as having the lowest category, stage 1 (analogous to RIFLE risk): 33% (37%) in m-allo, 23% (32%) in nm-allo and 8.3% (16.7%) in auto. Cox regression showed that categories higher than the intermediate stage were independent predictors of mortality in all three definitions. The areas under receiver operating characteristic curves showed that both definition systems had similar and significant ability to predict mortality (0.643–0.649 in m-allo and 0.734–0.766 in nm-allo, respectively). These abilities of the conventional graded criteria were comparable with those of the RIFLE criteria. The RIFLE criteria have greater sensitivity than the AKIN criteria to identify patients with AKI and therefore are more favorable as a uniform definition system for post-SCT AKI. However, the RIFLE criteria do not improve on the clinical relevance of the conventional graded criteria.

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Keywords: acute kidney injury; allogeneic myeloablative transplant; allogeneic nonmyeloablative transplant; autologous transplant; long-term mortality

Correspondence: Dr M Ando, Department of Nephrology, Tokyo Metropolitan Komagome Hospital, 3-18-22, Honkomagome, Bunkyo-Ku, Tokyo, Japan.
E-mails: nephrol@cick.jp or hdcn@cick.jp
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Introduction

Acute kidney injury (AKI) is a common early complication after hematopoietic SCT.[1,2] Recent reports have also shown that AKI associated with SCT results in poor long-term survival of affected patients.[3,4] At present, there are three major SCT modalities that include autologous SCT (auto), myeloablative allogeneic SCT (m-allo) and non-myeloablative allogeneic SCT (nm-allo), with selection depending on stem cell sources and preconditioning procedures. AKI is a common complication of all these SCT modalities, although the incidence, severity and impact on mortality differ between the three modalities. Schrier *et al.*[5] showed the frequency of AKI increased significantly from auto (21%) to nm-allo (40%) to m-allo (69%), and that the increased incidence of AKI correlated with a parallel increase in mortality 6–12 months after SCT from 7 to 34 to 58%, respectively. However, the clinical validity of such epidemiological data needs to be re-examined from the viewpoint of the contemporary paradigm for AKI, as these earlier studies were conducted according to conventional, but likely arbitrary definition systems, used at that time.

The lack of consensus concerning the quantitative definition of AKI has hindered clinical research as it confounds comparisons between studies. Thus, the Acute Dialysis Quality Initiative (ADQI) group has proposed a new graded definition for AKI, called the RIFLE criteria (risk, injury, failure, loss of kidney function, end-stage kidney disease), to establish a uniform standard for diagnosing and classifying AKI.[6] More recently, the acute kidney injury network (AKIN) proposed new diagnostic criteria and a three-staging system for AKI modified from RIFLE, with the aim of increasing the sensitivity of the classifications.[7] These criteria have now been evaluated in a number of clinical studies of critically ill patients with AKI and have been shown to be able to identify and classify the severity of AKI and monitor progression of the disorder, in addition to being a predictive index of in-hospital mortality.[8–10] It is possible that these new AKI criteria may have great utility in standardizing the definitions for establishing inclusion criteria and outcomes for post-SCT AKI. However, it is unknown currently whether discernible advantages exist between RIFLE, AKIN and the conventional graded criteria for classifying post-HCT AKI introduced by Parikh *et al.*[5,11,12]

The objectives of this study were (1) to determine the current incidence of post-SCT AKI according to the two new definition systems, and (2) to compare the performance of the RIFLE, AKIN and the conventional graded criteria for identifying AKI and predicting long-term all-cause mortality associated with post-SCT AKI in the three major SCT modalities

Patients and methods

The subjects were 249 Japanese patients (mean age, 41.7 ± 12.5 years; 157 males) who received SCT between August 2004 and December 2007 in the Department of Hematology at Tokyo Metropolitan Cancer Center Komagome Hospital. This cohort comprised 141 myeloablative allogeneic SCTs (m-allo), 60 non-myeloablative allogeneic SCTs (nm-allo) and 48 myeloablative autologous SCTs (auto). Candidates for allogeneic myeloablative SCT were screened for the presence of pre-existing comorbidities and deemed suitable for the procedure on the basis of normal kidney morphology and a 24 h timed urine creatinine (Cr) clearance $\geqslant$80 ml/min and albuminuria $\leqslant$300 mg/gCr. Patients older than $\geqslant$50 years or with other comorbidities such as hypertension, diabetes and hepatic dysfunction were excluded from the study. Patients who were not eligible for myeloablative SCT were considered for non-myeloablative SCT. Patients gave their written consent and were treated according to protocols approved by the institutional Ethics Committee.

The study was an observational cohort study. Preparative therapy was performed according to the primary disease and type of transplant. In general, patients with lymphoid malignancy were conditioned using a TBI (12 Gy)-containing regimen that included administration of cytarabine (8 g/m^2) and CY (120 mg/kg). Selective kidney shielding blocks were used during TBI and this reduced the renal dose to 10 Gy.[13] Conversely, patients with myeloid malignancy were conditioned using a non-TBI-containing regimen that included administration of BU (16 mg/kg) and CY (120 mg/kg). The plasma concentrations of BU were not monitored. TLI (7 Gy) was included in the BU/CY regimens in cases of mismatched or unrelated transplantation. Patients with severe aplastic anemia were also conditioned using a TLI-containing regimen. The preparative regimen for the non-myeloablative procedure consisted of CY (120 mg/kg) and fludarabine (125 mg/m^2). The GVHD prophylaxis regimen typically comprised a short course of MTX and CYA (CSP) or tacrolimus (FK). FK was used in cases involving either unrelated or mismatched transplantation. MTX was administered at 10 mg/m^2 i.v. on day +1 and at 7 mg/m^2/day on days +3, +6 and +11. Continuous i.v. infusion of CSP and FK was started on day −1 at dosages of 3 mg/kg/day and 0.03 mg/kg/day, respectively. The target blood concentrations were 450–550 ng/ml for CSP and 10–20 ng/ml for FK, with the dosage of both drugs being adjusted according to renal function and the grade of acute GVHD. If no GVHD was present, both drugs were administered orally approximately 2 months after SCT, followed by tapering of the dosages between 3 and 6 months. Acute and chronic GVHD were diagnosed and graded according to previously established criteria.[14,15] Tosulfloxacin and fluconazol were administered orally for 14 days before SCT. Trimethoprim–sulfamethoxazole (TMP 240 mg, SMX 1200 mg; 3 times per week) was also used to prevent pneumocystis pneumonia. CMV infection was monitored weekly by CMV antigenemia. Positive antigenemia, defined as >1 cell/65 000 cells, was treated using ganciclovir twice daily until negative CMV antigenemia was obtained.

Definition of post-SCT AKI

AKI was defined and classified into three categories according to two current AKI definitions, the RIFLE and AKIN criteria and also by the conventional graded system introduced by Parikh *et al.*,[11,12] hereafter denoted as 'the Grading criteria'. A comparison of these AKI definitions is shown in Table 1. AKI within the first 100 days after SCT was defined based on serum creatinine (Cr) and/or estimated glomerular filtration rate (eGFR) criteria proposed by each of the AKI definitions. The RIFLE criteria was determined on the basis of the most abnormal value of either Cr or eGFR criteria. Urine output criteria included in the RIFLE and AKIN definitions were not used, as we were unable to obtain accurate records of urine output from all the patients. The serum concentration of Cr was measured by an enzymatic method using an isotope-dilution mass spectrometry-traceable calibrator (N-assay L Creatinine Kit, Nittoubo Medical Co., Tokyo, Japan). eGFR was calculated by the modification of diet in renal disease formula, as modified by the Japanese Society of Nephrology: $\text{eGFR (ml/min/1.73m}^2) = 194 \times \text{Cr}^{-1.094} \times \text{age}^{-0.287} \times 0.739$ (if female).[16] 'Severe AKI' was defined as greater than the intermediate category, such as injury in RIFLE, $\geqslant$stage 2 in AKIN and $\geqslant$grade 2 in the Grading criteria.

