Table I. Characteristics and outcome data for 19 patients based on initial platelet count (PC) before eradication of *H. pylori* in 2001.

UPN	Gender	Age at diagnosis (years)	Age at treatment (years)	Steroid treatment	Splenectomy	Platelet count (PC) before treatment ($\times 10^9$/L)	PC 6 months after treatment ($\times 10^9$/L)	Response at 6 months	UTIB test after 8 years	PC 8 years after treatment ($\times 10^9$/L)	Comments PC ($\times 10^9$/L)
1	M	25	29	Y	Y	17	105	CR	Neg	321	
2	F	65	65	N	N	23	147	CR	Neg	173	
3	M	49	59	Y	N	28	176	CR	Neg	184	
4	M	63	73	N	N	30	88	R	ND	153	
5	F	45	65	Y	N	32	169	CR	Neg	207	
6	M	63	66	N	N	32	39	NR	Pos	24	CR after 2nd treatment.
7	F	53	53	N	N	32	235	CR	Neg	190	
8	F	60	66	N	N	37	212	CR	ND	152	
9	F	57	67	N	Y	43	126	CR	Pos	217	
10	M	30	44	N	N	43	127	CR	Neg	140	
11	F	30	50	Y	Y	44	23	NR	Neg	53	Liver cirrhosis
12	F	69	70	Y	N	45	206	CR	Neg	202	
13	M	46	53	N	N	45	142	CR	ND	ND	In 2006, PC was 165
14	M	41	46	N	N	46	29	NR	ND	16	MDS, transplanted.
15	M	53	55	N	N	78	64	NR	ND	82	
16	F	50	51	N	N	85	84	NR	Neg	100	
17	F	60	61	N	N	93	112	NR	Neg	56	
18	F	56	74	Y	N	119	109	NR	ND	ND	In 2006, PC was 192
19	F	48	76	Y	N	144	110	NR	Neg	135	

UPN, unique patient number; PC, Platelet count; F, female; M, male; Y, yes; N, no; CR, complete response; R, response; NR, no response; Neg, negative; Pos, positive; ND, not determined. See text for definition of response.

reappearance rate of *H. pylori* after eradication in patients with duodenal ulcer was 7% [9]. This low incidence of re-infection might be another possible reason for the excellent prognosis of patients with thrombocytopenia who responded to eradication treatment. By contrast, this would make it more difficult to examine the effect of recurrent exposure to *H. pylori* on platelet count. Nonetheless, the prognosis of patients who responded to eradication is excellent and the chance of relapse seems to be low.

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ORIGINAL ARTICLE

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

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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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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]

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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: $\text{eGFR}\ (\text{ml/min/1.73m}^2) = 194 \times \text{Cr}^{-1.094} \times \text{age}^{-0.287} \times 0.739$ (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 ⩾1.5 × baseline or decrease in GFR ⩾25%
Injury	Increase in serum Cr ⩾2.0 × baseline or decrease in GFR ⩾50%
Failure	Increase in serum Cr ⩾3.0 × baseline or decrease in GFR ⩾75% or an absolute serum Cr ⩾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 ⩾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 ⩾300% (⩾3-fold) from baseline or serum Cr ⩾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 ⩾2-fold from baseline but not requiring dialysis
Grade 3	Increase in serum Cr ⩾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 ⩾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 ⩾0.3 mg per 100 ml in the 48 h period after SCT. However, the ability to identify severe AKI (⩾injury or ⩾stage 2 or ⩾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.)	*Mortality no. (%)*	*AKIN (no.)*	*Mortality no. (%)*	*Grading (no.)*	*Mortality no. (%)*
M-allo (n=141)					
None (48)	17 (35.4)	None (54)	20 (37.0)	Grade 0 (48)	17 (35.4)
Risk (52)	23 (44.2)	Stage 1 (46)	20 (43.5)	Grade 1 (52)	23 (44.2)
Injury (21)	9 (42.9)	Stage 2 (21)	9 (42.9)	Grade 2 (38)	25 (65.8)
Failure (20)	18 (90.0)	Stage 3 (20)	18 (90.0)	Grade 3 (3)	2 (66.7)
Any category[b] (93)	50 (53.8)	Any stage (87)	47 (54.0)		50 (53.8)
				Any grade (93)	
Nm-allo (n=60)					
None (31)	5 (16.1)	None (36)	7 (19.4)	Grade 0 (31)	5 (16.1)
Risk (19)	5 (26.3)	Stage 1 (14)	3 (21.4)	Grade 1 (19)	5 (26.3)
Injury (5)	4 (80.0)	Stage 2 (5)	4 (80.0)	Grade 2 (7)	6 (85.7)
Failure (5)	5 (100)	Stage 3 (5)	5 (100)	Grade 3 (3)	3 (100)
Any category (29)	14 (48.3)	Any stage (24)	12 (50.0)	Any grade (29)	14 (48.3)
Auto (n=48)					
None (39)	5 (12.8)	None (43)	5 (11.6)	Grade 0 (39)	5 (12.8)
Risk (8)	0 (0)	Stage 1 (4)	0 (0)	Grade 1 (8)	0 (0)
Injury (1)	1 (100)	Stage 2 (1)	1 (100)	Grade 2 (1)	1 (100)
Failure (0)	0 (—)	Stage 3 (0)	0 (—)	Grade 3 (0)	0 (—)
Any category (9)	1 (11.1)	Any stage (5)	1 (20.0)	Any grade (9)	1 (11.1)

