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Analysis

Risk Factors and Predictive Scoring System For Post-Transplant Lymphoproliferative Disorder after Hematopoietic Stem Cell Transplantation

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A B S T R A C T

We analyzed data from 64,539 consecutive patients in the Japanese national transplant registry, including 40,195 after allogeneic hematopoietic stem cell transplantation (HSCT), 24,215 after autologous HSCT and 129 after syngeneic HSCT, of whom 299 developed Epstein-Barr virus-positive post-transplant lymphoproliferative disorder (PTLD). The probability of developing PTLD at 2 years post-HSCT was .79% after allogeneic transplantation, .78% after syngeneic transplantation, and .11% after autologous transplantation. The following variables were identified as risk factors after allogeneic HSCT in multivariate analysis: antithymocyte globulin (ATG) use in a conditioning regimen, ATG use for acute graft-versus-host disease (GVHD) treatment, donor other than an HLA-matched related donor, aplastic anemia, second or subsequent allogeneic HSCT, the most recent year of transplantation, and acute GVHD. The probability at 2 years increased particularly after 2009 (1.24%) than before 2009 (.45%). To stratify the risk of PTLD before allogeneic HSCT, we developed a novel 5-point scoring system based on 3 pretransplant risk factors: ATG use in a conditioning regimen (high dose, 2 points; low dose, 1 point), donor type (HLA-mismatched related donor, 1 point; unrelated donor, 1 point; cord blood, 2 points), and aplastic anemia (1 point). Patients were classified into 4 risk groups according to the summed points: low risk (0 or 1 point), intermediate risk (2 points), high risk (3 points), and very high risk (4 or 5 points) groups, with probabilities at 2 years of .3%, 1.3%, 4.6%, and 11.5%, respectively. Our scoring system is useful for predicting patients at high risk for PTLD. Careful observation and close monitoring of Epstein-Barr virus reactivation are warranted for these high-risk patients.

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INTRODUCTION

Post-transplant lymphoproliferative disorder (PTLD) following hematopoietic stem cell transplantation (HSCT) is a rare but life-threatening complication, resulting mostly from the outgrowth of donor-derived Epstein-Barr virus (EBV)-infected B cells [1]. After primary infection, which occurs in >90% of children worldwide, EBV remains latent in B cells for life [2]. In immunocompetent individuals, those infected B cells are controlled by EBV-specific cytotoxic T cells [3]. However, particularly in the early phase after HSCT, patients are immunocompromised because of a reduced number of T cells and T cell dysfunction resulting from conditioning chemotherapy and the use of immunosuppressive agents. This facilitates EBV reactivation, which may induce an increase in the number of infected B cells and result in the development of PTLD.

The reported incidence of patients who develop PTLD after allogeneic HSCT ranges widely from 1% to 17% [4-15]. The incidence of PTLD has increased in the last several decades due to increasing use of HSCT, introduction of new immunosuppressive agents and regimens, increasing awareness of PTLD, and improved accuracy of diagnosing PTLD [16]. PTLD typically develops within 6 to 12 months of HSCT, before the reconstitution of EBV-specific cytotoxic T cell immunity [6,8,17,18]. Several risk factors for the development of PTLD have been reported in the literature, and the risk of PTLD is principally dependent on the degree of T cell depletion (TCD) or dysfunction. The use of antithymocyte globulin (ATG) and ex vivo TCD are established risk factors for PTLD. The use of grafts from HLA-mismatched and unrelated donors increases the risk of PTLD, and the use of an umbilical cord blood (CB) graft is also a strong risk factor for PTLD because of marked T cell cytopenia and naivety [12,14,15,17,19]. However, many of the studies of PTLD reported to date are single-center, small-scale studies involving patients with different characteristics, and the roles of other risk factors, including primary diseases and patient age, have not yet been clarified in detail [16]. Furthermore, the risk of PTLD with respect to the stem cell source remains unknown.

To further evaluate the probability and risk factors associated with PTLD, we conducted a nationwide multicenter retrospective analysis of data collected from 71,904 patients who underwent HSCT in Japan.

METHODS

Study Design and Data Source

This study is a multicenter retrospective analysis of data from a Japanese nationwide transplant outcome registry database provided by the Japan Society of Hematopoietic Stem Cell Transplantation (JSHCT). The data were consecutively collected from the Transplant Registry Unified Management Program, as described previously [20,21]. This study was performed in accordance with the Declaration of Helsinki and was approved by the JSHCT Ethical Committee and the Institutional Review Boards of Kobe City Medical Center General Hospital and Shimane University Hospital.

Patients and Definitions

A total of 71,904 patients age ≥16 years who underwent HSCT between January 1990 and December 2016 were included. patients for whom information regarding the transplantation type or PTLD development was unavailable were excluded, which ultimately resulted in the analysis of 64,539 patients. A diagnosis of PTLD was established by attending physicians and hematologic pathologists at each institution. The degree of HLA matching between patient and donor was assessed by HLA-A, -B, and -DR compatibility at the antigen level in the graft-versus-host direction. Conditioning regimens were classified according to established criteria [22]. The effect of ATG was analyzed when used in a conditioning regimen, for graft-versus-host disease (GVHD) prophylaxis, or for acute GVHD treatment. The ATG used was either of 2 preparations of rabbit polyclonal immunoglobulins immunized with human thymocytes (Thymoglobulin) or with Jurkat T lymphoblastoid cells (ATG-F). In cases using ATG as a conditioning regimen, high-dose ATG was defined as a total dose of >2.5 mg/kg Thymoglobulin, which is based on the median dose of Thymoglobulin used in a conditioning regimen, and >5.0 mg/kg ATG-F, the dose equivalent to 2.5 mg/kg of Thymoglobulin based on health insurance approval in Japan, whereas low-dose ATG was defined as a total dose of ≤2.5 mg/kg of Thymoglobulin and ≤5.0 mg/kg of ATG-F. Acute GVHD was graded according to standard criteria [23].