Statistical analysis

Data are shown as the mean ± s.d. Comparisons between more than three groups were performed using analysis of variance for continuous variables and the χ^2-test for categorical variables. Patient follow-up was undertaken on 31 December 2007. All-cause mortality was determined at 1000 days following SCT. Cumulative survival curves were prepared by the Kaplan–Meier method and the log-rank test was used to analyze differences between the curves. Cox regression analysis was used to determine the association of each AKI category with mortality, followed by calculation of adjusted hazard ratio (HR) and 95% confidence intervals. The multivariate models incorporated a forward selection stepwise method using variables with a P-value of <0.20 in the univariate analyses. JMP version 7 (SAS Institute Japan, Cary, CA, USA) was used for all the statistical analyses. Values of $P<0.05$ were considered statistically significant.

Results

Demographics and baseline characteristics of patients following SCT

The baseline characteristics of the 141 m-allo patients, 60 nm-allo patients and 48 auto patients are summarized in Table 2. The age of the patients at the time of SCT was

Table 1 A comparison of post-SCT AKI[a] definition and classification according to serum Cr levels in the RIFLE, AKIN and grading criteria

(A) RIFLE	
Risk	Increase in serum Cr $\geqslant 1.5\times$ baseline or decrease in GFR $\geqslant 25\%$
Injury	Increase in serum Cr $\geqslant 2.0\times$ baseline or decrease in GFR $\geqslant 50\%$
Failure	Increase in serum Cr $\geqslant 3.0\times$ baseline or decrease in GFR $\geqslant 75\%$ or an absolute serum Cr $\geqslant 4.0$ mg/dl (354 μmol/l) with an acute rise of at least 0.5 mg/dl (44 μmol/l)
Loss	Persistent AKI >4 weeks
ESKD	ESKD >3 months
(B) AKIN	
Stage 1	Increase in serum Cr $\geqslant 0.3$ mg/dl (26.5 μmol/l) or increase to 150–199% (1.5–1.9-fold) from baseline
Stage 2	Increase in serum Cr to 200–299% (>2.0–2.9 fold) from baseline
Stage 3	Increase in serum Cr to $\geqslant 300\%$ ($\geqslant$3-fold) from baseline or serum Cr $\geqslant 4.0$ mg/dl (354 μmol/l) with an acute rise of at least 0.5 mg/dl (44 μmol/l).
(C) Grading	
Grade 0	Decrease in GFR <25% of baseline
Grade 1	Increase in serum Cr<2-fold from baseline with a decrease in GFR >25% but <50% of baseline
Grade 2	Increase in serum Cr $\geqslant$2-fold from baseline but not requiring dialysis
Grade 3	Increase in serum Cr $\geqslant$2-fold from baseline and need for dialysis

Abbreviations: GFR = glomerular filtration rate; RIFLE = risk, injury, failure, loss of kidney function, and end-stage kidney disease; AKIN = acute kidney injury network; Cr = creatinine; AKI = acute kidney injury; ESKD = end-stage kidney disease.
[a]Post-SCT AKI is defined within the first 100 days after SCT.

Table 2 Demographics and baseline characteristics of the patients following SCT

Variable	*M-allo (n = 141)*	*Nm-allo (n = 60)*	*Auto (n = 48)*
Age (years)	38.3 ± 10.8	42.9 ± 13.8†	50.4 ± 11.6*
Gender (M/F)	87/54	35/25	35/13
Baseline Cr μg/l	64.2 ± 17.6	66.3 ± 19.9	66.8 ± 17.3
Diagnosis			
ALL	37 (26.2%)	2 (3.3%)	0 (0%)
ANLL	53 (37.6%)	26 (43.3%)	5 (10.4%)*
CML	13 (9.2%)	5 (1.7%)	0 (0%)
MDS	23 (16.3%)	10 (16.7%)	0 (0%)
MM	3 (3.1%)	2 (2.5%)	29 (60.4%)*
AA	1 (0.7%)	12 (6.5%)	0 (0%)
NHL	5 (3.5%)	1 (1.7%)	11 (22.9%)*
Others	6 (4.3%)	3 (5%)	3 (6.3%)
Stem cell source			
BM	102 (72.3%)	42 (71.6%)	0 (0%)
PB	23 (16.3%)	12 (17.4%)	48 (100%)*
CB	16 (11.4%)	6 (11.0%)	0 (0%)
Related donor	53 (37.6%)	16 (26.7%)	—
Acute GVHD			
Grade 0–1	94 (66.7%)	37 (61.7%)	—
Grade $\geqslant$2	47 (33.3%)	23 (38.3%)	—
Chronic GVHD	18 (12.8%)	10 (16.7%)	—
Relapse	38 (27.0%)	12 (24.9%)	—

Abbreviations: M-allo = myeloablative allogeneic transplantation; Nm-allo = non-myeloablative allogeneic transplantation; Auto = autologous transplantation; Cr = serum creatinine before transplant; ALL = acute lymphocytic leukemia; ANLL = acute non-lymphocytic leukemia; CML = chronic myelocytic leukemia; MDS = myelodysplastic syndrome; MM = multiple myeloma; AA = aplastic anemia; CB = umbilical cord blood stem cell.
Asterisk (*) indicates a significant difference between the auto and other SCT modalities. Mark (†) indicates a significant difference between the m-allo and nm-allo groups.

significantly higher in the auto group than in the other two SCT modalities. There was also a significant difference in age between the m-allo and the nm-allo groups. The auto transplant group had the lowest proportion of acute non-lymphocytic leukemia and the highest proportion of multiple myeloma and peripheral blood SCT compared with the other two modalities. There were no significant differences in gender proportion, baseline Cr level, ratio of related donors, frequency of acute and chronic GVHD and frequency of relapse between the three modalities.

Incidence of AKI classified according to the RIFLE, AKIN and grading criteria

A comparison of the incidence data of post-SCT AKI according to the three different criteria is shown in Table 3. The current incidence of any AKI ranged between 62 and 66% in m-allo, between 40 and 48% in nm-allo and between 10 and 19% in auto transplants. We found small differences in the number of patients classified as having AKI between the AKIN and the other two criteria. The AKIN criteria had the lowest ability to identify patients with AKI of the three criteria for all three SCT modalities, because of reduced sensitivity to identify the lowest category of AKI. Only two patients were classified into stage 1 of AKIN, based on a rapid increase in serum Cr $\geqslant 0.3$ mg per 100 ml in the 48 h period after SCT. However, the ability to identify severe AKI ($\geqslant$injury or $\geqslant$stage 2 or $\geqslant$grade 2) was identical between the three criteria (29% in m-allo, 17% in nm-allo and 2% in auto). The sensitivity of the RIFLE criteria was comparable with that of the grading criteria. Two patients in the m-allo group and three patients in the nm-allo group were classified into stage 3 of AKIN as they were receiving dialysis treatment.

