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Results of therapy with anti-CD20 antibodies in EBV-related post-transplant lymphoproliferative disorder

Wyniki terapii przeciwciałami anty-CD20 u dzieci z EBV-zależnym poprzeszczepowym zespołem limfoproliferacyjnym

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Key words

Epstein-Barr virus, post-transplant lymphoproliferative disorder, hematopoietic stem cell transplantation, rituximab, risk factors, children

Słowa kluczowe

wirus Epsteina-Barr, poprzeszczepowy zespół limfoproliferacyjny, przeszczepienie komórek kriotwóczych, rituximab, czynniki ryzyka, dzieci

Summary

Introduction. Post-transplant lymphoproliferative disorder (PTLD) is a life-threatening malignant disease developing after hematopoietic stem cell transplantation (HSCT).

Aim. The objective of this study is the analysis of the outcome of EBV-related PTLD after a rituximab-based treatment in children after allo-HSCT.

Material and methods. 369 patients aged < 20 years after allo-HSCTs performed between 2005-2011 in 2 pediatric centres have been retrospectively analysed for PTLD, either biopsy-proven or probable disease.

Results. 20 PTLD cases were identified, indicating an overall EBV-PTLD frequency of 5.4%, ranging from 2.3% for matched-family donor, 5.6% in matched unrelated donor, to 16.2% in mismatched unrelated donor recipients. EBV-PTLD occurred at a median of 1.6 months (range, 0.7-8) after HSCT. 120-days survival from PTLD determined by Kaplan-Meier method was 75%. Univariate analysis showed that a poor response of PTLD to rituximab was associated with initial multiorgan involvement, acute/chronic GVHD, and increase of EBV-DNA-emia after one week therapy with rituximab. In multivariate analysis, poor response of PTLD to rituximab was associated with acute/chronic GVHD upon PTLD diagnosis. Immunosuppression tapering by at least 20% of dose was associated with a lower PTLD mortality (8 vs 50%).

Conclusions. Three-fourths of patients with EBV-PTLD survived after rituximab-based treatment. Reduction of immunosuppression was associated with improved outcome while multiorgan disease, acute/chronic GVHD, and increase of EBV-DNA-emia after one week therapy predicted poor outcome.

Streszczenie

Wstęp. Poprzeszczepowy zespół limfoproliferacyjny (PTLD) jest zagrażającym życiu powikłaniem po przeszczepieniu komórek krwiotwórczych (HSCT).

Cel pracy. Celem tej pracy jest analiza wyników terapii EBV-PTLD, opartej na zastosowaniu rituximabu u dzieci po allo-HSCT.

Materiał i metody. Retrospektywnej analizie poddano 369 pacjentów w wieku < 20 lat po allo-HSCT wykonanym w latach 2005-2011 w dwóch ośrodkach pediatrycznych w kierunku PTLD pewnej lub prawdopodobnej.

Wyniki. U 20 pacjentów rozpoznano EBV-PTLD. Częstość EBV-PTLD wyniosła 5,4%, w tym 2,3% dla HSCT od zgodnych dawców rodzinnych, 5,6% dla HSCT od zgodnych dawców niespokrewnionych oraz 16,2% dla HSCT od częściowo zgodnych dawców niespokrewnionych. Rozwój EBV-PTLD nastąpił w czasie 0,7-8 miesięcy (mediana 1,6) po allo-HSCT. Przeżycie 120-dniowe po rozpoznaniu PTLD wyznaczone metodą Kaplana-Meiera wyniosło 75%. W analizie jednowariantowej czynnikami wpływającymi na niepowodzenie terapii były: choroba wielosystemowa, ostra lub przewlekła GVHD oraz wzrost EBV-DNA-emii po pierwszym tygodniu terapii rituximabem. W analizie wielowariantowej jedynym czynnikiem wpływającym na niepowodzenie terapii była obecność ostrej lub przewlekłej choroby GVHD. Redukcja immunosupresji o co najmniej 20% dawki sprzyjała niższej śmiertelności z powodu PTLD (8 vs 50%).

