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MAJOR ARTICLE

Response to Rituximab-Based Therapy and Risk Factor Analysis in Epstein Barr Virus–Related Lymphoproliferative Disorder After Hematopoietic Stem Cell Transplant in Children and Adults: A Study From the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation

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Background. The objective of this analysis was to investigate prognostic factors that influence the outcome of Epstein-Barr virus (EBV)–related posttransplant lymphoproliferative disorder (PTLD) after a rituximab-based treatment in the allogeneic hematopoietic stem cell transplant (HSCT) setting.

Methods. A total of 4466 allogeneic HSCTs performed between 1999 and 2011 in 19 European Group for Blood and Marrow Transplantation centers were retrospectively analyzed for PTLD, either biopsy-proven or probable disease.

Results. One hundred forty-four cases of PTLD were identified, indicating an overall EBV-related PTLD frequency of 3.22%, ranging from 1.16% for matched-family donor, 2.86% for mismatched family donor, 3.97% in matched unrelated donors, and 11.24% in mismatched unrelated donor recipients. In total, 69.4% patients survived PTLD.

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Multivariable analysis showed that a poor response of PTLD to rituximab was associated with an age ≥30 years, involvement of extralymphoid tissue, acute GVHD, and a lack of reduction of immunosuppression upon PTLD diagnosis. In the prognostic model, the PTLD mortality increased with the increasing number of factors: 0–1, 2, or 3 factors being associated with mortality of 7%, 37%, and 72%, respectively ($P < .0001$). Immunosuppression tapering was associated with a lower PTLD mortality (16% vs 39%), and a decrease of EBV DNAemia in peripheral blood during therapy was predictive of better survival.

Conclusions. More than two-thirds of patients with EBV-related PTLD survived after rituximab-based treatment. Reduction of immunosuppression was associated with improved outcome, whereas older age, extranodal disease, and acute graft-vs-host disease predicted poor outcome.

Keywords. Epstein-Barr virus; post-transplant lymphoproliferative disorder; hematopoietic stem cell transplantation; risk factors; prognostic model.

Posttransplant lymphoproliferative disorder (PTLD) is a heterogenous group of malignant diseases presenting after transplant and caused by iatrogenic suppression of T-cell function. The most common form of PTLD is related to Epstein-Barr virus (EBV) disease (EBV-PTLD), which presents as B-cell proliferation and is referred to as post–allogeneic hematopoietic stem cell transplant (HSCT) EBV-PTLD.

Post-HSCT EBV-PTLD is a life-threatening complication and was estimated to affect approximately 4.3 in 1000 recipients a decade ago (78 of 18 014 patients from 234 transplant centers) with an attributable mortality of 84.6% [1]. Since then, 3 main therapeutic approaches have been advanced for the prevention and treatment of EBV-PTLD, summarized in the European Conference on Infections in Leukemia (ECIL) recommendations [2]. These include the administration of rituximab, reduction of immunosuppression (RI), and use of EBV-specific cytotoxic T lymphocytes (CTLs). However, CTLs are not available to most transplant centers, and tapering of immunosuppression has limited efficacy and is not always feasible in the presence of graft-vs-host disease (GVHD). Consequently, treatment with rituximab seems to offer the most promise. Recent reports indicate that treatment with rituximab of posttransplant EBV-PTLD is efficacious, particularly for recipients of solid organ transplant (SOT). However, the data for HSCT recipients are based mainly on limited series, anecdotal reports, and a single summary of reported cases [3–9]. Moreover, there has been no analysis of the prognostic factors for the outcome of treatment of PTLD after HSCT with rituximab-based regimens.

To address this, we undertook a retrospective analysis of patients who had been treated with rituximab for PTLD after allo-HSCT, so we could analyze the factors that might be associated with survival.

PATIENTS AND METHODS

We conducted a multicenter, retrospective analysis of 4466 allogeneic HSCTs performed in 19 pediatric and adult European Group for Blood and Marrow Transplantation transplant centers in Europe. Centers volunteered the data on each of their patients who had been treated with rituximab between 1999 and 2011 for PTLD by completing a questionnaire specifically designed for the purpose. The inclusion criteria were proven or probable PTLD diagnosis and rituximab treatment administered either alone or combined with other approaches. The clinical data were entered into a centralized database. The study was approved by the institutional review board of the Medical College, Nicolaus Copernicus University (Bydgoszcz, Poland).