Mortality of AKI classified according to the RIFLE, AKIN and grading criteria

Mortality according to the three different criteria is shown in Table 4. The mortality of patients with any AKI category was approximately 54% in m-allo, 48–50% in nm-allo and 11–20% in auto transplants. Mortality increased in parallel with increases in AKI category, with mortality for

Table 3 Incidence of AKI classified according to the RIFLE, AKIN and grading criteria

RIFLE	*No. (%)*	*AKIN*	*No. (%)*	*Grading*	*No. (%)*
M-allo (n=141)					
None	48 (34.0)	None	54 (38.3)	Grade 0	48 (34.0)
Risk	52 (36.9)	Stage 1	46 (32.6)	Grade 1	52 (36.9)
Injury	21 (14.9)	Stage 2	21 (14.9)	Grade 2	38 (27.0)
Failure	20 (14.2)	Stage 3	20 (14.2)	Grade 3	3 (2.1)
Any category[a]	93 (66.0)	Any stage	87 (61.7)	Any grade	93 (66.0)
Severe AKI[b]	41 (29.1)	Severe AKI	41 (29.1)	Severe AKI	41 (29.1)
Nm-allo (n=60)					
None	31 (51.7)	None	36 (60.0)	Grade 0	31 (51.7)
Risk	19 (31.7)	Stage 1	14 (23.3)	Grade 1	19 (31.7)
Injury	5 (8.3)	Stage 2	5 (8.3)	Grade 2	7 (11.7)
Failure	5 (8.3)	Stage 3	5 (8.3)	Grade 3	3 (5.0)
Any category	29 (48.3)	Any stage	24 (40.0)	Any grade	29 (48.3)
Severe AKI	10 (16.7)	Severe AKI	10 (16.7)	Severe AKI	10 (16.7)
Auto (n=48)					
None	39 (81.3)	None	43 (89.6)	Grade 0	39 (81.3)
Risk	8 (16.7)	Stage 1	4 (8.3)	Grade 1	8 (16.7)
Injury	1 (2.1)	Stage 2	1 (2.1)	Grade 2	1 (2.1)
Failure	0 (0)	Stage 3	0 (0)	Grade 3	0 (0)
Any category	9 (18.8)	Any stage	5 (10.4)	Any grade	9 (18.8)
Severe AKI	1 (2.1)	Severe AKI	1 (2.1)	Severe AKI	1 (2.1)

Abbreviations: RIFLE = risk, injury, failure, loss of kidney function, and end-stage kidney disease; AKIN = acute kidney injury network; M-allo = myeloablative allogeneic transplantation; Nm-allo = non-myeloablative allogeneic transplantation; Auto = autologous transplantation.
[a]Any category includes AKI ⩾risk or ⩾stage 1 or ⩾grade 1.
[b]Severe AKI is denoted as AKI ⩾injury or ⩾stage 2 or ⩾grade 2.

Table 4 Mortality[a] of AKI classified according to the RIFLE, AKIN and grading criteria

RIFLE	*No. (%)*	*AKIN*	*No. (%)*	*Grading*	*No. (%)*
M-allo (n=141)					
None	48 (34.0)	None	54 (38.3)	Grade 0	48 (34.0)
Risk	52 (36.9)	Stage 1	46 (32.6)	Grade 1	52 (36.9)
Injury	21 (14.9)	Stage 2	21 (14.9)	Grade 2	38 (27.0)
Failure	20 (14.2)	Stage 3	20 (14.2)	Grade 3	3 (2.1)
Any category[a]	93 (66.0)	Any stage	87 (61.7)	Any grade	93 (66.0)
Severe AKI[b]	41 (29.1)	Severe AKI	41 (29.1)	Severe AKI	41 (29.1)
Nm-allo (n=60)					
None	31 (51.7)	None	36 (60.0)	Grade 0	31 (51.7)
Risk	19 (31.7)	Stage 1	14 (23.3)	Grade 1	19 (31.7)
Injury	5 (8.3)	Stage 2	5 (8.3)	Grade 2	7 (11.7)
Failure	5 (8.3)	Stage 3	5 (8.3)	Grade 3	3 (5.0)
Any category	29 (48.3)	Any stage	24 (40.0)	Any grade	29 (48.3)
Severe AKI	10 (16.7)	Severe AKI	10 (16.7)	Severe AKI	10 (16.7)
Auto (n=48)					
None	39 (81.3)	None	43 (89.6)	Grade 0	39 (81.3)
Risk	8 (16.7)	Stage 1	4 (8.3)	Grade 1	8 (16.7)
Injury	1 (2.1)	Stage 2	1 (2.1)	Grade 2	1 (2.1)
Failure	0 (0)	Stage 3	0 (0)	Grade 3	0 (0)
Any category	9 (18.8)	Any stage	5 (10.4)	Any grade	9 (18.8)
Severe AKI	1 (2.1)	Severe AKI	1 (2.1)	Severe AKI	1 (2.1)

Abbreviations: RIFLE = risk, injury, failure, loss of kidney function, and end-stage kidney disease; AKIN = acute kidney injury network; M-allo = myeloablative allogeneic transplantation; Nm-allo = non-myeloablative allogeneic transplantation; Auto = autologous transplantation.
[a]Mortality was determined 1000 days after transplantation.
[b]Any category includes AKI ⩾risk or ⩾stage 1 or ⩾grade 1.

the highest category in the RIFLE and AKIN criteria increasing markedly in m-allo transplants and fatally in nm-allo transplants. The difference in mortality between AKI and no AKI according to the RIFLE criteria was marked and was especially apparent in the nm-allo group (48 versus 16%) compared with the m-allo group (54 versus 35%). This difference was not applicable to the auto group. Three patients in the m-allo group and three patients in the nm-allo group who required dialysis treatment died within 100 days of SCT. The Kaplan–Meier curves stratified by the RIFLE classification are shown in Figures (a), (b) and (c) and show clear differences in long-term survival between

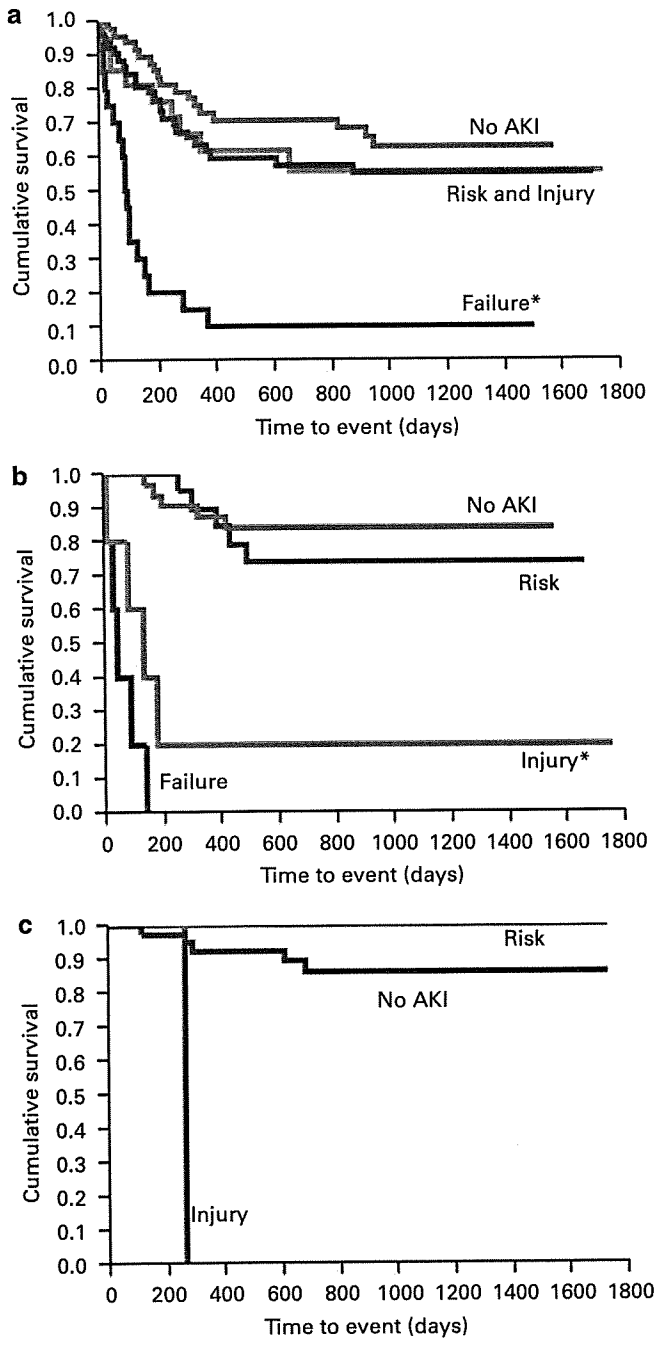

Figure 1 (a), (b) and (c) show cumulative survival curves after m-allo, nm-allo and auto transplants, stratified according to the RIFLE categories. The *x* axis represents the number of days post-hematopoietic cell transplantation (HCT) and the *y* axis represents cumulative survival. (a): myeloablative allo-SCT ($n=141$), (b): nonmyeloablative allo-SCT ($n=60$), (c): auto-SCT ($n=48$). *Indicates log-rank test, $P<0.0001$.

the various categories. The difference between curves 1000 days after SCT were significant for failure in m-allo transplants and for injury in nm-allo transplants, compared with no AKI (log-rank, $P<0.0001$). Similar curves of comparable significance were obtained when other criteria were used for stratification (data not shown).