Statistical Analysis

The probability of developing PTLD was estimated using the Kaplan-Meier method, and differences between groups were evaluated using the log-rank test. To identify risk factors for PTLD, univariate and multivariate analyses were performed using a Cox proportional hazard regression model for patients who underwent allogeneic HSCT, and hazard ratio (HR) with 95% confidence interval (CI) were calculated for each variable. In these regression models, ATG use for treating acute GVHD and the occurrences of acute and chronic GVHD were treated as time-dependent variables. To evaluate the effect of chronic GVHD on the probability of PTLD, a landmark analysis of day +100 was performed. A 2-sided P value of <.05 was considered significant. The following variables were evaluated: year of HSCT, patient age at the time of HSCT, patient sex, primary disease (acute myeloid leukemia and myelodysplastic syndrome versus acute lymphoblastic leukemia, chronic myelogenous leukemia and myeloproliferative disorder, aplastic anemia [AA], lymphoid malignancies, or other), conditioning regimen (myeloablative conditioning versus reduced-intensity conditioning [RIC]), donor type (HLA-matched related donor [MRD] versus HLA-mismatched-related donor [MMRD], HLA-matched-unrelated donor [MURD], HLA-mismatched-unrelated donor [MMURD], or CB), number of allogeneic HSCTs, GVHD prophylaxis (cyclosporine-based versus tacrolimus-based), ATG use in a conditioning regimen, ATG use for GVHD prophylaxis, ATG use for acute GVHD treatment, use of alemtuzumab, use of ex vivo TCD, and acute GVHD (grade 0-I versus grade II-IV). All statistical analyses were performed using Stata version 14.0 (StataCorp, College Station, TX).

RESULTS

Patient Characteristics

The 64,539 patients who underwent HSCT included 40,195 recipients with allogeneic HSCT, 24,215 recipients with autologous HSCT, and 129 recipients with syngeneic HSCT. Patient characteristics according to HSCT type are summarized in Supplementary Table S1. A total of 299 patients developed PTLD, including 267 who had received allogeneic HSCT, 31 who had received autologous HSCT, and 1 who had received HSCT. Table 1 summarizes characteristics of the allogeneic HSCT recipients. The cohort who experienced PTLD after allogeneic HSCT was approximately 60% male, with a median age of 44 years (range, 16 to 76 years). Patients who developed PTLD were more likely to have had AA as the primary disease.

The characteristics of the allogeneic HSCTs are summarized in Table 2. Patients who developed PTLD were more likely than those who did not develop PTLD to have undergone HSCT with a CB graft. In particular, receipt of ATG was more prevalent in the PTLD cohort compared with the no PTLD cohort (42% versus 10%). Characteristics of patients who received autologous or syngeneic HSCT are reported in Supplementary Table S2.

Probability of PTLD and Prognosis

The probability of PTLD at 2 years after HSCT was .79%, with a median onset at 131 days (range, 15 to 6356 days) in recipients of allogeneic HSCT, compared with .11%, with a median onset at 198 days (range, 21 to 4759 days) in recipients of autologous HSCT. Only 1 syngeneic HSCT recipient (.78%) developed PTLD, at 29 days post-transplantation. The probability of PTLD was significantly higher in the allogeneic HSCT recipients compared with the autologous HSCT recipients ($P < .001$; Supplementary Figure S1). PTLD occurred within 1 year in 212 of the 267 (79%) allogeneic HSCT recipients and in 18 of the 31 (58%) autologous HSCT recipients. Late-onset PTLD (ie, occurring >5 years post-HSCT) was observed in 17 (6%) allogeneic HSCT recipients and in 5 (16%) autologous HSCT recipients.

Regarding the year of HSCT, we determined the cutoff value for the year of HSCT as 2010 using a receiver operating characteristic curve analysis. In allogeneic HSCT recipients, the probability of PTLD at 2 years post-transplantation was higher

Table 1
Demographic Data

Characteristic	No PTLD Group (N = 39,928)	PTLD Group (N = 267)
Year of HSCT, n (%)		
1990-2009	21,812 (55)	98 (37)
2010-2015	18,116 (45)	169 (63)
Age at HSCT, yr, median (range)	46 (16-88)	44 (16-76)
Sex, n (%)		
Male	23,450 (59)	158 (59)
Female	16,470 (41)	109 (41)
Unknown	8 (0)	0 (0)
Disease, n (%)		
AML/MDS	20,364 (51)	110 (41)
ALL	6768 (17)	40 (15)
CML/MPD	2809 (7)	18 (7)
Lymphoid malignancies*	7778 (20)	46 (17)
AA	1284 (3)	43 (16)
Others	922 (2)	10 (4)
Unknown	3 (0)	0 (0)
Number of allogeneic HSCTs, n (%)		
One	34,814 (87)	221 (83)
Two or more	5107 (13)	45 (17)
Unknown	7 (0)	1 (0)

AML indicates acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MPD, myeloproliferative disorder.