Patients aged 18 years old or younger were defined as children. The diagnosis of EBV-PTLD was defined as proven or probable according to published definition [2]. Proven PTLD was diagnosed when EBV was detected in a specimen obtained from an organ by biopsy or other invasive procedure, with a test with appropriate sensitivity and specificity together with symptoms and signs from the affected organ. Probable PTLD was defined as significant lymphoadenopathy or other end-organ disease accompanied by a high EBV-DNA blood load, in the absence of other etiologic factors or established diseases [2, 9]. EBV DNAemia was measured by quantitative or qualitative polymerase chain reaction (PCR) in whole blood (n = 67), plasma (n = 64), or serum (n = 1) or in peripheral blood mononuclear cell–based assays in other cases. Repeat PCR testing was done at local sites using the same methodology throughout the study period. EBV DNA levels were determined before the beginning of therapy and 1 week after each dose of rituximab. PTLD occurring within the first 100 days after transplantation was defined as early-onset disease.

Reduction of immunosuppression (RI) was defined as a sustained decrease of at least 20% of the daily dose of immunosuppressive drugs with the exception of low-dose corticosteroid therapy, that is, ≤0.2 mg/kg in patients <40 kg of body weight or ≤10 mg/day in patients of >40 kg of body weight [10]. Response to given treatment was assessed on clinical level as complete remission, partial response, stable disease, or progressive disease, according to standard definition [11]. The virologic response was also assessed based on EBV DNAemia reduction. Failure of PTLD treatment was defined by death due to PTLD. The cause of death was reported as being related to PTLD or due to other causes.

Statistical Analysis

Descriptive statistics were used to show the patients' general characteristics. Percentages were reported for categorical variables and median and ranges for continuous variables, and overall survival (OS) was calculated from the date of PTLD diagnosis to the date of death from any cause or to the date of the latest follow-up. Death due to any cause was considered as an event. The probabilities of OS were estimated by the Kaplan-Meier method and univariate comparisons were performed using the log-rank test [12, 13]. PTLD mortality was analyzed in a competing risks framework. The time from the date of PTLD diagnosis to the date of death due to PTLD or death due to other causes or to the date of the latest follow-up was considered. PTLD-related death was considered as the event of interest, death due to other causes was considered as a competing event, and patients who did not develop an event were censored at their last follow-up. Mortality rates due to PTLD were estimated in terms of cumulative incidence curves using the proper nonparametric method. Univariate comparisons were performed by using the Gray test [14].

The impact of the following variables was analyzed: age at transplant (>30 vs <30 years), underlying diagnosis (malignant vs not malignant), stem cell source (peripheral blood vs bone marrow vs cord blood), acute GVHD at the time of PTLD diagnosis (≥ grade II vs < grade II or absent), PTLD organ involvement (extranodal vs nodal), initial EBV DNAemia ($\geq 10^4$ vs $<10^4$ genome copies/mL [gc/mL]), and RI upon PTLD diagnosis. Multivariable analysis for OS and PTLD mortality was performed by using the Cox regression in order to estimate hazards and cause-specific hazards, respectively, including as candidates only variables that resulted statistically significant at 0.15 level from univariate analysis [15].

In order to analyze the influence of the viral load after 1 and 2 weeks on OS and on PTLD mortality, a landmark analysis was performed using data on only those patients who survived up to 1 and 2 weeks after PTLD diagnosis. Absolute and logarithmic changes from baseline at week 0 were analyzed. A P value <.05 was regarded as statistically significant.

RESULTS

Frequency of PTLD

PTLD had been diagnosed in 144 cases, a median of 2 months after HSCT (range, 0.5–53 months). Early onset of PTLD affected 109 patients (75.7%). Eight patients (6%) developed PTLD in the first month, whereas 16 patients (11.1%) were diagnosed >6 months after HSCT. PTLD was proven by biopsy in 86 cases (59.7%), and the remaining 58 (40.3%) cases were considered probable disease. The results of therapy of 9 patients have been published previously as case series [16–20].

Table 1. Frequency of Posttransplant Lymphoproliferative Disorder and Hazard Risk Related to Type of Transplant

Type of Donor	Number of Allogeneic HSCT	No. of PTLDs	Frequency, %	Hazard Ratio (95% CI)	*P* Value
MFD	1902	22	1.16	1.00	
MMFD/ haplo	455	13	2.86	2.47 (1.17–5.17)	.015
MUD	1762	70	3.97	3.43 (2.07–5.74)	<.001
MMUD	347	39	11.24	9.72 (5.53–17.17)	<.001
Total	4466	144	3.22	2.79 (1.74–4.50)	<.001

Abbreviations: CI, confidence interval; HSCT, hematopoietic stem cell transplant; MFD, matched family donor; MMFD, mismatched family donor; MMUD, mismatched unrelated donor; MUD, matched unrelated donor; PTLD, posttransplant lymphoproliferative disorder.