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

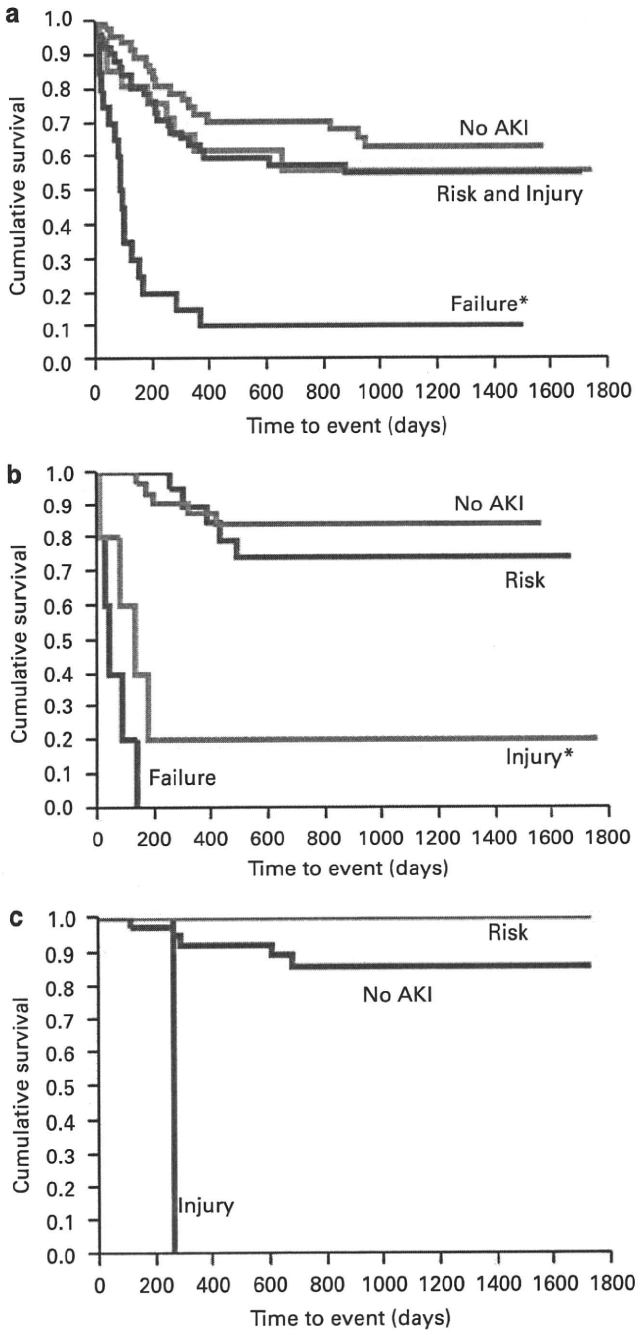

Figure 1 (**a**), (**b**) and (**c**) show cumulative survival curves after m-allo, nm-allo and auto transplants, stratified according to the RIFLE categories. The *x* axis represents the number of days post-hematopoietic cell transplantation (HCT) and the *y* axis represents cumulative survival. (**a**): 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 ($\geq$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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ORIGINAL ARTICLE

Safety and efficacy of high-dose ranimustine, cytarabine, etoposide and CY (MCVAC) regimen followed by autologous peripheral blood stem cell transplantation for high-risk diffuse large B-cell lymphoma

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The efficacy of high-dose chemotherapy followed by autologous hematopoietic SCT for relapsed diffuse large B-cell lymphoma (DLBCL) has been reported, but an optimal conditioning regimen has not been determined. This study was conducted to evaluate the safety and efficacy of the MCVAC regimen (consisting of ranimustine (MCNU), cytarabine, etoposide and CY) followed by autologous peripheral blood stem cell transplantation (PBSCT) for patients with high-risk or relapsed DLBCL. A total of 40 patients with DLBCL who received the MCVAC regimen followed by autologous PBSCT were retrospectively evaluated. Median follow-up duration of the surviving patients was 51.2 months (range, 5.4–151.2 months). At 5-year OS and PFS were 73.7% (95% confidence interval (CI), 58.6–88.8) and 62.5% (95% CI, 46.8–78.2), respectively. Although relapse remained the most frequent cause of treatment failure, late-onset adverse events were observed, including two cases of severe pulmonary impairment, and two cases of therapy-related myelodysplastic syndromes (MDS)/AML. In conclusion, the MCVAC regimen would be an effective and tolerable conditioning regimen without TBI for autologous PBSCT for high-risk or relapsed DLBCL. However, late-onset pulmonary toxicity and MDS/AML should be monitored.