* Lymphoid malignancies include both malignant lymphoma and multiple myeloma.

between 2010 and 2015 than between 1990 and 2009 ((1.24% versus .45%). The probability of PTLD at 2 years post-transplantation was also higher between 2010 and 2015 than between 1990 and 2009 inautologous HSCT recipients (.18% versus .06%). To evaluate this in more detail, we divided the time period by 5-year intervals from 1990 onward. The probability of PTLD at 2 years post-transplantation was 0% for 1990 to 1994, .43% for 1995 to 1999, .26% for 2000 to 2004, .63% for 2005 to 2009, and 1.24% for 2010 to 2015 in the allogeneic HSCT recipients and 0% for 1990 to 1994, .05% for 1995 to 1999, .03% for 2000 to 2004, .10% for 2005 to 2009, and .18% for 2010 to 2015 in the autologous HSCT recipients. Regarding prognosis, the overall survival of patients who developed PTLD at 2 years post-transplantation was 42.5% in the allogeneic HSCT recipients and 15.4% in the autologous HSCT recipients (Figure 1).

Risk Factors for Developing PTLD

Table 3 presents the results of the univariate and multivariate analyses conducted to evaluate risk factors for PTLD in patients who underwent allogeneic HSCT. In univariate analyses, significant variables were the year of HSCT, disease (ie, AA), conditioning regimen, donor type, number of allogeneic HSCTs, GVHD prophylaxis, ATG use in a conditioning regimen, ATG use for acute GVHD treatment, and acute GVHD grade II-IV. The other results of the univariate analyses are provided in Supplementary Table S3. In the multivariate analysis, the probability of PTLD was significantly higher in 2010 to 2015 compared with 1999 to 2009 (HR, 1.87; $P < .001$). ATG use in a conditioning regimen was strongly correlated with an increased risk of PTLD (HR, 6.13; $P < .001$), and ATG use for acute GVHD treatment was also significantly associated with an increased risk of PTLD (HR, 2.09; $P < .001$). Patients who underwent HSCT from an MMRD (HR, 4.39; $P < .001$), an MURD (HR, 4.08; $P < .001$), an MMURD (HR, 3.20; $P = .001$), and particularly CB (HR, 8.03; $P < .001$) were at significantly greater risk of developing PTLD compared with those who underwent HSCT from an MRD. Patients who underwent a second or subsequent HSCT (HR, 1.50; $P = .03$) and those who developed acute GVHD grade II-IV (HR, 1.93; $P < .001$) were at slightly increased risk of PTLD. Regarding underlying diseases, AA was the sole significant risk factor for PTLD in univariate (HR, 4.95; $P < .001$) and multivariate (HR, 5.19; $P < .001$) analysis. The percentages of patients who underwent allogeneic HSCT with each risk factor identified on the multivariate analysis are shown in Figure 2. The percentage of patients who underwent HSCT with MURD and CB grafts increased markedly over time, particularly after 2005. Notably, the percentage of patients who received ATG in a conditioning regimen increased from 6.8% between 2000 and 2009 to 12.7% between 2010 and 2015. The percentage of patients who underwent second or subsequent HSCT also steadily increased.

Table 2
Characteristics of Allogeneic HSCT

Variable	No PTLD Group (N = 39,928)	PTLD Group (N = 267)
Conditioning regimen, n (%)		
MAC	23,680 (60)	123 (47)
RIC	16,144 (40)	140 (53)
Unknown	104 (0)	4 (0)
Donor type, n (%)		
MRD	13,034 (33)	24 (9)
MMRD	4043 (10)	52 (19)
MURD	10,135 (25)	81 (30)
MMURD	1977 (5)	16 (6)
CB	10,030 (25)	88 (33)
Unknown	709 (2)	6 (2)
Stem cell source, (%)		
BM	20,063 (50)	126 (47)
PB	9660 (24)	52 (19)
CB	10,030 (25)	88 (33)
Other/unknown	175 (0)/3 (0)	1 (0)/0 (0)
GVHD prophylaxis, n (%)		
CSP-based	18,216 (46)	88 (33)
TAC-based	20,593 (52)	172 (64)
Other	637 (2)	4 (2)
None/unknown	274 (0)/208 (0)	0 (0)/3 (1)
Use of ATG, n (%)	3915 (10)	111 (42)
Conditioning only	3299 (8)	94 (35)
GVHD prophylaxis only	70 (0)	1 (0)
Acute GVHD treatment only	303 (1)	6 (2)
Two or more	234 (1)	10 (4)
No/unknown	35,870 (90)/152 (0)	152 (57)/4 (2)
Use of alemtuzumab, n (%)	45/38,895 (<1)	0/251 (0)
Use of ex vivo TCD, n (%)	291/38,766 (<1)	5/264 (2)
Acute GVHD grade II-IV (%)		
Yes	13,797 (35)	115 (43)
No/unknown	22,784 (57)/3347 (8)	142 (53)/10 (4)
Chronic GVHD		
Yes	12,150 (30)	88 (33)
No/unknown	18,298 (46)/9480 (24)	146 (55)/33 (12)

MAC indicates, myeloablative conditioning; BM, bone marrow; PB, peripheral blood; CSP, cyclosporine; TAC, tacrolimus.