The overall EBV-PTLD frequency in the participating centers was 3.22% (144/4466), and ranged from 1.16% in matched family donor (MFD), 2.86% in mismatched family donor (MMFD), 3.97% in matched unrelated donor (MUD), and 11.24% in mismatched unrelated donor (MMUD) recipients (Table 1). Overall, the frequency of PTLD after alternative donor (MMFD or unrelated donor) allogeneic HSCT was 4.75% (hazard ratio [HR] = 4.11; 95% CI, 2.55–6.69; $P < .001$) and the PTLD frequency after cord blood transplant was 4.06% (HR = 3.61; 95% CI, 1.74–7.46; $P < .001$).

Baseline Characteristics

The median age at transplant of 144 patients with PTLD was 25 years (range, <1–68 years). Baseline disease, patient characteristics, and univariate risk of PTLD mortality and OS are reported in Table 2. Clinically, 61 patients (42%) had extranodal PTLD involvement, including 15 (10%) with multiorgan (ie, >2 systems) involvement.

Treatment

Patients were treated with a median of 3 doses of rituximab (range, 1–16), administered at dosage of 375 mg/m^2 at weekly intervals, except in 6 patients who were treated at 3- to 6-day intervals, and a further single case that was treated every 10 days. Tapering off of immunosuppression was done in 51 cases. Chemotherapy was administered as a second-line therapy because of a partial response, or stable or progressive disease to 31 patients (21.5%), including 4 of 51 patients who had immunosuppression tapered and 27 of 93 without RI (Figure 1). Additional therapies included surgery in 4 cases and donor lymphocyte infusion in 11 cases. In 48 patients, antiviral agents were also used (mainly cidofovir), but it had no impact on survival from PTLD.

Survival After PTLD

One hundred (69.4%) patients survived after rituximab-based therapy, and 44 died due to PTLD. Only those who achieved a

Table 2. Baseline Patient and Disease Characteristics With Univariate Analysis

		PTLD-Related Mortality, Cumulative Incidence				Overall Survival			
Characteristic	Patients, No.	Events	Hazard Ratio (95% CI)	1-y PTLD-Related Mortality (95% CI)	*P* Value, Gray	Events	Hazard Ratio (95% CI)	3-y OS (95% CI)	*P* Value, Log-Rank
Patients									
Children	54	11	1.00	20.42 (12.05–34.60)	.04	21	1.00	59.29 (44.42–71.41)	.031
Adults	90	33	2.00 (1.01–3.95)	37.00 (28.20–48.55)		50	1.74 (1.04–2.90)	39.61 (28.37–50.63)	
Age, y									
<30	79	14	1.00	17.84 (11.10–28.70)	.0003	29	1.00	60.25 (47.80–70.63)	.0003
≥30	65	30	3.10 (1.64–5.85)	46.39 (35.67–60.33)		42	2.33 (1.45–3.75)	30.85 (18.81–43.71)	
Malignant disease									
No	32	5	1.00	15.63 (6.99–34.95)	.036	7	1.00	78.13 (59.52–88.92)	.001
Yes	112	39	2.54 (1.00–6.45)	35.06 (27.21–45.17)		64	3.41 (1.56–7.45)	37.63 (27.68–47.54)	
Source									
Peripheral blood	108	36	1.00 (.39–2.56)	33.49 (25.65–43.72)	.19	59	1.12 (.51–2.46)	41.07 (30.84–51.01)	.04
Bone marrow	22	3	0.36 (.09–1.50)	13.64 (4.76–39.03)		5	0.37 (.12–1.15)	76.36 (51.99–89.48)	
Cord blood	14	5	1.00	37.95 (18.77–76.73)		7	1.00	46.88 (19.16–70.65)	
Donor									
Other	122	41	1.00	33.80 (26.34–43.38)	.056	63	1.00	45.26 (35.65–54.36)	.14
Family matched	22	3	0.35 (.11–1.12)	13.64 (4.76–39.03)		8	0.58 (.28–1.21)	58.25 (31.97–77.41)	
Donor type									
Mismatched	52	18		35.04 (24.11–50.93)	.4	26	1.00	46.52 (31.33–60.37)	.7
Matched	92	26	0.79 (.43–1.44)	28.36 (20.48–39.27)		45	0.91 (.56–1.48)	47.53 (36.27–57.96)	
Reduced intensity conditioning									
No	74	22	1.00	29.82 (21.01–42.34)	.7	37	1.00	47.81 (35.58–59.04)	.9
Yes	70	22	1.11 (.62–2.01)	31.61 (22.37–44.68)		34	1.04 (.65–1.66)	45.88 (32.18–58.54)	
Antithymocyte globulin use									
No	28	7	1.00	25.00 (13.16–47. 49)	.4	14	1.00	46.08 (25.66–64.31)	.7
Yes	116	37	1.44 (.64–3.23)	32.17 (24.65–41.97)		57	1.14 (.64–2.05)	47.74 (37.79–57.02)	
Acute GVHD ≥II at PTLD diagnosis									
No	125	34	1.00	27.41 (20.57–36.51)	.046	57	1.00	50.81 (40.98–59.82)	.001
Yes	10	5	2.29 (.89–5.86)	50.00 (26.90–92.93)		9	3.00 (1.48–6.10)	10.00 (.57–35.81)	
Extensive chronic GVHD at PTLD diagnosis									
No	22	5	1.00	22.73 (10.52–49.11)	.5	9	1.00	58.18 (34.90–75.68)	.6
Yes	11	3	1.09 (.26–4.56)	27.27 (10.39–71.59)		6	1.29 (.46–3.64)	31.82 (5.32–63.95)	
Donor EBV serology									
Negative	21	6	1.00	28.57 (14.53–56.19)	.9	9	1.00	56.28 (32.59–74.47)	.5
Positive	110	34	1.14 (.48–2.71)	31.17 (23.57–41.22)		57	1.25 (.62–2.52)	43.72 (33.42–53.56)	
Recipient EBV serology									
Negative	24	5	1.00	20.83 (9.55–45.44)	.6	10	1.00	56.47 (34.03–73.86)	.3
Positive	111	36	1.65 (.65–4.22)	32.71 (25.01–42.79)		57	1.41 (.72–2.76)	44.59 (34.30–54.36)	
Year of HSCT									
<2009	92	32	1.00	34.80 (26.31–46.04)	.17	47	1.00	48.17 (37.52–58.03)	.8
≥2009	52	12	0.65 (.33–1.26)	23.30 (14.18–38.27)		24	1.05 (.64–1.74)	50.11 (34.79–63.63)	
PTLD onset									
Early (≤100 d)	35	9	1.00	25.83 (14.71–45.37)	.3	15	1.00	53.85 (34.75–69.59)	.16
Late (>100 d)	109	35	1.47 (.70–3.05)	32.20 (24.51–42.31)		56	1.50 (.85–2.66)	45.11 (34.88–54.79)	
Level of PTLD diagnosis									
Probable	58	14	1.00	24.14 (15.30–38.09)	.2	27	1.00	51.36 (37.28–63.76)	.5
Proven	86	30	1.47 (.78–2.76)	35.38 (26.51–47.22)		44	1.18 (.73–1.91)	44.93 (33.32–55.86)	