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Keywords: diffuse large B-cell lymphoma; autologous peripheral blood stem cell transplantation; high-dose chemotherapy; MCVAC regimen; pulmonary toxicity

Introduction

First-line treatment of diffuse large B-cell lymphoma (DLBCL) with CHOP or CHOP-like regimens can cure ~40–50% of patients with aggressive non-Hodgkin's lymphoma. The addition of rituximab significantly improved the remission rate and resulted in an improvement in PFS and OS by 15–20% over CHOP chemotherapy alone.[1–3] However, 20–60% of patients are refractory to initial therapy or relapse after achieving a CR.[4] Salvage chemotherapy was effective in 60–70% of patients with refractory or relapsed DLBCL, but could cure no > 10% of such patients.[5–9] High-dose chemotherapy followed by autologous hematopoietic SCT has been shown to be superior to salvage chemotherapy alone for patients with chemosensitive relapsed or refractory aggressive non-Hodgkin's lymphoma.[10–14]

BEAM, CY plus TBI (CY/TBI) and some other regimens have frequently been used as conditioning regimens for autologous hematopoietic SCT. However, an optimal conditioning regimen which produces the least toxicity and greatest therapeutic efficacy has not been determined.[15,16] High-dose methyl 6-[3-(2-chloroethyl)-3-nitrosoureido]-6-deoxy-α-D-glucopyranoside (MCNU; ranimustine), cytarabine, etoposide and CY (MCVAC) chemotherapy was first reported as a conditioning regimen in children, as it possessed a high antitumor activity with acceptable toxicity for lymphoid malignancies.[17] We herein reviewed a single institute experience in order to evaluate the safety and efficacy of the MCVAC regimen with autologous peripheral blood stem transplantation (PBSCT) in adult patients with relapsed or high-risk DLBCL.

Patients and methods

Patients

Patients who underwent autologous PBSCT following the MCVAC regimen between August 1994 and April 2005 at Keio University Hospital for the treatment of relapsed or high-risk DLBCL were identified from our transplant database, and their demographic as well as transplant data records were collected by chart review. Patients with disease

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transformation from low-grade B-cell lymphomas were excluded. High-risk DLBCL was defined as partial or no response to initial treatment or high-intermediate/high risk disease according to age-adjusted international prognostic index at initial diagnosis.[18] Clinical staging was performed by computed tomography scanning of the neck, thorax, abdomen and pelvis, BM biopsy, cerebrospinal fluid examination and other tools such as magnetic resonance imaging if indicated.

MCVAC regimen, PBSCT and its toxicities

The MCVAC regimen consisted of ranimustine (250 mg/m^2 on day −9 and 200 mg/m^2 on day −4), cytarabine (2.0 g/m^2 twice daily on days −8 to −5), etoposide (200 mg/m^2 twice daily on days −8 to −5) and CY (50 mg/kg on days −3 and −2) followed by unpurged PBSCT. On day 0, cryopreserved PBSCs were rapidly thawed at 37 °C and promptly infused into the patient through a central venous catheter. Neutrophil recovery was defined as the first day of three consecutive days with an ANC $>0.5 \times 10^9$/L. Platelet recovery was defined as the first of three consecutive days with an unsupported platelet count $>20 \times 10^9$/L.

Non-hematological toxicities without nausea and hair loss were graded according to the Common Terminology Criteria for Adverse Events v3.0.

Supportive care

All patients were managed in high-efficiency particulate air filtered-rooms. Bacterial, fungal, HSV and pneumocystis pneumonitis prophylaxes were given to all patients according to our institutional protocol. Granulocyte colony-stimulating factor was given intravenously from day +1 until neutrophil recovery. The patients were transfused with irradiated blood products to keep the hemoglobin level above 8.0 g/100mL and the platelet count above 20×10^9/L.

Response criteria

CR was defined as the disappearance of any clinically detectable signs of tumor by clinical and laboratory assessment. PR was defined as >50% reduction of detectable tumor. No response was defined as <50% reduction of detectable tumor. Progressive disease was defined as an increase in detectable tumor or the appearance of any new lesion.

Statistical analysis

OS was defined as the time from transplant until death because of any causes or the last follow-up. PFS was defined as the time from transplant until relapse or progression of lymphoma, or death from any causes, or the last follow-up if none of these events had occurred. TRM was defined as death from any causes other than lymphoma. Survival rates were estimated using the Kaplan–Meier method. Survival curves were compared applying the log-rank test. A P-value of <0.05 was considered statistically significant. Factors that were potentially predictive of OS ($P<0.05$) and PFS ($P<0.05$) were entered into a multivariate analysis using the Cox proportion hazards model.

Results

Patient characteristics

The characteristics of 40 patients at diagnosis and transplant are shown in Tables 1 and 2, respectively. The diagnosis included two patients with mediastinal DLBCL. The nine patients in the first CR at transplant were at high or high-intermediate risk according to age-adjusted international prognostic index at diagnosis.[18] Disease was chemosensitive in 18 patients not in CR at transplant, except for 1 patient. Median time from diagnosis to transplant was 13.9 months (range, 4.2–198.4). Of the 20 patients who had received radiation therapy before transplant, 7 patients received involved field radiation therapy after chemotherapy for an early-stage disease, 11 patients received radiation therapy for bulky or residual disease after chemotherapy and 2 patients with mediastinal

Table 1 Patient characteristics at diagnosis ($N=40$)

Median age, years (range)	49 (22–59)
Sex, no. of male/no. of female	16/24
Stage	
I–II	10
III–IV	30
B symptoms	
A	27
B	13
LDH	
Normal	12
Elevated	25
Unknown	3
No. of extranodal sites	
0–1	10
1	17
2 or upper	13
Performance status	
0–1	26
2–4	13
Unknown	1
International prognostic index	
Low risk	14
Low-intermediate risk	7
High-intermediate risk	13
High risk	5
Unknown	1
Bulky disease	
Yes	8
No	32
BM involvement	
Yes	30
No	9
Unknown	1
CNS involvement	
Yes	2
No	32
Unknown	6

Abbreviations: CNS = central nervous system; LDH = lactate dehydrogenase.