Adjusted association of each AKI category with mortality and discriminative ability of each classification in allogeneic SCT

Cox regression analysis, adjusted for age at SCT, baseline serum Cr, gender (male), absence or presence of acute GVHD ⩾grade 2 and chronic GVHD, an unrelated donor and relapse of underlying diseases, showed that each category (⩾injury, ⩾stage 2 and ⩾grade 2) independently predicted mortality in both of the m-allo and nm-allo groups (Table 5). A large stepwise increment in hazard ratio for mortality was observed with increases in AKI category for all three classification systems. The discriminative ability for mortality was almost comparable in the three definitions. In the m-allo group, the AuROC curve was 0.649 ($P=0.0005$) with the RIFLE criteria, 0.643 ($P=0.0007$) with the AKIN criteria and 0.629 ($P=0.0058$) with the grading criteria. In the nm-allo group, the corresponding AuROC values were 0.766 ($P=0.0006$) with the RIFLE criteria, 0.734 ($P=0.0008$) with the AKIN criteria and 0.765 ($P=0.0006$) with the grading criteria (Table 5).

Discussion

The present study obtained the most recent data regarding the incidence and mortality of AKI following SCT, according to two new and one conventional AKI definition systems. Compared with the RIFLE and grading criteria, the AKIN criteria had the disadvantage of relatively poor sensitivity to identify the lowest category of AKI in any type of transplantation modality. All three criteria were found to have comparable utility for stratifying post-SCT patients with AKI according to mortality risk.

Our study showed that the current incidence of post-SCT AKI was, at most, 66% in m-allo, 48% in nm-allo and 19% in auto transplants and that mortality was, at most, 54% in m-allo, 50% in nm-allo and 20% in auto transplants. The incidence rates for each of the allogeneic types were markedly higher than those of auto transplants, a finding that is comparable with those reported in previous studies.[1,2,5] The current incidence rates were considerably lower than those reported previously by Parikh *et al.* and Caliskan *et al.* using the grading criteria within the first 100 days after SCT (91–92% in m-allo, 90% in nm-allo and 52–57% in auto).[2,11,12,17] In contrast, the present mortality rate was almost the same as previous estimations using the grading criteria (56–58% in m-allo transplants and 43% in nm-allo transplants, but no reference mortality in auto transplants alone).[2,4,11,18] Most recently, Lopes *et al.* showed the incidence of AKI (40%) and 3-year mortality (48.8%) according to the AKIN criteria in m-allo, and the incidence (53.6%) and 5-year mortality (58.4%) in reduced intensity conditioning SCT according to the RIFLE criteria.[3,19] Compared with our results, the incidences and mortality rates reported were lower in m-allo transplants and higher in RIC transplants. However, there were the following differences between the two studies: their cohort of m-allo transplants included 35.8% of myeloablative auto transplants and the length of the follow-up periods was longer than in our study. Unfortunately, this earlier study

Table 5 Adjusted association of each AKI category with mortality[a] and discriminative ability of each classification in allogeneic transplantation

AKI category	*Adjusted HR[b] (95% CI)*	P-*value*	*AuROC*	P-*value*
Myeloablative (n = 141)				
RIFLE			0.649	0.0005
Failure	8.802 (4.720–16.467)	<0.0001		
Injury	2.590 (1.252–5.151)	0.0114		
Risk	1.639 (0.939–2.897)	0.0822		
None (reference)	1.00			
AKIN			0.643	0.0007
AKIN stage 3	7.950 (4.359–14.413)	<0.0001		
AKIN stage 2	2.332 (1.149–4.503)	0.0202		
AKIN stage 1	1.409 (0.800–2.460)	0.2319		
None (reference)	1.00			
Grading			0.629	0.0058
Grade 3	10.04 (3.226–26.160)	0.0004		
Grade 2	4.333 (2.494–7.652)	<0.0001		
Grade 1	1.670 (0.956–2.952)	0.0715		
Grade 0 (reference)	1.00			
Non-myeloablative (n = 60)				
RIFLE			0.766	0.0006
Failure	123.9 (16.74–1228)	<0.0001		
Injury	34.51 (4.813–277.4)	0.0005		
Risk	1.246 (0.298–3.428)	0.7589		
None (reference)	1.00			
AKIN			0.734	0.0008
AKIN stage 3	110.3 (16.07–1036)	<0.0001		
AKIN stage 2	28.20 (4.277–202.4)	0.0007		
AKIN stage 1	0.822 (0.157–9.041)	0.7950		
None (reference)	1.00			
Grading			0.765	0.0006
Grade 3	609.3 (48.81–11813)	<0.0001		
Grade 2	52.85 (8.859–399.9)	<0.0001		
Grade 1	1.316 (0.310–5.843)	0.7059		
Grade 0 (reference)	1.00			

Abbreviations: AKI = acute kidney injury; HR = hazard ratio; RIFLE = risk, injury, failure, loss of kidney function, and end-stage kidney disease; AKIN = acute kidney injury network; AuROC = the area under the receiver operator characteristic curve for long-term mortality.
[a]Mortality was determined 1000 days after transplantation.
[b]Adjusted for age, acute GVHD, chronic GVHD, unrelated donors, relapse and stem cell sources.

did not include a comparative assessment of their data with the grading criteria. Further epidemiological studies on post-SCT AKI according to the new AKI criteria are needed to estimate the recent incidence and mortality of AKI following SCT.

The Kaplan–Meier curves showed that the separation between the 'risk' and 'injury' curves in the m-allo group appeared to be obscure, compared with that seen in the nm-allo group. In addition, Cox regression analysis indicated that the HR of 'injury' for long-term mortality was significant but rather lower in the m-allo group (HR; 2.59) than in the nm-allo group (HR; 34.5). These differences suggest that the nm-allo group may be more vulnerable for exposure to the 'intermediate' level of AKI in the long term than the m-allo group. There was a significant difference in age at the time of SCT between the m-allo and nm-allo groups (38.3 versus 42.9 years). The older age of the nm-allo group may therefore have contributed to their higher risk of mortality after they were exposed to the 'intermediate' level of AKI.