Table 2 continued.

Characteristic	Patients, No.	PTLD-Related Mortality, Cumulative Incidence				Overall Survival			
		Events	Hazard Ratio (95% CI)	1-y PTLD-Related Mortality (95% CI)	*P* Value, Gray	Events	Hazard Ratio (95% CI)	3-y OS (95% CI)	*P* Value, Log-Rank
Histology									
Polyclonal	17	4	1.00	23.53 (9.99–55.44)	.2	8	1.00	51.76 (26.16–72.37)	.5
Monoclonal/ HD-like	72	29	1.84 (.65–5.22)	40.99 (30.94–54.30)		40	1.27 (.59–2.71)	40.30 (28.07–52.20)	
PTLD CD20 status									
Negative	10	6	1.00	60.00 (36.17–99.53)	.12	7	1.00	20.00 (1.36–54.72)	.18
Positive	77	26	0.49 (.20–1.20)	34.26 (25.06–46.83)		40	0.58 (.26–1.30)	44.49 (32.40–55.89)	
Extranodal involvement									
No	83	18	1.00	21.76 (14.46–32.76)	.007	40	1.00	48.04 (36.10–59.01)	.4
Yes	61	26	2.22 (1.22–4.05)	43.14 (32.24–57.73)		31	1.24 (.77–1.98)	46.64 (32.99–59.19)	
Initial EBV DNAemia ≥10 000 gc/mL									
No	33	4	1.00	12.12 (4.84–30.38)	.013	14	1.00	53.07 (33.33–69.39)	.2
Yes	106	36	3.31 (1.18–9.29)	34.09 (26.14–44.45)		52	1.46 (.81–2.64)	48.43 (38.05–58.04)	
Reduction of IS therapy									
No	93	36	1.00	38.71 (29.98–49.99)	.006	53	1.00	40.89 (30.38–51.12)	.024
Yes	51	8	0.36 (.17–.78)	16.19 (8.55–30.65)		18	0.55 (.32–.93)	59.86 (43.38–72.96)	

Abbreviations: CI, confidence interval; EBV, Epstein-Barr virus; GVHD, graft-vs-host disease; HD, Hodgkin disease; HSCT, hematopoietic stem cell transplant; IS, immunosuppressive; OS, overall survival; PTLD, posttransplant lymphoproliferative disorder.