Table 2 Patient characteristics at transplant ($N=40$)

Median age, years (range)	50.5 (23–61)
Median time from diagnosis to transplant, months (range)	13.9 (4.2–198.4)
Stage	
CR	22
1st CR	9
2nd CR	13
Non-CR	18
Performance status	
0–1	38
2	2
Previous radiation therapy	
Yes	20
No	20
No. of regimens before transplant	
1	4
2	28
>3	8
Previous rituximab therapy	
Yes	8
No	32

DLBCL received whole-lung radiation therapy. Eight of the patients had received rituximab before transplant.

MCVAC regimen and transplant procedures

In all, 39 patients received the MCVAC regimen as scheduled. One patient did not receive the second dose of CY on day −2 because of progressive impaired performance status due to severe ataxia caused by cytarabine. The median number of infused CD34-positive cells was 4.16×10^6/kg (range, $1.76–39.0 \times 10^6$/kg). The median post transplant days of neutrophil recovery and platelet were 9 days (range, 8–19) and 11 days (range, 7–19), respectively.

Toxicities and TRM

Non-hematological treatment-related toxicities within the first 100 days after transplant are shown in Table 3. Prominent toxicities were stomatitis, diarrhea, liver toxicity and infection. Infections included febrile neutropenia ($N=30$), sepsis ($N=4$), catheter-related bacteremia ($N=3$), pneumonia ($N=1$), cellulitis ($N=1$) and CMV diseases ($N=2$). Other less common severe toxicities (grades 3–5) were subdural hematoma ($N=1$), renal impairment ($N=1$), myocardial toxicity ($N=1$), ataxia ($N=1$) and skin damage ($N=2$). Veno-occlusive disease of the liver was not observed in any of the patients. Late-onset adverse events included two cases of serious restrictive pulmonary impairment due to pulmonary fibrosis diagnosed at 61.4 and 69.2 months after transplant. One case received radiation therapy to the whole lungs before transplant. Two other patients developed therapy-related myelodysplastic syndrome/AML (MDS/AML), which was diagnosed at 20.3 and 94.7 months after transplant.

Early (within 100 days post transplant) and 4-year overall TRM was 5.0% (95% confidence interval (CI), 0–11.7%) and 9.0% (95% CI, 0–19.0%), respectively.

Table 3 Toxicities within 100 days after transplantation ($N=40$)

	Grade[a]					
	0	1	2	3	4	5
Stomatitis	0	1	8	31	0	0
Diarrhea	1	2	18	19	0	0
Liver	1	9	15	14	1	0
CNS hemorrhage	39	0	0	1	0	0
Ataxia	39	0	0	1	0	0
Cardiac	38	0	0	1	0	1
Renal	16	20	3	1	0	0
Cystitis	22	16	2	0	0	0
Skin	38	0	0	2	0	0
Infection						
Febrile neutropenia		—	0	30	0	0
Sepsis		—	0	4	0	0
Catheter-related bacteremia		—	0	3	0	0
Pneumonia		—	0	1	0	0
Cellulitis		—	0	1	0	0
CMV disease		—	0	1	0	1[b]

Abbreviation: CNS = central nervous system.
[a]Grades were determined according to the Common Terminology Criteria for Adverse Events v3.0.
[b]CMV pneumonitis.

The causes of TRM included myocardial toxicity in one patient (day +14), CMV pneumonitis in one patient (day +65) and therapy-related MDS/AML in two patients (day +3119 and +830).

Survival

Median follow-up duration of 29 patients surviving at the time of the analysis was 51.2 months (range, 5.4–151.2 months). Four patients died of the treatment-related complications described above, and six patients died of disease progression. At 4-years, OS and PFS were 75.0% (95% CI, 55.8–94.2) and 60.1% (95% CI, 38.3–81.9) in patients in CR ($N=22$), and 72.5% (95% CI, 49.2–95.8) and 61.1% (95% CI, 38.6–83.6) in patients in non-CR ($N=18$), respectively. The differences were NS (Figure 1).

In a univariate analysis for factors affecting OS and PFS, only radiation therapy before transplant was significantly associated with unfavorable OS (Table 4). It was also significant with a relative risk of 5.46 (95% CI, 1.2–25.5; $P<0.05$) by a multivariate analysis. No other factors, including clinical stage, international prognostic index and lactate dehydrogenase values at diagnosis and relapse, number of previous chemotherapy regimens and previous rituximab therapy, had a significant influence on OS and PFS.

Discussion

The findings of this retrospective study showed that the MCVAC regimen followed by autologous PBSCT in patients with relapsed or high-risk DLBCL was generally well tolerated and yielded about a 60% or higher cure rate of the disease with a low TRM rate (9%). Disease status at transplant has been reported to be the most important prognostic factor influencing outcome in aggressive