The sensitivity of the AKIN criteria to identify patients with the lowest category of AKI was less than the other two criteria for all three transplant modalities. The AKIN criteria require at least two Cr values within a 48 h period rather than referring to a baseline Cr value in Stage 1. A rapid, small increase ($\geqslant$0.3 mg per 100 ml) in serum Cr within 48 h could be under-recognized and often overlooked in the context of SCT. In fact, the 48 h time frame definition was not available in almost all post-SCT patients as we were not able to measure serum Cr frequently during the 100 days after SCT. This is most likely associated with the lower sensitivity of the AKIN criteria to identify stage-1 AKI, possibly leading to misclassification of AKI stages. In addition, Cox regression analysis showed that each of the categories higher than the intermediate stage were significant independent predictors of mortality in all three AKI definition systems. The AuROC curves for mortality showed equal significance for all the AKI definition systems. These results show that the RIFLE and AKIN classifications have almost the same predictive utility for mortality in patients with post-SCT AKI, but that they do not substantially improve the ability of the grading criteria to predict mortality in the context of SCT. Taken together, our results suggest that the RIFLE criteria are more favorable than the AKIN criteria as a uniform identification system for post-SCT AKI, and that clinical significance of previous epidemiological data according to the grading criteria remain valid today.

This study has some limitations. Firstly, we were not able to use urine output criteria in the new AKI criteria. When

applying these new definitions to post-SCT AKI, it proved difficult to obtain accurate records of urine output in every patient throughout the SCT period. In addition, the volume status of patients during the acute period of SCT may have varied widely according to infusion therapy, whereas urinary tract obstruction sometimes occurred because of hemorrhagic cystitis induced by local adenoviral infections or as a side effect of administration of high dose of CY. These conditions may have resulted in a biased assessment of the true burden of post-SCT AKI using the two new criteria. The difficulties in practicing the urine output criteria are inherent in the assessment of the new AKI criteria especially in the setting of SCT. Secondly, we used serum Cr level and Cr-based estimates of GFR according to previous literature.[1,2] However, a challenge in the study of AKI in the post-SCT population is that serum Cr is a less effective measure of GFR in the setting of co-morbid illness. Cr-based estimates of GFR above 60 ml/min/1.73 m^2 are considered imprecise even in the general population.[16] Thirdly, although non-relapse mortality would add important information to this study, the current data set was not adequate to perform statistical analysis for non-relapse mortality in all types of SCT in accordance with that described in previous studies.[2,3] Finally, this was a single-center study on a relatively small cohort of SCT patients.

In summary, the variability in incidence of AKI following SCT most likely reflects the lack of a standard definition for post-SCT kidney disease, differences in the types of transplants investigated and variability in the length of the follow-up periods. It is, therefore, worthwhile to establish a uniform standard for diagnosing and classifying post-SCT AKI in comparative studies and carrying out robust epidemiological investigations internationally. The RIFLE criteria have shown promise as a uniform standard to identify and classify post-SCT patients with various degrees of AKI and to predict the mortality of these patients. However, this definition system does not substantially exceed the abilities of the conventional grading criteria. The incidence of AKI that we observed appears to be lower than that reported in previous studies, although the mortality of patients with higher categories of AKI still remains high in m-allo and nm-allo transplants. Emerging evidence suggests that even minor changes in serum Cr are associated with increased mortality.[7–10] Despite the significant progress made in understanding the biology and mechanism of AKI in animal models, application of this knowledge into improved management and outcomes for patients has been limited. However, immediate fluid therapy for potential circulatory deficits, avoidance and minimizing the use of nephrotoxic agents and dosage adjustment of medication according to kidney function are sometimes effective for preventing worsening of the early stages of AKI.[20] Transplant physicians and nephrologists need to work together in the treatment of patients receiving SCT to identify early renal disease and also to examine small changes in serum creatinine concentration or promising new urinary biomarkers.[6,21] Further efforts will be required to decrease the frequency of life-threatening AKI, with initial focus on m-allo and nm-allo transplants.

Conflict of interest

The authors declare no conflict of interest.

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Transmission of chromosomally integrated human herpesvirsus 6 (HHV-6) variant A from a parent to children leading to misdiagnosis of active HHV-6 infection

T. Mori, K. Tanaka-Taya, H. Satoh, Y. Aisa, R. Yamazaki, J. Kato, Y. Ikeda, S. Okamoto. Transmission of chromosomally integrated human herpesvirsus 6 (HHV-6) variant A from a parent to children leading to misdiagnosis of active HHV-6 infection.
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Abstract: Only a handful of cases of chromosomally integrated human herpesvirus 6 (CI-HHV-6) have been reported, suggesting that this phenomenon is rare. We here present a familial case of HHV-6 variant A (HHV-6A) transmission through a generation, which was identified in the setting of allogeneic hematopoietic stem cell transplantation (HSCT). A 31-year-old man with myelodysplastic syndrome underwent allogeneic HSCT from a human leukocyte antigen-identical sibling, and was found to be continuously yielding high copy numbers of HHV-6A DNA in plasma evaluated by real-time polymerase chain reaction (PCR). Antiviral therapy with ganciclovir or foscarnet failed to decrease the copy numbers. HHV-6A DNA was detected in the patient's buccal mucosa and hair follicles, and was also detected in the plasma, whole blood, and buccal mucosa of the patient's father and 2 siblings, but not in his mother. The sequences of HHV-6A DNA isolated from all family members were identical. Since monitoring of HHV-6 by PCR has been widely introduced to the field of HSCT, transplant physicians should be aware of such an alternative form of HHV-6 transmission, particularly when HHV-6A is detected.

T. Mori[1], K. Tanaka-Taya[2], H. Satoh[2], Y. Aisa[1], R. Yamazaki[1], J. Kato[1], Y. Ikeda[1], S. Okamoto[1]

[1]Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo, Japan, [2]Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan

Key words: human herpesvirus 6 (HHV-6); HHV-6 variant A; hematopoietic stem cell transplantation; chromosomally integrated HHV-6; polymerase chain reaction

Correspondence to:
Takehiko Mori, MD, PhD, Division of Hematology, Department of Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
Tel: + 81 3 3353 1211 (ext 62385)
Fax: + 81 3 3353 3515
E-mail: tmori@sc.itc.keio.ac.jp

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Human herpesvirus 6 (HHV-6), a member of the beta-herpesvirinae subfamily, possesses biological properties similar to those of cytomegalovirus and HHV-7 (1). After primary infection, such as exanthema subitum during early childhood, HHV-6 persists extra-chromosomally in latently infected host cells and could reactivate in immunocompromised patients (2). HHV-6 is widespread in the human population, and seroprevalence studies suggest that >80–90% of adults are seropositive for HHV-6, without differences among ethnicities (3, 4). HHV-6 is classified into 2 variants, variant A (HHV-6A) and variant B (HHV-6B), and they differ in genetic, epidemiological, and pathological properties (1). Our previous study demonstrated a higher incidence of detecting HHV-6B in throat swabs of children, suggesting that high seroprevalence for HHV-6 is chiefly due to HHV-6B (4). HHV-6B is well recognized as the etiologic agent for exanthema subitum and febrile illness in children and universally infects the general population. In contrast, HHV-6A exhibits a low prevalence and has no association with any diseases, and little is known of its natural course, including the time of first infection (1). Salivary transmission is the most common route of HHV-6 infection, primarily evaluated with HHV-6B, although some investigators have suggested the possibility of intrauterine or perinatal transmission (1, 5, 6).

HHV-6 is the only HHV known to be integrated into human chromosomes and to transmit from parents to offspring. Thus, an alternative form of HHV-6 persistence is chromosomally integrated HHV-6 (CI-HHV-6). Luppi et al. (7) first reported 3 unrelated cases of CI-HHV-6 in 1993. The phenomenon is characterized by persistent high copy numbers of HHV-6 DNA in whole blood, cell-free plasma, or serum, and even in other cells including throat swabs and hair follicles without viral replication (7–13). We have accidentally identified a patient with CI-HHV-6 variant A during the monitoring of HHV-6 reactivation soon after

allogeneic hematopoietic stem cell transplantation (HSCT). The patient's family members, including his father and 2 siblings, were also identified as having CI-HHV-6 variant A, indicating the transmission of CI-HHV-6 from father to children. To the best of our knowledge, no case of familial transmission of CI-HHV-6 variant A has been documented except for 1 case reported in our previous study (9), and thus this is the second reported case of the genetic inheritance of CI-HHV-6 variant A.