complete remission survived from PTLD. The overall cumulative incidence of mortality due to PTLD was 30.8% (95% CI, 24.0%–39.3%; Figure 2*A*). PTLD resolved in 43 of 51 (84%) patients who received both rituximab and RI, and in 57 of 93 patients (61%) in whom immunosuppression tapering was not done ($P = .006$; Figure 1 and Figure 2*C*). RI reduced the risk of death due to PTLD 2.8-fold in univariate analysis (Table 2). Despite chemotherapy as second-line treatment, only 40.9% of the patients without RI survived (Figure 1). No differences in PTLD mortality was found in proven vs probable PTLD categories, both for patients with RI (18.6% vs 14.8%; $P = .91$) and without RI (41.9% vs 32.3%; $P = .45$).

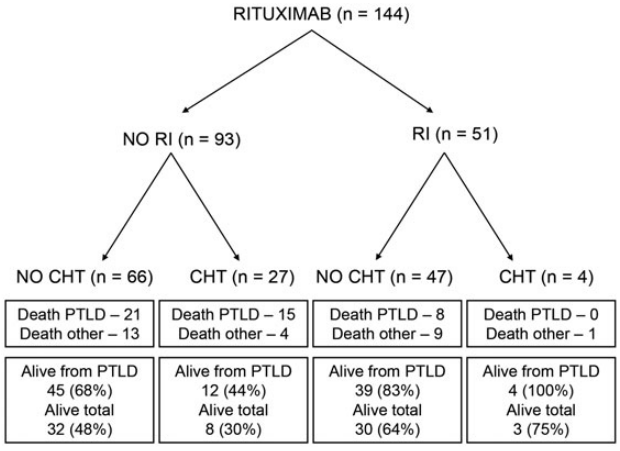

Figure 1. Initial therapy and outcomes of posttransplant lymphoproliferative disorder (PTLD). Treatment patterns of each patient with PTLD are reported according to therapy received: reduction of immunosuppression (RI) and chemotherapy (CHT). Therapy received is described as rituximab based, that is, single-agent rituximab or rituximab ± RI ± CHT. Outcome is expressed as death, PTLD-related or other, or alive. Abbreviations: CHT, chemotherapy; PTLD, posttransplant lymphoproliferative disorder; RI, reduction of immunosuppression.

Mortality due to PTLD was lower in children than in adults (20% vs 37%; $P = .04$). There was no difference in PTLD mortality with respect to the type of transplant (3/22 deaths after MFD, 5/13 after MMFD/haplo, 23/70 after MUD, 13/39 after MMUD). The level of diagnosis expressed as proven or probable had no impact on PTLD mortality ($P = .2$). Monomorphic vs polymorphic PTLD disease also had no difference in outcome (Table 2).

Factors predicting poor outcome of PTLD therapy by univariate analysis were age >30 years, initial malignant diagnosis, acute GVHD ≥ grade II, extranodal involvement, initial EBV DNAemia ≥ 10^4 gc/mL, and no RI at PTLD diagnosis (Table 2). Reduction of immunosuppression was statistically not related to occurrence of acute GVHD at PTLD diagnosis ($P = .76$).

Multivariable analysis for PTLD-related mortality was performed by using the significant prognostic factors identified in univariate analysis. Four variables remained that had prognostic significance for PTLD mortality: (1) age at transplant >30 years, (2) extranodal involvement, (3) acute GVHD ≥ grade II