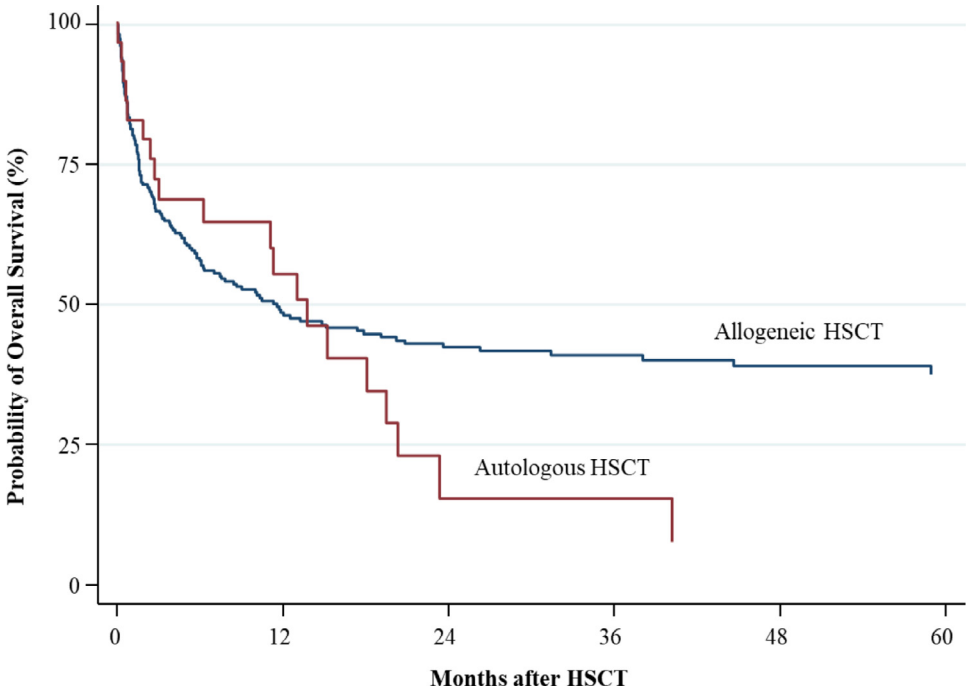

Figure 1. Overall survival of patients who developed PTLD. The survival rates for patients who developed PTLD after allogeneic HSCT and autologous HSCT were 48.6% and 55.3%, respectively, at 1 year and 42.5% and 15.4%, respectively, at 2 years after the development of PTLD.

We also performed univariate analysis to evaluate risk factors for PTLD in patients who underwent autologous HSCT (Supplementary Table S3). Year of HSCT, age at HSCT (≥50 years), sex (male), disease (malignant lymphoma), and conditioning regimen (including melphalan) were identified as significant variables.

Impact of Total ATG Dose on the Probability of PTLD

ATG was used in 4026 patients (10%) who underwent allogeneic HSCT, mainly as part of a conditioning regimen (Table 2). Approximately one-half of the patients who received ATG in a conditioning regimen underwent HSCT from an MMRD (47%), followed by an MURD (18%), an MRD (16%), CB (10%), and an MMURD (9%). Univariate analysis revealed a significantly higher probability of PTLD in patients who received ATG in a conditioning regimen (HR, 7.76; $P < .001$). The probability of PTLD at 2 years post-transplantation was 3.8% in patients who received ATG in a conditioning regimen, compared with .5% in those who did not (Figure 3A).

We also evaluated the effects of the total ATG dose on the probability of developing PTLD in patients who received ATG in a conditioning regimen. The median total doses of Thymoglobulin and ATG-F were 2.5 mg/kg and 8 mg/kg, respectively. A total of 1205 patients (33%) received high-dose ATG, 1566 (43%) received low-dose ATG, and information on total ATG dose was not available for the remaining 862 (24%). The probability of developing PTLD at 2 years post-transplantation was significantly higher in the patients who received high-dose ATG (6.0%; HR, 13.6; $P < .001$) and those who received low-dose ATG (2.8%; HR, 5.83; $P < .001$) than in those who did not receive ATG (.5%) (Figure 3B). High-dose ATG was associated with a 2.3-fold higher risk of developing PTLD compared with low-dose ATG. The impact of high-dose and low-dose ATG use on PTLD development remained significant (HR, 9.84 and 4.13, respectively) even after adjustments for the following risk factors: year of HSCT, disease, conditioning regimen, donor type, number of allogeneic HSCTs, GVHD prophylaxis, ATG use for acute GVHD treatment, and acute GVHD grade II-IV.