Patients and methods

Patients and transplant procedures

A 31-year-old man with myelodysplastic syndrome underwent allogeneic peripheral blood stem cell transplantation from a human leukocyte antigen (HLA)-identical sibling donor. The conditioning regimen consisted of total body irradiation and cytarabine administration. Cyclosporine and short-term methotrexate were used as prophylaxis against graft-versus-host disease (GVHD). Intravenous acyclovir (750 mg/day) was given as prophylaxis against herpes simplex virus from 3 days before transplant until day 14 post transplant. His post-transplant course was remarkable for moderate, acute GVHD involving his skin and the gastrointestinal tract; this was successfully treated with prednisolone. HHV-6 reactivation was monitored by using real-time polymerase chain reaction (PCR) as reported previously (14). High copy numbers of HHV-6 DNA of variant A were repeatedly detected in plasma, ranging between 2×10^3 and 2×10^4/mL of plasma without any association with development of GVHD or the doses of prednisolone. Although, the patient remained asymptomatic, intravenous ganciclovir was initiated at a daily dose of 10 mg/kg and continued for 3 weeks, which failed to reduce HHV-6A DNA copy numbers in the plasma. Because of its myelotoxicity, ganciclovir was replaced with foscarnet, which also failed to reduce the copy numbers of HHV-6A DNA. Therefore, samples (plasma, peripheral blood mononuclear cells [PBMCs], buccal mucosa, and hair follicles) were collected not only from the patient but also from the patient's family members, including his parents, sister, and brother. The samples were applied to further investigation.

Serological examination for HHV-6

A fluorescent antibody method was used to detect anti-HHV-6 antibody in serum as described previously (14). Briefly, sera were first diluted 10 times, then serially diluted 2-fold in phosphate-buffered saline, after which they were reacted with HHV-6 antigen fixed on a slide. HHV-6 antigen was prepared from NP40-solubilized lymphocytes infected with the virus. After incubation for 1 h at 37°C, the slides were washed and then reacted with fluorescein isothiocyanate-conjugated rabbit anti-human immunoglobulin (Ig) G antibody (Dako, Glostrup, Denmark). Fluorescence was detected by a fluorescent microscope, and the endpoint titer was determined as the reciprocal of the maximum dilution at which fluorescence was detected. Titers >1:10 were deemed positive.

Real-time PCR to detect HHV-6 in plasma and whole blood

Real-time PCR was performed basically as reported previously (14). Primers were designed to distinguish HHV-6A from HHV-6B. Briefly, DNA extracted from plasma or whole blood using a QIAamp Blood Mini Kit (Qiagen, Valencia, California, USA) was subjected to PCR. Sequences of PCR primers and the probe for HHV-6A were selected from the U89 immediate-early (IE) protein region of HHV-6, and those for variant B (HHV-6B) were selected from the U90 IE-A transactivator region. The sequences of forward primers were 5′-GTACAGCCTCAGTGACAGATC TG-3′ for both variant A and variant B; the reverse primers were 5′-AGGAACCATCTTGTTCTGTCCCTT-3′ for HHV-6A and 5′-GGTCATACCAGGAAGCGTTTCG-3′ for HHV-6B. The TaqMan probe selected between the primers was dual-labeled with FAM (6-carboxyfluorescein) at the 5′-end as a reporter dye and with TAMRA (6-carboxy-teremethyl-rhodamine) at the 3′-end as a quenching dye, whose sequence was 5′-CAGCCCCGATAAAAGGTCACAGAC AAAAGA-3′. The PCR reaction was performed by using TaqMan Universal PCR Master Mix (PE Biosystems, Foster City, California, USA), and nuclease degradation of the probe was detected as an increase in fluorescent intensity by ABI PRISM 7700 (PE Biosystems). The quantification of HHV-6 was carried out with a serially diluted standard ranging from 10 to 1×10^7 copies, and the gene copy numbers were calculated by Sequence Detection System ver. 1.6.3 software (PE Biosystems). The sensitivity of this assay was 200 copies/mL of plasma or whole blood.

PCR to detect HHV-6 in PBMCs, buccal mucosa, and hair follicles

HHV-6 DNA in PBMCs, buccal mucosa, and hair follicles was amplified by nested PCR basically as described previously (4, 9). The PCR was designed to amplify the IE regions of HHV-6A and HHV-6B. The sizes of the first PCR amplification products of HHV-6A and HHV-6B were 325 and 553 bp, and those of the nested double PCR amplification products were 195 and 421 bp, respectively. The primers

for the first PCR were 5′-TTCTCCAGATGTGCCAGGGAAATCC-3′ and 5′-CATCATATGTTATCGTTTCACTCTC-3′. Those for nested double PCR were 5′-AGTGACAGATCTGGGCGGCCCTAATAACTT-3′ and 5′-AGGTGCTGAGTGATCAGTTTCATAACCAAA-3′.

DNA sequencing of the PCR products

The PCR products were completely sequenced by the dideoxynucleotide chain termination methods. DNA similarity searches were performed by using the FASTA network service.

Results

Detection of HHV-6 DNA in plasma and whole blood by real-time PCR

All 5 family members were HHV-6 seropositive, with titers of anti-HHV-6 IgG ranging from 1:40 to 1:160 (Table 1). HHV-6A DNA was detected in the plasma and whole blood of the patient as well as in those of other family members, except for the patient's mother (Table 1). HHV-6B DNA was not detected in any samples obtained from the patient or his family members. The copy number of HHV-6A DNA ranged between 6×10^2 and $2 \times 10^4/\mu L$ of plasma, and between 4×10^5 and $4 \times 10^6/\mu L$ of whole blood, respectively.

Detection of HHV-6A DNA in PBMCs, buccal mucosa, and hair follicles by nested PCR

A PCR product of 195 bp, which is specific for HHV-6A DNA, was detected in PBMCs and buccal mucosa of the patient and other family members except for the patient's mother (Table 1). HHV-6A DNA was also detected in the patient's hair follicles. The sequences of PCR products from each member were completely identical to each other, and showed a high homology with the prototypes of HHV-6A (ranging between 94.12% and 95.59%), including GenBank accession nos. AY245914, X83413, AF015298, M73681, and L21759. The sequences were different from that of HHV-6B (accession no. AB021506).

Discussion

We have identified a case of CI-HHV-6 variant A that misled us to make a diagnosis of active HHV-6 infection after allogeneic HSCT. CI-HHV-6 was confirmed by the persistence of high copy numbers of HHV-6A DNA in peripheral blood and by the detection of HHV-6 DNA in the buccal mucosa and hair follicles (11). Detection of HHV-6 DNA not only in blood cells but also in other tissues reflects germline inheritance, although it was not confirmed by fluorescent *in situ* hybridization analysis. Instead, further examination identified all of the patient's family members except his mother as having CI-HHV-6 variant A, indicating the transmission of HHV-6A genetically from father to children. The phenomenon of CI-HHV-6 is considered rare, and cases of CI-HHV-6 have only sporadically been reported in the literature (7–13). Genetic inheritance of HHV-6 through generations was first reported by Daibata et al. (8), and was later substantiated by our previous report (9). Although HHV-6 variants were not necessarily specified in these reports, there has been only 1 case of inheritance of CI-HHV-6 variant A, and this was in our previous report (7). Thus, the present case is the second documentation of inheritance of CI-HHV-6 variant A.