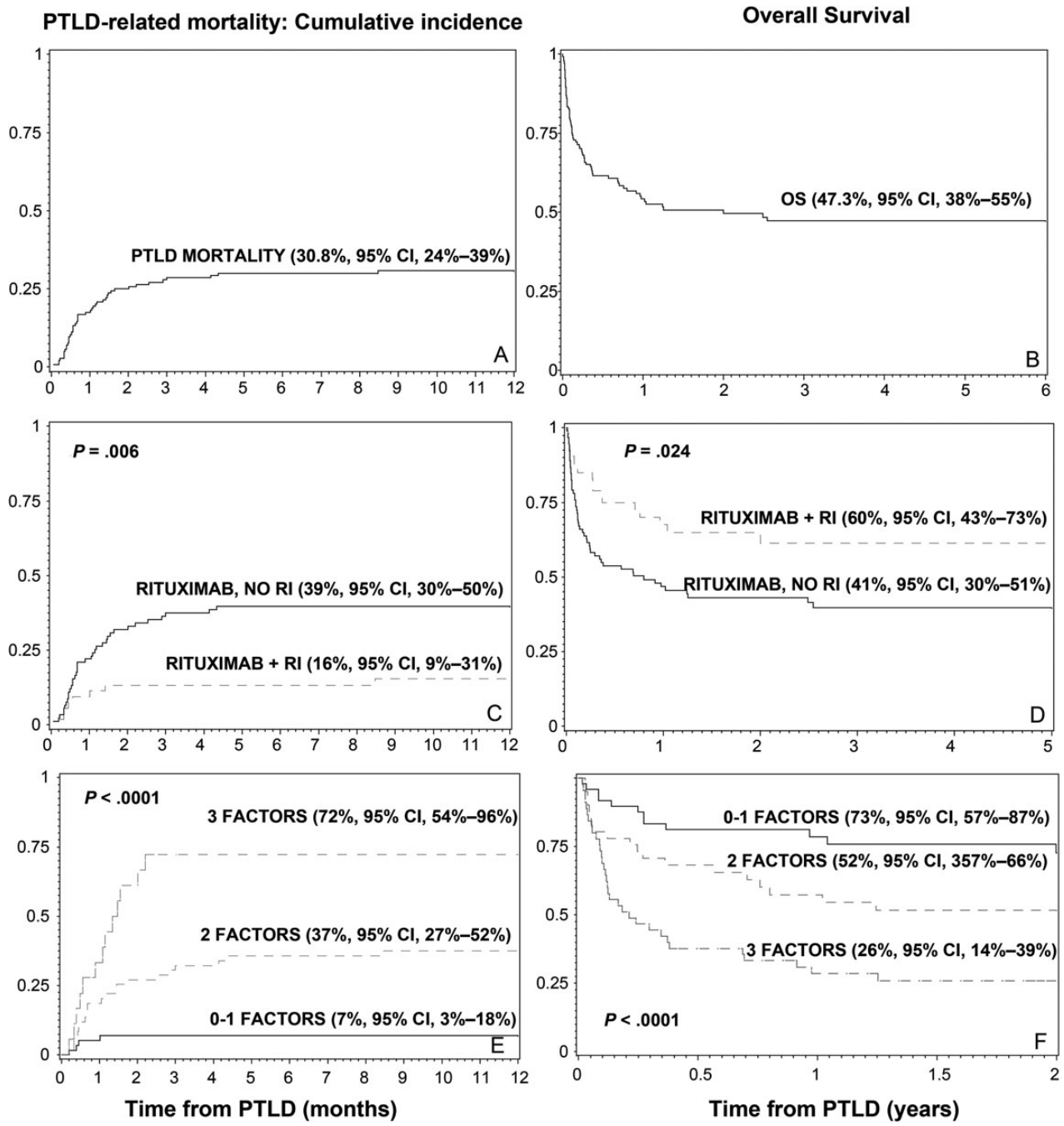

Figure 2. Posttransplant lymphoproliferative disease (PTLD)–related mortality (PRM), overall survival (OS), and prognostic survival model. *A*, Cumulative incidence of PRM in 144 patients with PTLD treated with rituximab. *B*, Probability of OS (estimate and confidence interval at 3 years) in the same cohort. *C*, Cumulative incidence of PRM of the 51 patients who could reduce immunosuppression therapy upon PTLD diagnosis (RI) compared with the 93 patients who could not reduce immunosuppression (no RI). *D*, Probability of OS in patients with RI compared with patients with no RI. Combined approach with rituximab and RI was associated with significantly reduced PRM ($P = .006$) and improved OS ($P = .024$). *E*, Cumulative incidence of PRM according to the number of the following risk factors for each patient: age ≥ 30 years at transplant, extranodal involvement, acute graft-vs-host disease (GVHD) ≥ grade II at PTLD diagnosis, and no RI. The higher the number of risk factors, the worse the outcome: PRM with 0–1, 2, or 3 factors were 7%, 37%, and 72%, respectively ($P < .0001$), and the number of events was 4 of 58, 22 of 59, and 13 of 18, respectively. No patient presented with 4 factors simultaneously. *F*, Probability of OS according to the number of the following risk factors for each patient: age ≥30 years at transplant, malignant disease, acute GVHD ≥grade II at PTLD diagnosis, and no RI. A higher number of risk factors was associated with an increasingly poor prognosis: 3-year OS rates with 0–1, 2, or 3 factors were 73%, 52%, and 26%, respectively ($P < .0001$), and the number of events was 12 of 49, 19 of 41, and 35 of 45, respectively. No patient presented with 4 factors simultaneously. Abbreviations: CI, confidence interval; OS, overall survival; PTLD, posttransplant lymphoproliferative disorder; RI, reduction of immunosuppression.