Scoring System

Based on the results of the multivariate analysis, we selected 3 pretransplant risk factors that were strongly associated with the development of PTLD—ATG use in a conditioning regimen, donor type, and AA—to create a simple scoring system that predicts the risk of developing PTLD at the time of allogeneic HSCT. We assigned 2 points for high-dose ATG use and receipt of a CB graft and 1 point for low-dose ATG use, AA, and receipt of an MMRD, MURD, or MMURD graft by referring to the HR of each variable in the multivariate analysis. Patients who underwent allogeneic HSCT were then classified into 4 risk groups according to the summed points as follows: 0 or 1 point, low-risk group (n = 25,869); 2 points, intermediate-risk group (n = 11,104); 3 points, high-risk group (n = 1129); and 4 or 5 points, very-high-risk group (n = 312). The probability of PTLD at 2 years post-HSCT according to risk group increased markedly with increasing number of points (Figure 4): .3%, 1.3%, 4.6%, and 11.5%, respectively. We also evaluated the probability of PTLD according to the number of identified risk factors [12]. The probability of PTLD in our cohort using the Landgren socre was greater in patients with 2 or more risk factors compared with those with 0 or 1. However, it was inversed in those with 3 or more (2.15%) as compared with those with 2 factors (6.33%) (Supplementary Figure S2).

DISCUSSION

Although some previous studies have investigated the incidence of and risk factors for PTLD, only a few large-scale studies have assessed risk factors for PTLD, and these risk factors have varied widely among the studies [5-8,12,24-27]. Thus, we conducted the present multicenter retrospective analysis using data from a large transplant registry. Our series was sufficiently large to allow us to extensively evaluate risk factors for PTLD.

Table 3
Univariate and Multivariate Analyses for the Development of PTLD

Variable	Univariate Analysis			Multivariate Analysis		
	HR	(95% CI)	*P*	HR	(95% CI)	*P*
Year of HSCT						
1990–2009	1.00			1.00		
2010–2015	2.77	(2.13-3.61)	<.001	1.87	(1.38-2.52)	<.001
Disease						
AML/MDS	1.00			1.00		
ALL	.99	(.69-1.44)	.98	1.08	(.75-1.57)	.68
CML/MPD	.94	(.56-1.57)	.81	1.55	(.89- 2.69)	.12
Lymphoid malignancies	1.24	(.88-1.75)	.22	1.33	(.92-1.92)	.13
AA	4.95	(3.47-7.07)	<.001	5.19	(3.32-8.11)	<.001
Others	1.91	(.97-3.76)	.06	1.94	(.97-3.89)	.06
Conditioning regimen						
MAC	1.00			1.00		
RIC	2.00	(1.56-2.55)	<.001	.82	(.60-1.12)	.22
Donor type						
MRD	1.00			1.00		
MMRD	10.4	(6.35-17.1)	<.001	4.39	(2.39-8.07)	<.001
MURD	4.89	(3.07-7.79)	<.001	4.08	(2.39-6.99)	<.001
MMURD	5.46	(2.88-10.3)	<.001	3.20	(1.58-6.47)	.001
CB	7.24	(4.56-11.5)	<.001	8.03	(4.72-13.7)	<.001
Number of allogeneic HSCT						
Two or more	2.15	(1.56-2.97)	<.001	1.50	(1.05-2.15)	.03
GVHD prophylaxis						
CSP-based	1.00			1.00		
TAC-based	2.07	(1.59-2.69)	<.001	.82	(.59-1.12)	.21
ATG in a conditioning regimen						
Yes	7.76	(6.03-9.99)	<.001	6.13	(4.33-8.68)	<.001
ATG for GVHD treatment*						
Yes	6.87	(4.00-11.8)	<.001	2.09	(1.17-3.72)	.01
Acute GVHD grade II-IV*						
Yes	1.83	(1.43-2.35)	<.001	1.93	(1.48-2.52)	<.001

* ATG for GVHD treatment and acute GVHD grade II-IV were treated as time-dependent variables.