Wnioski. Trzy czwarte pacjentów z EBV-PTLD uzyskało remisję PTLD po zastosowaniu terapii opartej na rituximabie. Redukcja immunosupresji w momencie rozpoznania PTLD była związana z lepszymi wynikami terapii, podczas gdy choroba wielosystemowa, ostra lub przewlekła GVHD oraz wzrost EBV-DNA-emii po pierwszym tygodniu terapii były niekorzystnymi czynnikami prognostycznymi.

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INTRODUCTION

Post-transplant lymphoproliferative disorders (PTLD) is a life-threatening malignant disease developing after transplantation, caused by iatrogenic suppression of T-cell function. The most common form of PTLD after allogeneic hematopoietic stem cell transplantation (allo-HSCT) is related to Epstein-Barr virus disease (EBV-PTLD). Fifteen years ago, EBV-PTLD was reported to have an attributable mortality of 84.6% (1). Since then, therapeutic approaches have been advanced for the prevention and treatment of EBV-PTLD, summarised in the ECIL recommendations (2, 3). These include the administration of rituximab, reduction of immunosuppression (RI) and cellular therapy, such as the use of EBV-specific cytotoxic T-lymphocytes (EBV-CTL). 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-versus-host disease (GVHD). Consequently treatment with rituximab seems to be the most promising method of prevention and therapy of PTLD after HSCT (4-6).

Within Infectious Diseases Working Group (IDWP) of European Group for Blood and Marrow Transplantation (EBMT) a multicenter, retrospective analysis of 4466 allogeneic HSCTs performed in 19 pediatric and adult EBMT transplant centers in Europe was conducted (7). Finally 144 pediatric and adult patients who had been treated with rituximab for PTLD after allo-HSCT were analyzed, and the factors that might be associated with survival were taken into account. In final report, children and adults were pooled together as one group (7). However, it has been suggested that these two age populations were different in outcome. To address this, a retrospective analysis was undertaken to find out specific aspects of results of therapy in children.

AIM

Thus, the objective of this study was the analysis of the outcome of EBV-related PTLD after a rituximab-based treatment in children after allo-HSCT performed in two Polish pediatric transplant centrs.

MATERIAL AND METHODS

We retrospectively analyzed 369 allogeneic HSCTs performed in 2 pediatric transplant centers between 2005 and 2011 for PTLD. The following inclusion criteria were used for the study: proven or probable PTLD diagnosis and rituximab treatment administered either alone or combined with other therapeutic approaches. The study was approved by the Institutional Review Board of the Medical College, Nicolaus Copernicus University, Bydgoszcz.

The diagnosis of EBV-related 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 endorgan disease accompanied by a high EBV-DNA blood load, in the absence of other etiologic factors or established diseases (2, 4). EBV-DNA-emia was measured by quantitative or qualitative PCR in peripheral blood. Repeated 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 one 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, i.e. $\leq$ 0.2 mg/kg in patients $<$ 40 kg of body weight or $\leq$ 10 mg/day in patients with $>$ 40 kg of body weight (8). Response to given treatment was assesed on clinical level as complete remission, partial response, stable or progressive disease, according to standard definition (9). The virologic response was also assessed based on EBV-DNA-emia 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

The probability of survival from PTLD (PS) were estimated by the Kaplan-Meier method and univariate comparisons were performed using the log-rank test (10, 11). The time from the date of PTLD diagnosis to the date of death due to PTLD, death due to other causes or to the date of the latest follow-up was considered. Uni- and multivariate analysis for survival from PTLD was performed by using the Cox regression (12). In order to analyse the influence of the viral load after 1 and 2 weeks on survival from PTLD, a landmark analysis was performed using data on only those patients who survived up to 1 and 2 weeks after PTLD diagnosis. A P-value below 0.05 was regarded as statistically significant.