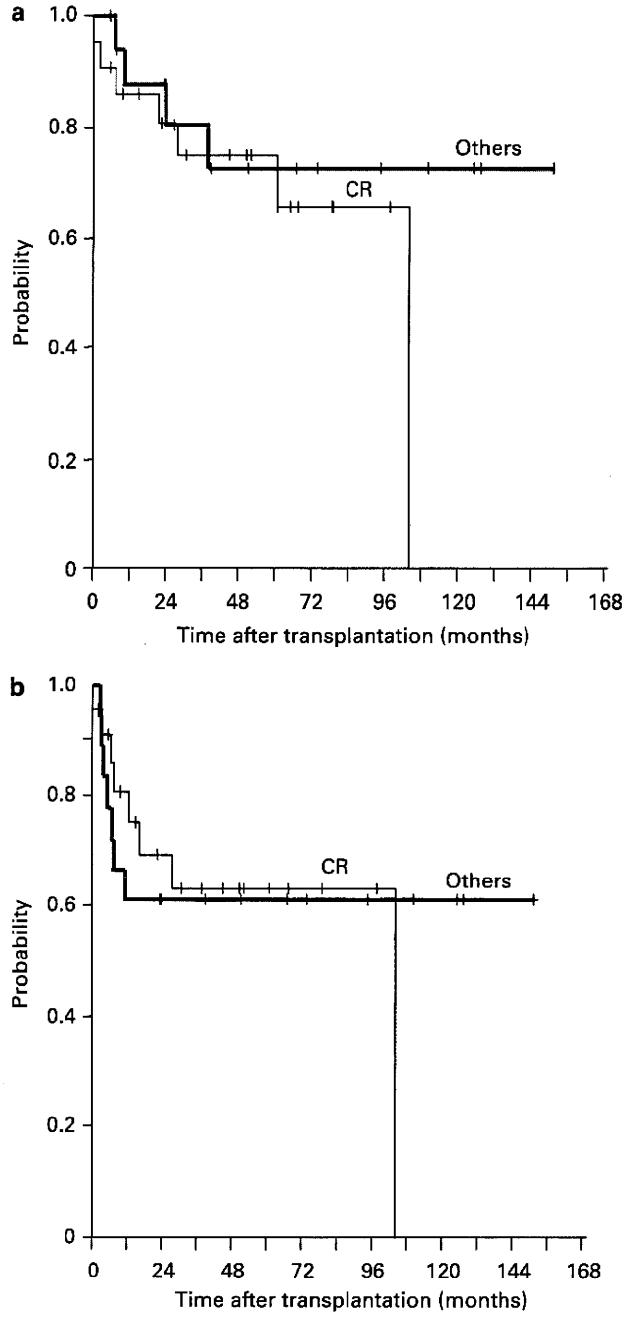

Figure 1 (a) OS and (b) PFS after PBSCT in patients in CR ($N=22$) or others ($N=18$).

non-Hodgkin's lymphoma.[16,19] However, disease status at transplant did not have a significant influence on survival in the present study. Although there were a small number of patients and they were evaluated in a retrospective manner, the outcomes suggest that the MCVAC regimen had potential highly curative anti-lymphoma activity for not only high-risk, but also relapsed DLBCL. Furthermore, disease was chemosensitive in 17 of 18 patients not in CR at transplant, which could also contribute to a high survival rate particularly in patients not in CR.

Table 4 Univariate analysis for factors affecting OS and PFS

Category at transplant	*Patients No.*	*4-year OS*			*4-year PFS*		
		Probability	*s.e.*	P-*value*	*Probability*	*s.e.*	P-*value*
Disease status							
CR	22	75.0	9.8	0.390	60.1	11.1	0.799
Non-CR	18	72.5	11.9		61.1	11.5	
Performance status							
0–1	38	74.8	7.9	0.608	61.4	8.2	0.751
⩾2	2	50.0	35.4		50.0	35.4	
Gender							
Male	16	60.2	14.5	0.250	57.5	13.7	0.683
Female	24	81.6	8.3		62.5	9.9	
Age at transplant, years							
<50	20	79.3	9.2	0.562	63.6	11.1	0.968
50<	20	68.7	11.9		58.2	11.4	
Previous radiation therapy							
Yes	20	57.8	12.5	0.035	45.3	12.1	0.072
No	20	89.1	7.3		74.7	9.8	
Previous rituximab therapy							
Yes	8	75.0	15.3	0.692	85.7	13.2	0.231
No	32	74.6	8.4		57.4	9.0	

Early toxicities were generally tolerable, and the incidence of TRM within 100 days was limited to 5%, which was identical to those reported in other regimens (3.0–14.8%).[16,19–21] Late-onset adverse events, however, included therapy-related MDS/AML and severe pulmonary toxicity. Therapy-related MDS/AML has been a well-recognized complication after high-dose chemotherapy, mainly in the setting of autologous hematopoietic SCT for non-Hodgkin's lymphoma. Conditioning regimens, particularly those containing TBI, were initially thought to be responsible for the development of this complication. The agents and intensity of pretransplant chemotherapy have also been reported to be major contributing factors as well as the type of conditioning regimen used for transplant. In a multicenter case–control study, a higher relative risk for developing therapy-related MDS/AML was observed in proportion to the total dosage and duration of pretransplant therapy with alkylating agents.[22] In our study, the two patients who developed therapy-related MDS/AML had both received two or three lines of salvage chemotherapy and also radiation therapy before the transplant. The incidence of therapy-related MDS/AML (cumulative incidence of 3.3%) seemed identical to those of other studies.[20,23]

In two patients, severe pulmonary impairment developed 61.4 and 69.2 months after transplant. Pulmonary toxicity has been well recognized as a complication related to the use of BCNU-containing regimens. The incidence of non-infectious pulmonary complications associated with BCNU-containing conditioning regimens for autologous hematopoietic SCT such as interstitial pneumonitis has been reported to be 11–26% (Patti *et al.*,[24] Mills *et al.*,[25] Stiff *et al.*,[26] Alessandrino *et al.*[27]). BCNU has been

implicated as one of the main causes of pulmonary toxicity, particularly in association with previous radiation therapy. In the present study, MCNU, which also has a potent pulmonary toxicity,[17] was used instead of BCNU. In contrast, the pulmonary toxicity of MCNU-containing conditioning regimens for autologous hematopoietic SCT has not been evaluated so far. In the present study, one patient received radiation therapy to the whole lungs as a previous therapy, whereas the other patient did not. Therefore, MCVAC without the effect of radiation therapy also has the potential to cause pulmonary toxicity. An accumulation of such cases is needed to elucidate the risk factors for the development of pulmonary toxicity after the MCVAC regimen.