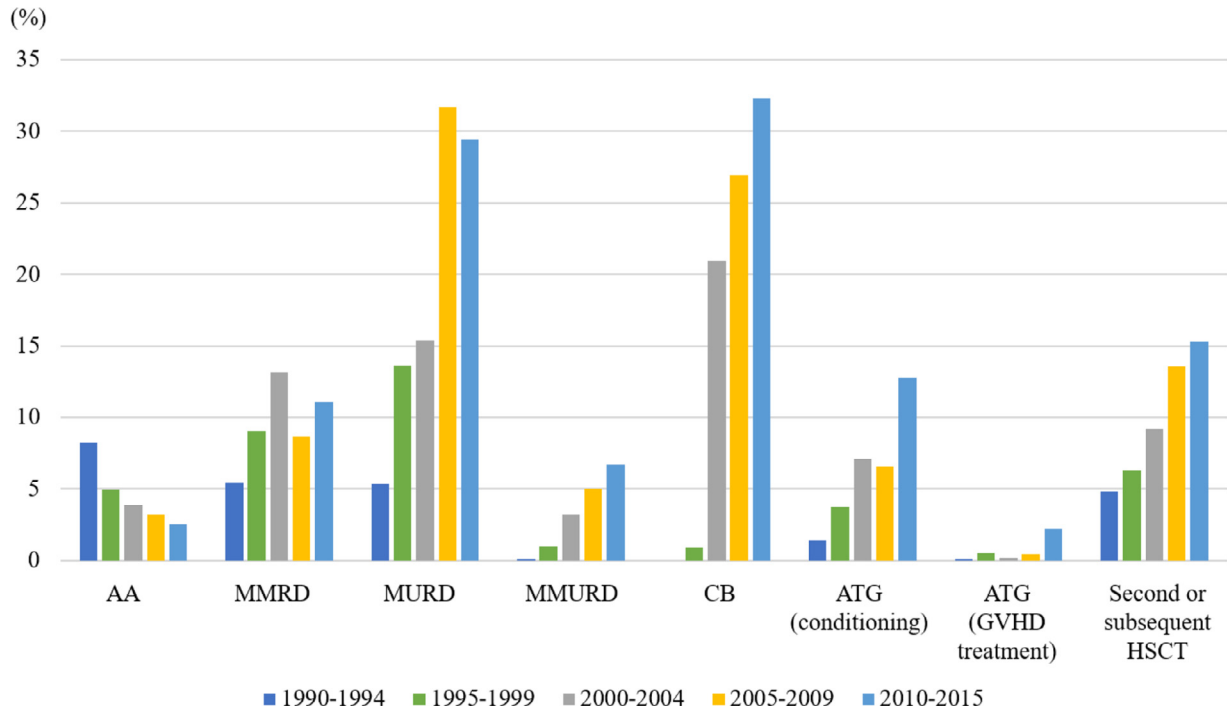

Figure 2. Prevalence of risk factors during the study period. The percentages of patients who underwent allogeneic HSCT with MMURD and CB grafts continuously increased. The percentages of patients who underwent HSCT with an MURD graft and who received ATG steadily increased.

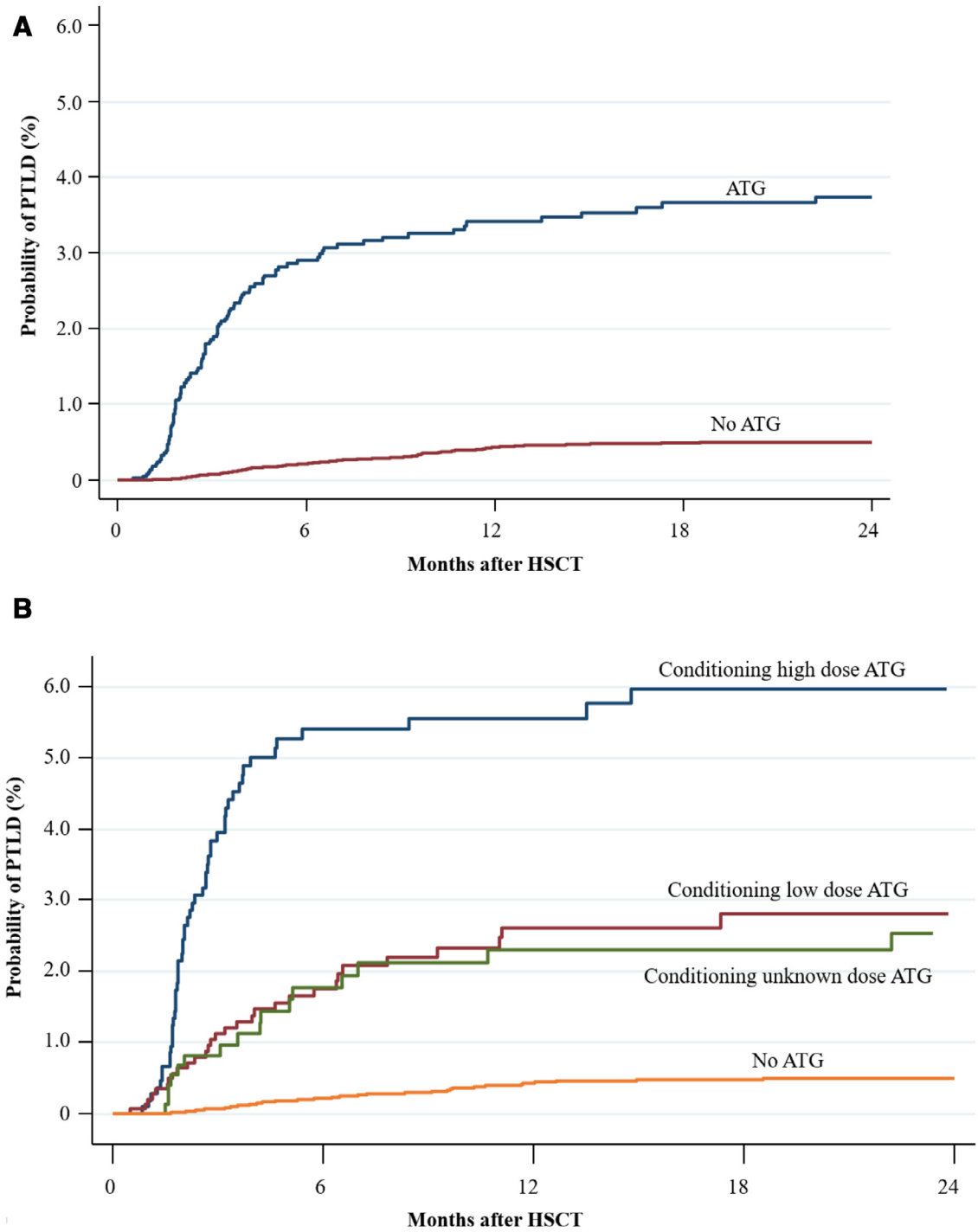

Figure 3. Probability of PTLD with the use of ATG in conditioning. (A) The probability of PTLD was significantly higher in patients who received ATG. (B) Receipt of high-dose ATG (total dose >2.5 mg/kg Thymoglobulin or >5.0 mg/kg ATG-F) was associated with a significantly higher risk of developing PTLD. By 2 years after HSCT, PTLD developed in 6.0% of patients who received high-dose ATG and in 2.8% of those who received low-dose ATG.