Table 3. Multivariable Analyses of Prognostic Factors

	PTLD-Related Mortality			Overall Survival		
Prognostic Factor	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Age ≥30 y vs <30 y	3.8	1.8–8.1	.0007	2.2	1.3–3.6	.0039
aGVHD ≥II at PTLD diagnosis vs aGVHD absent or grade I	6.3	2.0–20.1	.0019	3.2	1.5–6.7	.0022
Extranodal involvement vs nodal involvement only	3.3	1.6–6.6	.0010			NS
Malignant disease vs nonmalignant disease			NS	2.6	1.1–5.7	.0233
Immunosuppression reduction upon PTLD diagnosis vs no reduction	0.2	.1–.7	.0091	0.6	.3–1.0	.0471

Abbreviations: aGVHD, acute graft-vs-host disease; CI, confidence interval; HR, hazard ratio; NS, not significant; PTLD, posttransplant lymphoproliferative disorder.

at PTLD diagnosis, and (4) no RI at PTLD diagnosis (Table 3). An increasing number of independent variables was associated with markedly different PTLD-related mortality (PRM); with 0–1, 2, or 3 factors the PRM was 7%, 37%, and 72%, respectively ($P < .0001$; Figure 2*E*). No patient presented with all 4 adverse risk factors.

This model maintained its predictivity when it was repeated in the 2 subgroups of patients with proven and probable diagnosis. Mortality due to PTLD in patients with proven diagnosis was 4%, 42%, and 69% for 0–1, 2, or 3 factors, respectively ($P < .0001$), whereas in patients with probable diagnosis the PTLD mortality was 10%, 28%, and 80% for 0–1, 2, or 3 factors, respectively ($P = .01$). The prognostic models defined in the study substantially retained their discriminant capacity when applied separately in subgroups of children and adults.

Overall Survival

The probability of 3-year OS was 47.3% (95% CI, 38.3%–55.7%). Twenty-seven patients died due to causes unrelated to PTLD (5 due to relapse of the underlying disease, 16 due to infection, 4 due to GVHD, and 2 due to unknown causes). Factors predicting OS by univariate analysis were age >30 years, malignant disease, stem cell source, acute GVHD ≥ grade II, and no RI (Table 2). Four variables remained prognostic for OS after multivariable analysis: (1) age at transplant >30 years, (2) malignant disease, (3) acute GVHD ≥ grade II at PTLD diagnosis, and (4) no RI upon PTLD diagnosis (Table 3 and Figure 2*B*, 2*D*, and 2*F*). Reduction of immunosuppression upon PTLD diagnosis was an independent prognostic factor not only for survival from PTLD, but also for OS.

Response to Therapy With Respect to Blood Viral Load

Initial EBV DNAemia was analyzed before the beginning of the therapy and a week after each dose of rituximab. A decrease of EBV DNAemia improved the PTLD prognosis, whereas an increase of EBV DNAemia after 1 or 2 weeks of therapy was a predictor of poor response and increased the risk of death from PTLD by 3.1- and 3.7-fold in univariate analysis, respectively (Table 4).

DISCUSSION

We succeeded in identifying risk factors for PTLD-related mortality after rituximab-based therapy—namely, age ≥ 30 years, extranodal involvement, presence of acute GVHD ≥ grade II, and no RI upon PTLD diagnosis were associated with increased PTLD-related mortality. Moreover, age ≥ 30 years, malignant disease, extranodal involvement, presence of acute GVHD ≥ grade II, and no RI upon PTLD diagnosis were associated with a lower OS after PTLD diagnosis. Factors related to OS were

Table 4. Hazards and Posttransplant Lymphoproliferative Disorder–Related Mortality According to Viral Load Response

Viral Load Response[a]	Patients, No.	Events	HR (95% CI)	PTLD-Related Mortality (95% CI)	*P* Value
Week 1					
Decreased	84	13	1.00	15.61 (9.47–25.73)	.002
Increased or stable	40	16	3.13 (1.51–6.52)	40.11 (27.45–58.63)	
Week 2					
Decreased	92	12	1.00	13.18 (7.78–22.33)	.006
Increased or stable	18	7	3.70 (1.45–9.41)	38.89 (21.79–69.40)	

Univariate HR by viral load variations at 1 and 2 weeks after beginning of rituximab therapy and cumulative incidence of PTLD-related mortality at 1 and at 2 weeks. Viral load was analyzed at 1 and 2 weeks in patients who were alive at each time point (landmark analysis).

Abbreviations: CI, confidence interval; HR, hazard ratio; PTLD, posttransplant lymphoproliferative disorder.

[a] Logarithmic change in viral load was employed. Change in Epstein-Barr virus DNA load of at least 1 log of magnitude was considered significant.

partially similar to those associated with PTLD. This underlines the adverse impact of PTLD mortality on overall survival after HSCT.