RESULTS

Baseline characteristics

PTLD had been diagnosed in 20 cases, at a median of 1.6 months after HSCT (range: 0.7-8). The median age at transplant of 20 patients with PTLD was 12 years (range: 0.7-19). Three patients (15%) developed PTLD in the first month, whereas only 1 patient was diagnosed for PTLD later than 4 months after HSCT. PTLD was proven by biopsy in 6 cases and the remaining 14 cases were considered probable disease. The overall EBV-PTLD frequency was 5.4%, and ranged from 2.3% for matched-family donor, 5.6% in matched unrelated donor, to 16.2% in mismatched unrelated donor recipients (tab. 1). No PTLD was diagnosed after cord blood or haploidentical transplantation. Overall, the frequency of PTLD after unrelated donor allo-HSCT was 7.6% (OR = 3.5, 95% CI = 0.7-21, P = 0.137). Clinically, 10 patients (50%) had extranodal PTLD involve-

ment, including 2 (10%) with multiorgan (i.e. more than 2 systems) involvement.

Treatment

Patients were treated with a median of 3 doses of rituximab (range, 1-9), administered at dosage of 375 mg/m^2 at weekly intervals. Tapering off of immunosuppression was done in 11 cases. Chemotherapy was administered as a second line therapy because of a partial response, or stable or progressive disease to 3 patients. Additional therapies included surgery in 1 case, and antiviral agents (mainly cidofovir) in 9 patients. The use of antivirals had no impact on survival from PTLD.

Survival after PTLD

Fifteen (75%) patients survived after rituximab-based therapy, and 5 died due to PTLD. Only those who achieved a complete remission survived from PTLD (fig. 1). PTLD resolved in 11 of 12 (92.5%) patients who received both rituximab and RI, and in 4 of 8 patients (50%) in whom immunosuppression tapering was not done (P = 0.038). RI reduced the risk of death due to PTLD (OR = 7, P = 0.078). No differences in PTLD mortality was found between proven vs probable PTLD categories.

Factors predicting poor outcome of PTLD therapy by univariate analysis were: acute/chronic GVHD, multiorgan involvement, and increase of EBV-DNA-emia after 1 week of therapy (tab. 2 and 3).

Response to therapy with respect to blood viral load. Initial EBV-DNA-emia was analysed before the beginning of the therapy and a week after each dose of rituximab. A decrease of EBV-DNA-emia improved the PTLD prognosis, while an increase of EBV-DNA-emia after one week of therapy was a predictor of poor response and increased the risk of death from PTLD by 16-fold in univariate analysis (tab. 3).

Multivariate analysis for PTLD-reated mortality was performed by using the significant prognostic factors identified in univariate analysis. Only one variable remained prognostic significance for PTLD mortality: acute/chronic GVHD at PTLD diagnosis (tab. 4).

Table 2. Survival from PTLD.

Characteristics	Patients	Events	100-days PTLD survival (%)	P
Age, years				
< 10	7	0	100	0.072
≥ 10	13	5	61	
PTLD onset				
< 60 days after HSCT	5	3	40	0.056
> 60 days after HSCT	15	2	87	
Acute GVHD ≥ II at PTLD diagnosis				
No	18	3	83	< 0.001
Yes	2	2	0	
Acute GVHD ≥ II or cGVHD at PTLD diagnosis				
No	17	2	88	< 0.001
Yes	3	3	0	
Multiorgan involvement				
No	18	3	83	0.004
Yes	2	2	0	
Initial EBV-DNA-emia ≥ 10^5 gc/mL				
No	12	0	100	< 0.001
Yes	8	5	37.5	
Reduction or cessation of IS therapy				
No	8	4	50	0.038
Yes	12	1	92.5	

PTLD – Post-Transplant Lymphoproliferative Disorder, HSCT – Hematopoietic Stem Cell Transplantation, GVHD – Graft-versus-Host-Disease, EBV – Epstein-Barr virus, IS – Immunosuppressive, CI – confidence interval

DISCUSSION

PTLD presenting after HSCT is charcterized by an early development, extensive dissemination, aggressive course and high fatality rate. 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 (13). Such treatment strategies require detailed knowledge about risk factors for PTLD development and prognostic factors for PTLD outcome. Recognized major risk factors reported for developing PLTD following HSCT include: unrelated or HLA-mismatched transplant, T-cell depletion *in vitro* or *in vivo*, and serologic EBV incompatibility between donor and recipient (14, 15). The current analysis sought to identify prognostic factors for outcome with respect to recommended rituximab-based therapy for PTLD.