MCVAC regimen was associated with early and late adverse events, which were almost identical to those reported in other regimens, such as BCNU-containing regimens, and considered to provide a high-survival rate in high-risk or relapsed DLBCL patients even in patients not in CR at transplant. Furthermore, as BCNU has been in short supply, MCVAC regimen using MCNU instead of BCNU could be a promising high-dose regimen for high-risk or relapsed DLBCL. We conclude that the MCVAC regimen would be a tolerable, effective and curative conditioning regimen of autologous PBSCT for high-risk or relapsed DLBCL. However, late-onset MDS/AML and adverse effects on the lungs in long-term survivors can occur, and should be carefully monitored.

Conflict of interest

The authors decalre no conflict of interest.

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CRITICAL CARE

RESEARCH Open Access

Evaluation of pathogen detection from clinical samples by real-time polymerase chain reaction using a sepsis pathogen DNA detection kit

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Abstract

Introduction: Sepsis is a serious medical condition that requires rapidly administered, appropriate antibiotic treatment. Conventional methods take three or more days for final pathogen identification and antimicrobial susceptibility testing. We organized a prospective observational multicenter study in three study sites to evaluate the diagnostic accuracy and potential clinical utility of the Septi*Fast* system, a multiplex pathogen detection system used in the clinical setting to support early diagnosis of bloodstream infections.

Methods: A total of 212 patients, suspected of having systemic inflammatory response syndrome (SIRS) caused by bacterial or fungal infection, were enrolled in the study. From these patients, 407 blood samples were taken and blood culture analysis was performed to identify pathogens. Whole blood was also collected for DNA Detection Kit analysis immediately after its collection for blood culture. The results of the DNA Detection Kit, blood culture and other culture tests were compared. The chosen antimicrobial treatment in patients whose samples tested positive in the DNA Detection Kit and/or blood culture analysis was examined to evaluate the effect of concomitant antibiotic exposure on the results of these analyses.

Results: Septi*Fast* analysis gave a positive result for 55 samples, while 43 samples were positive in blood culture analysis. The DNA Detection Kit identified a pathogen in 11.3% (45/400) of the samples, compared to 8.0% (32/400) by blood culture analysis. Twenty-three pathogens were detected by Septi*Fast* only; conversely, this system missed five episodes of clinically significant bacteremia (Methicillin-resistant *Staphylococcus aureus* (MRSA), 2; *Pseudomonas aeruginosa*, 1; *Klebsiella spp*, 1; *Enterococcus faecium*, 1). The number of samples that tested positive was significantly increased by combining the result of the blood culture analysis with those of the DNA Detection Kit analysis (P = 0.01). Among antibiotic pre-treated patients (prevalence, 72%), Septi*Fast* analysis detected more bacteria/fungi, and was less influenced by antibiotic exposure, compared with blood culture analysis (P = 0.02).

Conclusions: This rapid multiplex pathogen detection system complemented traditional culture-based methods and offered some added diagnostic value for the timely detection of causative pathogens, particularly in antibiotic pre-treated patients. Adequately designed intervention studies are needed to prove its clinical effectiveness in improving appropriate antibiotic selection and patient outcomes.

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Introduction

Sepsis is a serious medical condition frequently found in transplant patients, in patients with hematological neoplasms or in patients admitted to the intensive care unit (ICU) after surgery. Rapid pathogen identification and appropriate chemotherapy are important to improve patient prognoses. In the United States, more than 750,000 cases of sepsis are reported annually [1]. The fatality rate is 28% to 50% for severe sepsis and as high as 90% when the causative agent is *Aspergillus* [1-3]. For most cases of suspected sepsis, blood culture analysis is performed for pathogen detection, and empirical treatment with broad-spectrum antibiotics is immediately started without waiting for the result of pathogen identification. This is because, in many cases, positive pathogen identification, and pathogen drug sensitivity analysis, using blood culture analysis, requires from three days to a week for common bacteria and a few weeks for fungi [4,5]. Therefore, choosing the appropriate antibiotic chemotherapy according to evidence-based medicine (EBM) is currently difficult in many sepsis cases. Moreover, in some cases, inappropriate antibiotic selection not only annuls the effects of chemotherapy but also promotes the emergence of drug-resistant bacteria.

Because of these problems with sepsis diagnosis, highly sensitive sepsis-pathogen detection methods using nucleic acid amplification techniques such as PCR have been recently studied for the purpose of rapid testing and the subsequent choosing of appropriate chemotherapy. However, the development of a diagnostic reagent to simultaneously detect a wide range of sepsis pathogens has been difficult using conventional genetic technology.

A new assay, termed Septi*Fast* (Roche Diagnostics, Mannheim, Germany), enables rapid, multiplex testing for micro-organisms using a real-time polymerase chain reaction that is coupled to melting curve analysis. This kit can identify up to 25 organisms from four different microbial groups, in a single sample, in about 4.5 hours [6].