Furthermore, the data obtained contain information on various donor types, thereby enabling effective comparisons. We confirmed that ATG use in a conditioning regimen strongly contributed to the development of PTLD [6,12]. Our results are also consistent with previous findings showing that HSCT from an unrelated donor or HLA-mismatched donor, second or subsequent HSCT, and acute GVHD were all associated with an increased risk of PTLD [12,14,15]. The novel results of the present study include identification of AA as the sole significant risk factor for PTLD among the underlying diseases examined and identification of CB as more strongly associated with an increased risk of PTLD compared with grafts from other donor types. Moreover, patients who received ATG in a conditioning regimen were at an increased risk of developing PTLD in a dose-dependent manner. We also created a novel scoring system that allowed us to readily estimate the risk of PTLD before HSCT.

To date, only a few studies have reported risk classification for PTLD based on various risk factors [12,15]. The risk factors

Risk factor	Point(s)
ATG use	
High-dose	2
Low-dose	1
Donor type	
MMRD	1
MURD	1
MMURD	1
CB	2
Disease	
AA	1

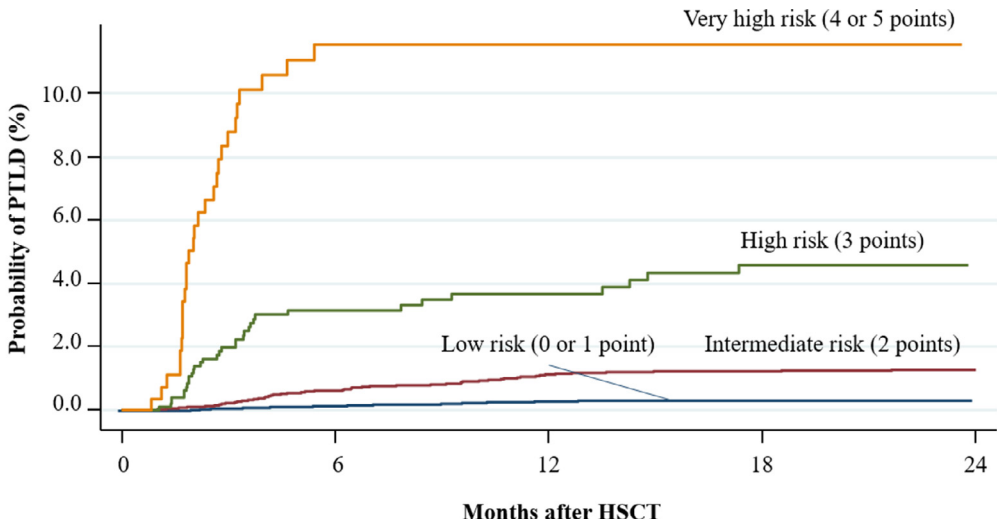

Figure 4. Probability of PTLD by the risk scoring system. Points were assigned for each risk factor as follows: high-dose ATG use, 2 points; low-dose ATG use, 1 point; MMRD, 1 point, MURD, 1 point; MMURD, 1 point; CB, 2 points, and AA, 1 point. The sum of points was used to classify risk groups: 0 or 1 point, low risk; 2 points, intermediate risk; 3 points, high risk; and 4 or 5 points, very high risk. The very-high-risk and high-risk groups had a markedly greater risk of developing PTLD, with probabilities of PTLD at 2 years after HSCT in these risk groups of 11.5% and 4.6%, respectively.

used in those studies have included pre-transplantation and post-transplantation parameters; however, post-transplantation parameters are not useful for predicting PTLD before HSCT. We have created a simple scoring system to estimate the risk of PTLD that has not been reported previously. The risk factors used in this scoring system are restricted to pretransplantation risk factors identified by a multivariate analysis. The risk strata are defined to give different weights to each risk factor and a sum of points according to each factor's HR. Patients are classified into 4 groups to identify those predicted to be at higher risk. Careful observation and close monitoring of EBV reactivation using EBV-DNA are necessary for an early diagnosis of PTLD in these patients. This will contribute to improved prognostic prediction in affected patients and earlier initiation of preemptive treatments, such as rituximab and EBV-specific cytotoxic T cells [28-32].