We succeeded in identifying risk factors for PTLD-related mortality after rituximab-based therapy: extran-

Table 1. Frequency of PTLD and odds ratio related to type of transplant.

Type of donor	Number of allo-HSCT	Number of PTLD	Frequency (%)	Odds Ratio (95% CI)	P
MFD	86	2	2.3	1.00	–
MMFD/haplo	29	0	0.0	ND	ND
MUD	213	12	5.6	2.7 (0.6-17)	0.245
MMUD	37	6	16.2	6.8 (1.1-53)	0.023
Total	369	20	5.4	2.5 (0.6-15)	0.277

PTLD – Post-Transplant Lymphoproliferative Disorder, MFD – matched family donor, MMFD – mismatched family donor, MUD – matched unrelated donor, MMUD – mismatched unrelated donor, CI – confidence interval, ND – not determined

Table 3. Survival from PTLD according to viral load response.

One-Week Viral Load Response*	Patients	Events	OR (95% CI)	100-days PTLD survival (%)	P
Decreased	17	2	1.00	88	0.007
Increased or stable	1	1	16 (1.1-263)	0	–

Univariate HR by viral load variations at 1 week after beginning of rituximab therapy and survival from PTLD at 1 week. Viral load was analysed at 1 week in patients who were alive at this time-point (land-mark analysis).
PTLD – Post-Transplant Lymphoproliferative Disorder, OR – Odds Ratio, CI – confidence interval
*Logarithmic change in viral load was employeed. Change in EBV-DNA load of at least 1 log of magnitude was considered significant.

Table 4. Uni- and multivariate analyses of prognostic factors.

Prognostic Factor	Univariate analysis			Multivariate analysis		
	Odds Ratio	95% CI	P	Odds Ratio	95% CI	P
PTLD onset < 60 days after HSCT	4.8	0.8-28	0.087	–	–	ns
aGVHD ≥ II or cGVHD at PTLD diagnosis	11.8	1.9-72	0.008	11.8	1.9-72	0.008
Multiorgan involvement	10.1	1.4-72	0.022	3.2	0.4-29	0.309
Immunosuppression reduction upon PTLD diagnosis	0.2	0.02-1.2	0.078	–	–	ns

PTLD – Post-Transplant Lymphoproliferative Disorder, GVHD – Graft-versus-Host-Disease, CI – confidence interval, ns – not significant