We organized a clinical performance research group to investigate the potential clinical utility of Septi*Fast* analysis by comparing with those obtained using the currently used routine blood culture analysis. We also compared the effect of antibiotic treatment on detection of pathogens by DNA Detection Kit and blood culture analysis, and we analyzed the number of pathogens that could be detected when the results of both assay methods were combined.

Materials and methods

We conducted a prospective multicenter study in Japan of Septi*Fast* (Roche Diagnostics GmbH, Mannheim, Germany) analysis, which detects sepsis pathogens in whole blood. Septi*Fast* is currently used as an *in vitro* diagnostic reagent in Europe. Table 1 lists the bacteria and fungi that are detectable by DNA Detection Kit analysis. When *S. aureus* was detected, Septi*Fast* mecA kit was used to confirm whether this *S. aureus* was MRSA or not.

This study was conducted at Keio University, Osaka University and Nagasaki University from May 2007 to April 2008, with the approval of the Institution Review Board at each site.

Patient selection

Patients selected for the study all provided informed consent. Included in the study were a total of 407 samples from 212 treated or untreated patients in the departments of surgery, hematology, emergency, cardiopulmonary and ICU, who were suspected of having systematic inflammatory response syndrome (SIRS) caused by bacterial or fungal infection, and for whom blood culture was considered to be required for identification of the causative pathogens. Table 2 shows the underlying diseases of the patients studied. The total number of underlying diseases exceeds the total number of enrolled patients since all underlying diseases were counted when a patient had multiple diseases. Of the 407 samples assayed, 277 samples from 156 patients were assessed as SIRS. SIRS was defined as a condition that fulfilled two or more of the following criteria [7]: temperature > 38°C or < 36°C; heart rate > 90 beats per minute; respiratory rate > 20 breaths per minute or $PaCO_2$ < 32 mmHg; white blood cell count < 4,000 or > 12,000 cells/μL; or ≥ 10% immature bands.

Blood culture analysis

BacT/ALERT 3D (BioMerieux Hazelwood, MO, USA) and BACTEC 9240 systems (Becton, Dickinson Co., Franklin Lakes, NJ, USA) were used for blood culture analysis. Blood administration was followed according to each instruction manual. When the result of blood culture analysis was positive, the sample was identified using each site's identification system. Moreover, we collected the blood culture bottles whose results were positive, and sent them to one commercial laboratory to confirm the validation of the identification microorganisms.

Blood collection

EDTA-2K vacuum blood collection tubes (Insepack II-D, Sekisui Chemical Co. Ltd., Tokyo, Japan) were used to collect whole blood for Septi*Fast* analysis. Ten milliliters of whole blood were collected for DNA Detection Kit analysis immediately after blood collection for microbial culture. 1.5 mL were used for the assay for

Table 1 Pathogens listed in the SeptiFast PCR menu

Gram-positive bacteria	Gram-negative bacteria	Fungi
Staphylococcus aureus	*Escherichia coli*	*Candida albicans*
Coagulase negative Staphylococcus	*Klebsiella (pneumoniae/oxyt.)*	*Candida tropicalis*
Streptococcus pneumonia	*Serratia marcescens*	*Candida parapsilosis*
Streptococcus spp.	*Enterobacter (cloacae/aerog.)*	*Candida krusei*
Enterococcus faecium	*Proteus mirabilis*	*Candida glabrata*
Enterococcus faecalis	*Pseudomonas aeruginosa*	*Aspergillus (fumigatus)*
	Acinetobacter baumanii	
	Stenotrophomonas maltophilia	

Drug-resistant bacteria. mecA (MRSA).

DNA Detection Kit. The blood for DNA Detection Kit was stored at -20°C for up to 72 hours before testing. The storage did not affect the assay performance. The detection sensitivity of SeptiFast is 30 colony-forming units per mL (CFU/mL), except for coagulase-negative *Staphylococci* (CoNS), *Streptococcus spp.* and *Candida glabrata*, for which the detection sensitivity is 100 CFU/mL [6]. Blood culture was performed at the three sites according to the usual protocol.

DNA extraction

There are four different SeptiFast kits: SeptiFast Lys M^{Grade}, SeptiFast Prep M^{Grade}, LightCycler SeptiFast M^{Grade} and LightCycler SeptiFast mecA M^{Grade} kits (Roche Diagnostics GmbH, Mannheim, Germany). The SeptiFast-Lys and Prep kits were used for DNA extraction. The extraction condition for Gram-negative, Gram-positive, and fungi was the same. The assay was performed according to the manufacturer's instructions [6]. To prevent contamination, DNA was extracted in a safety cabinet, M^{GRADE} disposables were used, and DNA extraction and amplification were performed in separate rooms. Negative control extraction was performed concurrently with sample extraction. An internal control (IC) was added to each sample to check for false-negatives.