Serology of human herpesvirus (HHV)-6 and detection of HHV-6A DNA in the patient and the family members

	Patient	Brother (donor)	Sister	Father	Mother
HHV-6 IgG	1:160	1:40	1:160	1:40	1:40
HHV-6 copy number μL^1					
Plasma	2×10^4	5×10^3	6×10^2	1×10^4	Negative
Whole blood	Not examined	4×10^5	4×10^6	4×10^5	Negative
Polymerase chain reaction					
Peripheral blood mononuclear cells	Positive	Positive	Positive	Positive	Negative
Buccal mucosa	Positive	Positive	Positive	Positive	Negative
Hair follicles	Positive	Not examined	Not examined	Not examined	Not examined

[1]HHV-6 copy number was quantitatively measured by real-time polymerase chain reaction.
IgG, immunoglobulin G.

Table 1

Only a few studies, including our previous report, have examined the prevalence of CI-HHV-6 among patients or healthy individuals, and it ranged between 0.21% and 2.8% (9, 12, 13). In contrast to its primary infection or reactivation, which is universally caused by HHV-6B, it is notable that a high proportion of CI-HHV-6 is due to variant A (9, 13). A recent report evaluated congenital HHV-6 infection by detecting HHV-6 DNA in cord blood cells, and showed that its incidence was 1% and that one-third of congenital HHV-6 infections were with variant A (15). Although the authors did not mention the possibility of CI-HHV-6, the incidence of HHV-6 detection and the high proportion of variant A are similar to those in the reports of CI-HHV-6 (11, 14). It is conceivable that most or all congenitally transmitted HHV-6 is chromosomally inherited. In addition, based on these observations, the main route of HHV-6A transmission is considered congenital, contributing to the development of CI-HHV-6, while HHV-6B is the etiology of CI-HHV-6 as well as primary infection and reactivation.

In regards to HSCT recipients, HHV-6 is known to cause various manifestations such as skin rash, pneumonitis, myelosuppression, delayed platelet engraftment, and central nervous system disorders (1, 2, 16). Thus, to prevent life-threatening HHV-6 infection, several studies have evaluated the significance of monitoring HHV-6 reactivation after allogeneic HSCT (2, 14, 17). Based on the recognition of such features, it is highly possible that the high viral load detected in individuals with CI-HHV-6 could mislead transplant physicians into administering potentially toxic antiviral agents. To avoid the administration of unnecessary antiviral agents, CI-HHV-6 should promptly be distinguished from active HHV-6 infection by clinically available methods, such as detection of a constant level of HHV-6 DNA in peripheral blood, simultaneous detection of HHV-6 DNA in non-blood cells (oral swab or hair follicle cells), and distinction of the variants.

In conclusion, because of the indistinguishable results of PCR obtained in individuals with CI-HHV-6 and active HHV-6 infection, the possibility of CI-HHV-6 should always be kept in mind by transplant physicians, particularly when HHV-6A is identified. Furthermore, continued follow-up and accumulation of cases of CI-HHV-6 are needed, as the natural history of CI-HHV-6 individuals is still poorly understood.

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ORIGINAL ARTICLE

Drug interaction between voriconazole and calcineurin inhibitors in allogeneic hematopoietic stem cell transplant recipients

T Mori[1], Y Aisa[1], J Kato[1,2], Y Nakamura[1], Y Ikeda[1] and S Okamoto[1]

[1]*Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo, Japan and* [2]*Novartis Pharma Program for the Clinical Therapeutics of Hematologic Malignancies, Keio University School of Medicine, Tokyo, Japan*

Although voriconazole has been shown to interact with calcineurin inhibitors, this interaction has not been thoroughly examined. The purpose of this study was to evaluate the drug interaction between voriconazole and calcineurin inhibitors among recipients of allogeneic hematopoietic stem cell transplantation (HSCT). Twenty-one recipients of allogeneic HSCT were evaluated. Those recipients had been on CsA ($n=10$) or tacrolimus ($n=11$) when voriconazole (400 mg per day orally, or 8 mg/kg per day, i.v.) was initiated. Trough concentrations of calcineurin inhibitors were measured before and periodically after initiating voriconazole to determine the concentration/dose (C/D) ratio of calcineurin inhibitors. Median C/D ratio significantly increased by initiating voriconazole: from 86.0 (range, 43.5–178.8) to 120.2 (range, 86.1–379.4) in CsA ($P<0.05$), and from 595.9 (range, 51.3–1643.3) to 890.7 (range, 94.1–4658.3) (ng/ml)/(mg/kg) in tacrolimus ($P<0.01$). Median increases in the C/D ratio did not differ significantly between CsA and tacrolimus (82.1%, ranging from −9.4 to 266.9% vs 115.6%, ranging from 25.4 to 307.6%). These results indicate that voriconazole alters the blood concentration of calcineurin inhibitors with a wide range of interindividual variability after allogeneic HSCT. Dose adjustment of calcineurin inhibitors on initiating voriconazole should not be decided uniformly, but determined on an individual basis by close monitoring of their blood concentrations.

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Introduction

Patients with hematological malignancies receiving chemotherapy, particularly those undergoing hematopoietic stem cell transplantation (HSCT), are at high risk of developing invasive fungal disease (IFD). Fluconazole has been routinely recommended for the prophylaxis and treatment of IFD after HSCT.[1] However, because of the increasing incidence of mold infection, mainly *Aspergillus* species, effective prophylaxis and treatment of *Aspergillus* species has been required.[2,3] Voriconazole, a novel triazole antifungal agent, has a potent activity against a broad spectrum of fungi, including yeasts and molds. Voriconazole has been reported to improve the survival of patients with invasive aspergillosis as compared with amphotericin B, and has become the first option for the treatment of invasive aspergillosis.[4,5]

Voriconazole is metabolized by cytochrome P-450 (CYP) enzymes, namely CYP 2C9, 2C19 and 3A4.[5] *In vitro* studies have demonstrated that voriconazole could be a substrate as well as an inhibitor of these enzymes.[6] Therefore, its drug interaction with a variety of agents metabolized by these enzymes, including immunosuppressive agents (CsA, tacrolimus and sirolimus), has been recognized. Its drug interaction with CsA and tacrolimus is especially problematic, because calcineurin inhibitors have a narrow therapeutic window.[5–8] However, the drug interaction between voriconazole and calcineurin inhibitors has been systematically examined only in a limited number of renal transplant recipients (CsA) and in healthy subjects (tacrolimus), and documented sporadically in a few case reports.[7–12] In particular, the drug interaction between voriconazole and tacrolimus has not been systematically evaluated in patients to this date. In spite of such limited data, a uniform dose reduction of calcineurin inhibitors on initiating voriconazole (1/2 for CsA, 1/3 for tacrolimus) has been recommended by the manufacturer.[13] Although the evaluation of drug interaction between voriconazole and calcineurin inhibitors is highly relevant for clinical practice because of the increase in the use of voriconazole after allogeneic HSCT, there has been no such evaluation in recipients of allogeneic HSCT except for one case report.[12] This prompted us to study the drug interaction between voriconazole and calcineurin inhibitors in 21 recipients of allogeneic HSCT to confirm the appropriateness of uniform

Correspondence: Dr T Mori, Division of Hematology, Department of Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.
E-mail: tmori@sc.itc.keio.ac.jp
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dose modification of calcineurin inhibitors on initiating voriconazole in this patient population.

Patients and methods

Patient selection and drug administration

Recipients of allogeneic HSCT who had already been on a steady dose of calcineurin inhibitors (CsA or tacrolimus), and were started on oral or i.v. voriconazole for the treatment or prophylaxis of aspergillosis, were included in this study. Patient's blood levels of CsA or tacrolimus were stable when voriconazole was initiated. Oral voriconazole was administered under fasting conditions at a maintenance dose of 200 mg per body every 12 h after two initial doses of 300 mg per body 12 h apart. The median maintenance dose of voriconazole was 4.0 mg/kg per day (range, 3.1–4.6) every 12 h in patients who received voriconazole orally. Patients who received voriconazole orally did not have gastrointestinal symptoms. Voriconazole (i.v.) was administered at a dose of 4 mg/kg every 12 h after two initial doses of 6 mg/kg 12 h apart.