There are substantial differences concerning histologic, immunobiologic, and molecular characteristics between PTLD after HSCT and SOT [21]. In the SOT setting, younger donor age, multiple organ transplant, and high intensity of immunosuppressive therapy represent the risk factors for PTLD development, and the early use of rituximab seems to improve the outcome [22–26]. However, in the HSCT setting, early development, extensive dissemination, aggressive course, and high fatality rate characterized the PTLD. The doubling time for EBV is estimated to be about 2–3 days and with the rapid development and progression of PTLD, there is a need for strategies for early treatment [27]. Such treatment strategies require detailed knowledge about risk factors for PTLD development and prognostic factors for PTLD outcome.

Recognized risk factors reported for developing PLTD following HSCT include unrelated or human leukocyte antigen–mismatched transplant, cord blood transplant, T-cell depletion in vitro or in vivo, use of thymoglobulin or anti-CD3 antibodies, serologic EBV incompatibility between donor and recipient, and splenectomy [28, 29]. The current analysis sought to identify prognostic factors for outcome with respect to recommended rituximab-based therapy for PTLD.

Standard laboratory test results such as lactate dehydrogenase, albumin level, hemoglobin level, or leukocyte count have been correlated with outcome of PLTD in SOT but not applicable to HSCT, as these are influenced by many factors including conditioning, neutropenia, and GVHD [26].

Two-thirds of patients with PTLD after HSCT survived after rituximab-based treatment. Age >30 years, extranodal disease, acute GVHD ≥ grade II at PTLD diagnosis, and lack of RI were related to poor prognosis. Survival from PTLD after rituximab-based therapy was better in children than in adults.

Extranodal PTLD usually corresponds to disseminated type of the disease. As with the disseminated stage III and IV of lymphomas, a higher mortality rate can be expected in these patients compared to those with less advanced disease. Age and extranodal involvement are usually regarded as adverse risk factors for the successful therapy of lymphomas [30, 31].

The beneficial effect of RI in the preemptive therapy of EBV DNAemia has already been shown and is also included in the ECIL recommendations [2, 10, 32]. Reduction of immunosuppressive therapy is recommended for all patients diagnosed with PTLD, whenever possible [2]. In our analysis of patients treated with rituximab, PTLD-associated mortality was significantly higher when immunosuppressive therapy was not reduced.

Acute GVHD ≥ grade II requires intensive immunosuppression, thus limiting the possibilities of RI. Advanced GVHD, both acute and chronic, is also influenced by significant immunologic impairment. It is also important that rituximab given for PTLD treatment may decrease severity of GVHD [33, 34].

Prognostic factors for the outcome of PTLD treated with rituximab were found. In multivariable analyses, we identified age >30 years, acute GVHD ≥ grade II, extranodal disease, and lack of RI upon PTLD diagnosis as the most significant prognostic variables. Consistent with PTLD in SOT recipients, by using these factors, we developed a survival prognostic model [26].

High EBV load, as defined by number of viral DNA copies in blood or serum, might be a new factor, as far as response to therapy in PTLD is concerned. With the development of quantitative analysis of EBV DNAemia, viral load can be regarded as a risk factor not only at diagnosis, but also as an initial response to rituximab-based therapy. An increase of EBV DNAemia after 1–2 weeks of therapy with rituximab was related to poor prognosis. This allows us to propose a definition of early molecular response as a decrease of EBV DNAemia after 1 or 2 weeks of rituximab-based therapy.

In summary, we found among a large multicenter cohort of patients with PTLD after HSCT that the use of rituximab-based therapy in conjunction with RI was associated with significantly improved survival compared with prior reports. This may be related to the use of rituximab-based therapy as first-line therapy, RI, and improved supportive care measures. Survival from PTLD after rituximab-based therapy was 69% in our study, whereas only 15 years ago the mortality rate of this disease exceeded 84%. Multivariable analysis identified variables predictive of outcome: risk-stratified survival from PTLD after rituximab-based therapy ranged from 28% to 93%. Furthermore, we identified strong adverse prognostic factors in PTLD patients after allo-HSCT, treated with rituximab. Clinical and tissue-based studies with prospective evaluation of rituximab-based therapy and prognostic factor analyses in multicenter and multinational collaborations are warranted.

Notes

Author contributions. J. S. had primary responsibility for study design, data analysis and interpretation. Study design: J. S., L. G., H. E., and S. C. Data collection: J. S., L. G., P. L., J. P. D., W. vdV., H. O., R. M., C. H., M. F., K. T., K. K., P. H., S. S., C. N., F. F., S. M., M. A. D., M. M., A. B., A. T., R. dlC., and S. C. Data analysis and interpretation: J. S., L. G., G. T., P. L., R. M., A. vB., J. H., and S. I. Writing: J. S. and L. G. Tables and figures: J. S., G. T., and S. I. Statistical analysis: G. T. and S. I.

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