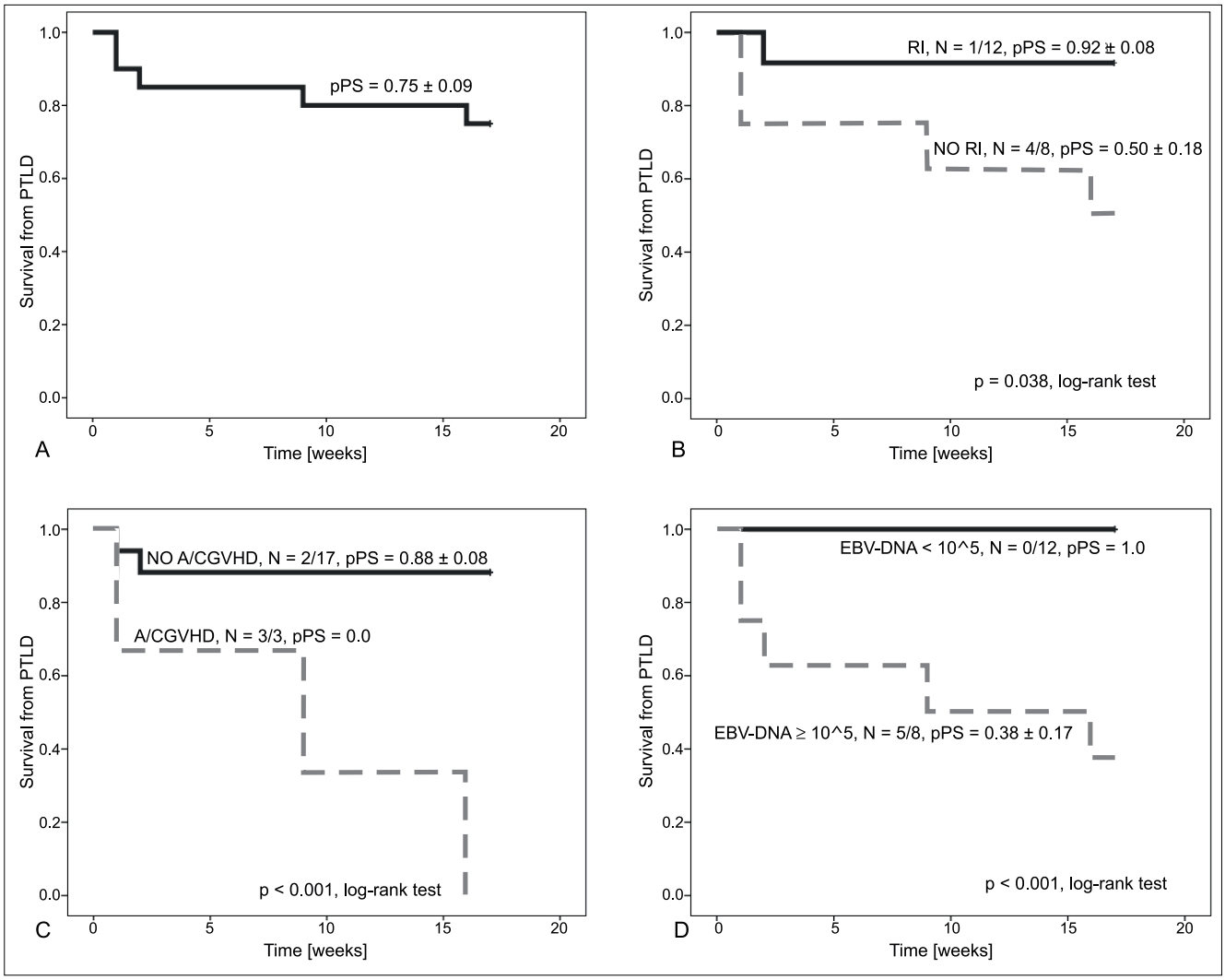

Fig. 1. Survival from PTLD. (A) All patients treated with rituximab. (B) Patients with reduction of immunosuppression therapy upon PTLD diagnosis (RI) compared with patients with no RI. (C) Patients with acute ≥ II or chronic GVHD at PTLD diagnosis. (D) Patients with EBV-DNA-emia ≥ 10^5 gc/mL at PTLD diagnosis.

odal involvement, presence of aGVHD ≥ grade II and and increase of EBV-DNA-emia after 1 week of therapy were associated with increased PTLD-related mortality. 75% of patients with PTLD after HSCT survived after rituximab-based treatment.

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 (16, 17).

The beneficial effect of RI in the pre-emptive therapy of EBV-DNA-emia has already been shown and is also included in the ECIL recommendations (2, 8, 18). 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 (19, 20).

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-DNA-emia, 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-DNA-emia 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-DNA-emia after one- or two weeks of rituximab-based therapy.

CONCLUSIONS

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, reduction of immunosuppression and improved supportive care measures. Survival from PTLD after rituximab-based therapy was 75% in our study, while only 15 years ago the mortality rate of this disease exceeded 84%. 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.

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