Amplification and detection

For detection of Gram-positive and Gram-negative bacteria, and for detection of fungi, 50 μL of each DNA extract was used. The LightCycler SeptiFast kit and LightCycler 2.0 (Roche Diagnostics GmbH, Mannheim, Germany) were used for DNA amplification and detection respectively. The amplification region used was an internal transcribed spacer (ITS) region. This region lies between the 16 S and 23 S ribosomal spacer in bacteria and between the 18 S and 5.8 S ribosomal spacer in fungi and is often used to detect bacterial/fungal genes [8,9]. For bacterial/fungal DNA identification after amplification, the DNA of each strain was identified and four different fluorescent nucleotide probes were followed by melting curve analysis. Negative control and

Table 2 Patients' background

		Number of positive samples (%)	
	The number of samples	Blood Culture	SeptiFast
Infectious disease	135	22(16.3)	27(20.0)
Blood Stream Infection	104	7(6.7)	5(4.8)
Tumor	51	0(0.0)	4(7.8)
Immune deficiency	33	2(6.1)	5(15.2)
Wound	14	3(21.4)	4(28.6)
Diabetes	48	3(6.3)	7(14.6)
Liver disease	13	0(0.0)	2(15.4)
Kidney disease	11	2(18.1)	2(18.1)
Heart disease	15	1(6.7)	2(13.3)
Pancreatic disease	11	1(9.1)	4(36.3)
Ulcer of the stomach	10	0(0.0)	2(20.0)
Hypertension	9	2(22.2)	2(22.2)
Influenza encephalopathy	7	2(28.6)	1(14.3)
Others	34	4(11.8)	8(23.5)
Total	495	49(9.9)	75(15.2)

the reagent control provided in the kit were used as controls.

MRSA detection

The presence of MRSA in samples was assayed using the Septi*Fast* mecA kit. MRSA was only assayed in samples in which *S. aureus* was detected, and CoNS was not detected since CoNS-derived mecA genes may compromise MRSA detection [10]. In the samples in which *S. aureus* was detected, but CoNS was not, the presence of mecA genes was confirmed using the LightCycler Septi*Fast* mecA kit and 50 μL of DNA extract, which were prepared using the Septi*Fast* Prep kits.

Definition of pathogens

It remains difficult to determine whether the organisms detected by the DNA Detection Kit are true pathogens. This also applies, although to a much lesser degree, to conventional blood culture analysis. However, detected organisms were considered to be pathogens if the results of culture tests from samples of the suspected infectious sites coincided with the results of DNA Detection Kit or blood culture analysis. The culture data of sputum, urine, pus and drainage fluid were used to define the pathogens.

A decision as to whether an identified organism was a pathogen was taken based on the decision tree shown in Figure 1. Thus, when the same organism was detected by both DNA Detection Kit and blood culture analysis, the detected organism was considered an infectious pathogen. If there was a discrepancy between the organism that was detected by Septi*Fast* analysis and that detected by blood culture analysis, or if an organism was only detected in one of these tests, then other samples taken from the infection site were analyzed. If this second culture test of the suspected infectious site revealed the presence of the same organism, this organism was considered to be a pathogen. If the microbial strain was only detected once for a sample, we then checked the second culture results in the suspected infectious sites. If this result identified the same strain as that identified by Septi*Fast* analysis then it was decided that this strain was a pathogen. However, if the strain was still only detected in some of the assays, we next determined if the patient involved suffered from sepsis. Sepsis is defined as SIRS caused by infection. The definition of sepsis that we used was based on the International Sepsis Forum Definition of Infection at the ICU Consensus Conference [7]. However, if the underlying disease is acute lymphoma leukemia (ALL), malignant lymphoma (ML), or acute myelogenous leukemia (AML), the definition of infection is defined as the ability to detect infectious organisms by blood culture analysis. If the patient was not defined as having sepsis when whole blood was administered to the patient, we decided that the strain detected by subsequent DNA Detection Kit or blood culture analysis was not a pathogen.

Samples were defined as negative for pathogens if a pathogen could not be detected by any method of analysis within seven days, and if another type of culture test did not detect this pathogen but could detect other organisms.

CoNS bacteria, which are represented by the *Staphylococcus epidermidis (S. epidermidis)* and *Streptococcus spp.* are indigenous bacteria and often cause contamination in assays of pathogens. Therefore, when CoNS or *Streptococcus spp.* were detected by blood culture and Septi*Fast* analysis, the following criteria were applied to define whether these strains represented a pathogenic infection: (1) Tests were performed at least twice within 48 hours before and after CoNS were detected by blood culture or Septi*Fast* analysis; (2) CoNS or *Streptococcus spp.* were detected in two different blood culture tests that were separately performed twice within 48 hours; and, (3) CoNS or *Streptococcus spp.* were detected twice or more in tests that were performed three times [11-15]. If a sample's results met any of these three criteria, then the sample was evaluated as a pathogen.

The distinction between pathogen and contamination was also determined for CoNS or *Streptococcus spp.* from the crossing point (Cp) obtained using the LightCycler analysis software v4.05. The Cp represents the point in the amplification cycle where the amplification curve crosses the detection threshold. When CoNS or *Streptococcus spp.* were detected using the LightCycler analysis software v4.05, a Cp of less than 20 was defined as indicating a pathogen and a Cp of over 20 was defined as contamination by checking the amplification curve.

Antibiotic administration survey

Antibiotic administration to patients at the time of blood collection was checked and it was confirmed that the spectrum of the antibiotic used corresponded to the organism detected in the blood analyses. The antibiotic spectra were determined based on information regarding susceptible organisms provided by the pharmaceutical company that marketed each antibiotic.

Statistical analysis

McNemar's test was conducted at a significance level of 5% to compare DNA Detection Kit and blood culture detection of pathogens. A two-sample test for equality of proportions was conducted at a significance level of 5% to compare detection of pathogens when DNA Detection Kit and blood culture results were combined.