Determination of concentration/dose ratio of calcineurin inhibitors

Whole blood levels of CsA and tacrolimus were measured using standard fluorescence polarization immunoassay and microparticle enzyme immunoassay, respectively. Blood levels of calcineurin inhibitors were measured just before and every 1–2 days after initiating voriconazole for 7–10 days. Each physician decreased doses of the calcineurin inhibitors in response to rising levels every 1–2 days. The concentration/dose (C/D; (ng/ml)/(mg/kg)) ratio of calcineurin inhibitors was calculated 7–10 days after initiating voriconazole when the increased blood levels of calcineurin inhibitors had stabilized. The increase in the C/D ratio after initiating voriconazole was determined in comparison with that just before initiating the drug.

Statistical analysis

The Wilcoxon signed-rank test was used to compare the difference in the C/D ratio before and after initiating voriconazole. The Mann–Whitney *U*-test was used to compare the difference in percentage of the increase in C/D ratio between CsA- and tacrolimus-administered patients. *P*-values less than 0.05 were accepted as statistically significant.

Results

Patients

A total of 21 patients were evaluated, and their characteristics are shown in Table 1. Of them, 10 patients had been on CsA, and 11 patients had been on tacrolimus. The median post transplant day when voriconazole was initiated was 106 (range, 10–580). Voriconazole was given orally in 11 patients, and i.v. in 10 patients. All patients had a stable renal and hepatic function during the administration of voriconazole.

Table 1 Patient characteristics

	Total (n = 21)	*CsA (n = 10)*	*Tacrolimus (n = 11)*
Median age (range)	51 (23–59)	50 (41–59)	46 (23–57)
Gender			
Male/female	13/8	7/3	6/5
Median body weight, kg (range)	51.3 (42.0–80.3)	56.9 (45.4–67.6)	46.5 (42.0–80.3)
Underlying diseases			
Myelodysplastic syndrome	10	7	3
Acute leukemia	9	3	6
Malignant lymphoma	1	0	1
Myeloproliferative disease	1	0	1
Stem cell donor			
Related	9	8	1
Unrelated	12	2	10
Conditioning regimen			
Myeloablative	16	7	9
Reduced intensity	5	3	2
AcuteGVHD			
Grades 0–I	5	2	3
Grades II–IV	16	8	8
Route of voriconazole administration			
Oral	11	7	4
I.v.	10	3	7

Effect of voriconazole administration on C/D ratio of calcineurin inhibitors

Blood levels of CsA and tacrolimus increased steadily after initiating voriconazole in all patients except for one patient in whom voriconazole did not affect the levels of CsA. The median C/D ratio of CsA after initiating voriconazole was 120.2 (ng/ml)/(mg/kg) with a range of 86.1–379.4, which was significantly higher than that before initiating voriconazole (86.0 (ng/ml)/(mg/kg) with a range of 43.5–178.8; $P<0.05$; Table 2). The median C/D ratio of tacrolimus after initiating voriconazole was 890.7 (ng/ml)/(mg/kg) with a range of 94.1–4658.3, which was significantly higher than that before initiating voriconazole (595.9 with a range of 51.3–1643.3; $P<0.01$; Table 2). Median increases were 82.1% (range, −9.4–266.9) and 115.6% (range, 25.4–307.6) in CsA- and tacrolimus-administered patients, respectively. The difference in increases between CsA and tacrolimus was not significant ($P=0.14$). Neither the route of voriconazole administration (i.v. or oral) nor the gender significantly affected the increase in C/D ratio ($P=0.12$ and 0.60, respectively). No significant adverse effects associated with increased level of calcineurin inhibitors were observed.

Discussion

In this study, we demonstrated that orally or i.v. administered voriconazole exerts a clinically significant drug interaction with calcineurin inhibitors in allogeneic HSCT recipients, resulting in a significant increase in the blood concentration of calcineurin inhibitors. The results were consistent with those of two previous reports showing its drug interaction with CsA in 7 renal transplant recipients and with tacrolimus in 14 healthy individuals.[7,8] In spite of the limited number of subjects in the previous studies, uniform dose reduction of calcineurin inhibitors has been recommended for patients on these drugs who are initiating voriconazole; the purpose is to prevent the toxicity of calcineurin inhibitors from reaching the toxic threshold.[13] In decreasing the dose of calcineurin inhibitors according to the drug interaction, physicians should always weigh the risks of the toxicity of calcineurin inhibitors and the development of GVHD or graft rejection in solid organ transplantation. However, our results showed that there was considerable interpatient variability in the magnitude of drug interaction in terms of increases in the C/D ratio of calcineurin inhibitors. Therefore, we think that the dose reduction of calcineurin inhibitors should not be decided uniformly, but should instead be determined on an individual basis by careful and periodic monitoring of their blood concentrations.

Previous studies have assessed the magnitude of drug interaction between voriconazole and calcineurin inhibitors by comparing the concentrations of calcineurin inhibitors before and after initiating voriconazole, in some cases using the area under the concentration–time curve, or by presenting the dose reduction rate of calcineurin inhibitors determined by each physician.[7–12] However, such approaches are unable to evaluate the exact drug interaction quantitatively, because they focused either on the concentration or the dose of calcineurin inhibitors. In contrast, we used the C/D ratio ((ng/ml)/(mg/kg)) for the quantitative evaluation of drug interaction, which reflects both the concentration and dose of calcineurin inhibitors. We believe that the present results using this method could provide data of more clinical relevance.

The manufacturer's recommendation sets uniform dose reduction rates for calcineurin inhibitors on initiating voriconazole. This recommendation is based on the results of two small studies performed separately.[7,8] As opposed to the two previous studies, we found that the impact of voriconazole on the concentration of CsA and tacrolimus did not differ significantly. Thus, the manufacturer's recommendation gave the physicians the misleading impression that voriconazole had a greater effect on tacrolimus than on CsA.

The reasons accounting for the notable interindividual difference in the drug interaction between voriconazole and calcineurin inhibitors remains to be elucidated. One possible explanation is the difference in the activity of CYP among patients. Voriconazole is metabolized by three separate enzymes, CYP 2C9, 2C19 and 3A4.[5] The CYP 2C19, the major enzyme responsible for the metabolism of voriconazole, exhibits genetic polymorphisms, so that voriconazole could be metabolized to a greater or lesser extent among different individuals, resulting in significant differences in its concentration of voriconazole. Because voriconazole acts as an inhibitor as well as a substrate of CYP3A4, it is also plausible that the higher the concentration of voriconazole, the more the activity of CYP 3A4 is reduced. Together with the interindividual difference in the blood concentration of voriconazole documented in allogeneic HSCT recipients,[14] it is suggested that the interindividual difference in the metabolism of voriconazole is critical in its drug interaction with calcineurin inhibitors.

Table 2 Effect of voriconazole administration on the blood levels of calcineurin inhibitors

	Median C/D ratio of calcineurin inhibitors[a]		*Median increase of C/D ratio*
	Before voriconazole	*After voriconazole*	(%)
CsA, $n=10$ (range)	86.0 (43.5–178.8)	120.2[b] (86.1–379.4)	82.1 (−9.4–266.9)
Tacrolimus, $n=11$ (range)	595.9 (51.3–1634.3)	890.7[b] (94.1–4658.3)	115.6[c] (25.4–307.6)

[a]C/D indicates concentration/dose (ng/ml)/(mg/kg).
[b]Significantly higher than that before voriconazole ($P<0.05$, <0.01, respectively).
[c]Not significantly different as compared with CsA.