The reported probability of PTLD after allogeneic HSCT varies widely between 1% and 17% depending on patient characteristics, stem cell source, degree of HLA mismatch, and conditioning regimen [6,8,9,11,13]. In the present study, the overall probability of PTLD after allogeneic HSCT was .79%, slightly lower than that reported previously. The smaller percentage of patients who receive ATG in this cohort might affect the low incidence of PTLD in Japan. However, the probability of PTLD at 2 years after allogeneic HSCT increased to 1.24% after 2010, possibly related to the increased use of HLA-mismatched donors, unrelated donors, or CB (Figure 2). In particular, the use of CB, which was identified as the strongest risk factor among donor types, increased sharply after 2010. In addition to the increase in the use of HLA-mismatched or unrelated donors, there was also an increase in the use of ATG, which was also strongly associated with an increased probability of PTLD. Other possible reasons are the increased number of patients who underwent second or subsequent HSCT and the increased use of RIC regimens, which have been reportedly correlated with an increased risk of PTLD [15]. However, we could not verify a correlation between RIC and the development of PTLD in our cohort. Some studies have reported an increase in the number of older HSCT recipients as a factor in the increasing incidence of PTLD [16,19,33,34]; however, in the present study, recipient age was not identified as a significant risk factor for PTLD. Although the use of haploidentical donor HSCT has been identified as a risk factor for PTLD, we did not evaluate this association in the present study because of the small number of patients (n = 172) who underwent haploidentical HSCT (data not shown).

Owing to the increased prevalence of HSCT from HLA-mismatched or unrelated donors, rabbit ATG is being increasingly used for the conditioning of HSCT [35]. The use of ATG is a risk factor for EBV reactivation, which has been linked to the development of PTLD [9,12,36,37]; however, the effects of the total ATG dose on the development of PTLD has not yet been clinically evaluated. Our data identify the use of ATG in conditioning as a strong risk factor for the development of PTLD. Moreover, in patients who received ATG as part of their conditioning regimen, the use of high-dose ATG was associated with a 2.3-fold increase in the risk of PTLD compared with the use of low-dose ATG, even after adjustments for other risk factors. This finding suggests that the use of ATG increases the risk of PTLD in a dose-dependent manner; thus, the ATG dose administered should be as low as possible to be efficacious while preventing the development of PTLD.

CB is being increasingly used in allogeneic HSCT in both children and adults with hematologic diseases in Japan. Although CB has been associated with an increased risk of PTLD [17,33], with an reported incidence of 2.0% to 4.5% [38-42], an detailed comparison of incidence between CB and other sources in the same cohort has not been performed. Therefore, we evaluated the probability of PTLD with respect to donor types, including CB, and subsequently found a high probability of PTLD in patients who underwent CB transplantation (CBT). The use of CB was associated with a 1.5- to 2-fold higher risk of developing PTLD compared with the use of an HLA-mismatched or unrelated donor. The higher probability of PTLD after CBT may be attributed to a low number of infused donor T cells, T cell naivety, or delayed antigen-specific cellular immune reconstitution in the early period after CBT [43]. In the present study, the probability of PTLD at 2 years after CBT was .9%, which was slightly lower than that reported previously, possibly because of the lower rate of ATG use in our cohort. In previous studies, most patients received ATG in a conditioning regimen, suggesting that the reported probability of PTLD was strongly affected by ATG use.

Acute GVHD has been reported as a risk factor for PTLD in the literature [6]. Immune dysfunction is well recognized as a consequence of acute GVHD because of depressed cytotoxic T cell and T helper cell function caused by a proinflammatory cytokine environment [44-46]. Therefore, T cell impairment likely contributes to the risk for PTLD. In this study, we also identified acute GVHD as a risk factor for PTLD; however, we found no correlation between chronic GVHD and the development of PTLD. Because information on treatment for chronic GVHD was not available, the effect of immunosuppressive treatment on PTLD development was not evaluated.

Here we report for the first time that AA is associated with an increased risk of PTLD independent of the use of ATG in conditioning. EBV is highly reactivated in patients with AA after immunosuppressive therapy [36,47]. EBV reactivation has been reported in 88% to 100% of EBV-seropositive patients with AA after ATG-based immunosuppressive therapy [36]. In addition, previous findings suggest that the use of ATG before HSCT increases the risk of PTLD in patients with AA [10]. Further studies are needed to confirm the impact of AA on the development of PTLD, including the use of ATG as an immunosuppressive treatment before HSCT.

Regarding other variables, even though ex vivo TCD is an established risk factor for the development of PTLD [12], we were unable to validate this finding in our cohort. This might be related to our low proportion of patients (<1%) who underwent ex vivo TCD. In contrast, alemtuzumab reportedly reduces the risk of PTLD by removing T and B cells [12]; however, we were unable to evaluate the effects of alemtuzumab on PTLD because only approximately .1% of our allogeneic HSCT recipients received alemtuzumab, and none developed PTLD.

Another limitation of the present study is the lack of detailed data on PTLD. Information on histological subtypes, treatment, and EBV reactivation was not available from our registry data, and diagnoses were based on those recorded by the attending physicians at each institution. We were not able to validate the risk scoring system using another dataset, such as that from another country. In addition, the incidence of PTLD itself might have been underestimated, particularly for PTLD occurring late after HSCT. However, approximately 80% of PTLD cases developed in the early phase within 1 year after allogeneic HSCT, suggesting a low degree of underestimation. The PTLD cases that developed in the late phase occurred as a simple second malignancy with EBV infection. The complete differentiation of such second malignancies from PTLD was difficult, however.

In conclusion, the incidence of PTLD has been increasing over the past few decades. PTLD is a severe complication after HSCT, and an early diagnosis is important. Our novel risk scoring system enables us to identify patients at greatest risk before HSCT, allowing for more careful evaluation of these patients after HSCT. Further research is warranted to improve the morbidity and mortality of PTLD in HSCT recipients.

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SUPPLEMENTARY MATERIALS

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