Retrospective evaluation of serum Epstein Barr virus DNA levels in 406 allogeneic stem cell transplant patients

Eeva Juvonen, Sanna Aalto, Jussi Tarkkanen, Liisa Volin, Klaus Hedman, Tapani Ruutu

From the Department of Medicine, Helsinki University Central Hospital, Helsinki Finland (EJ, LV, TR); Department of Virology, Haartman Institute, University of Helsinki and Helsinki University Central Hospital (SA, KH); Department of Pathology, Haartman Institute, University of Helsinki and Helsinki University Central Hospital (JT).

Funding: this study was financially supported by HUCH Research Fund (EVO), the Blood Disease Research Foundation, and the Finnish Cancer Research Foundation.

Manuscript received September 10, 2006.
Manuscript accepted March 16, 2007.

Correspondence:
Eeva Juvonen, Helsinki University Central Hospital, Department of Medicine, P.O.Box 340, 00029 HUS, Helsinki, Finland.
E-mail: eeva.juvonen@hus.fi

ABSTRACT

Background and Objectives

An HLA-mismatched donor and a T-cell-depleted graft are known risk factors for Epstein-Barr virus (EBV) reactivation after stem cell transplantation. We studied the frequency and outcome of serum EBV DNA levels in patients transplanted with an unmanipulated graft from an HLA-identical donor.

Design and Methods

Overall, 5479 serial serum samples from 406 consecutive allogeneic stem cell transplant recipients were analyzed retrospectively for EBV DNA with quantitative polymerase chain reaction (PCR).

Results

EBV DNA was found in the serum of 56 of the 406 patients (14%). EBV positivity was seen in 9% of the recipients of a graft from a sibling donor and in 29% of those with an unrelated donor. EBV-PCR positivity resolved without specific treatment in a third of the cases, in another third the copy number increased progressively or was high in the last serum sample, and in the remaining third the copy numbers were low in all positive sera including the last sample. In multivariate analysis antithymocyte globulin given for any reason and grade III–IV acute graft-versus-host disease were the only statistically significant risk factors for EBV reactivation. Only 8/56 patients with EBV-DNA positivity were alive at the time of the present analysis. The outcome of EBV-PCR positivity could not be predicted by the copy number or the timing of the first positive sample.

Interpretation and Conclusions

EBV reactivation was a common phenomenon in allogenic stem cell transplant recipients. In many patients the viremia resolved without EBV-directed treatment. Severe acute graft-versus-host disease and antithymocyte globulin given for any reason were risk factors for EBV viremia.

Key words: EBV, reactivation, viremia, predictive factors, PTLD.

Haematologica 2007; 92:819-825

Epstein Barr virus (EBV) establishes a latent infection in the majority of healthy adults. The cellular immune response of T-lymphocytes is crucial for controlling latent EBV-infected B cells.[1,2] T-lymphocyte dysfunction caused by immunosuppressive treatment may allow uncontrolled proliferation of B cells hosting EBV.[3] Following hematopoietic stem cell transplantation, EBV-associated post-transplant lymphoproliferative disorder (PTLD) is a serious complication that occurs usually within the first year after transplantation.[4-6] The overall incidence of PTLD after stem cell transplantation has been reported to be < 1%, but HLA disparity, T-cell depletion of the graft, severe acute graft-versus-host disease (GVHD), and prophylactic or therapeutic antilymphocyte globulin (ATG) may increase the incidence to 15-25%.[3-8] The diagnosis of PTLD after allogeneic stem cell transplantation is difficult because of the heterogeneity of clinical manifestations, which vary from an infectious mononucleosis-like illness with lymphadenopathy to a febrile illness with leukopenia with or without lymphadenopathy.[5,9] Almost any organ may be affected. PTLD has been described after conventional conditioning[7,10] or reduced intensity conditioning[9-11] and also after cord blood stem cell transplantations[12,13] and, in rare cases, after autologous stem cell transplantation.[14,15] The prognosis of PTLD was very poor before the era of rituximab with the mortality rate being 70-90%.[7,8,16,17] Serial polymerase chain reaction (PCR)-based monitoring of EBV DNA levels from mononuclear cells or plasma of transplant patients at risk now enables early detection of viral reactivation and EBV DNA load-guided pre-emptive or prompt treatment after stem cell or organ transplantation.[18-22]

In the present study a large number of serial serum samples from 406 consecutive allogeneic stem cell transplant recipients were analyzed retrospectively for EBV DNA with quantitative PCR to assess the frequency and outcome of, and risk factors for, EBV reactivation in patients transplanted with an unmanipulated graft from an HLA-matched donor. The majority of the patients in this series were transplanted before the molecular diagnostic tools and present treatment options had become available.

Design and Methods

Patients

Altogether 409 adult patients were treated with hematopoietic stem cell transplantation for hematologic malignancy or severe aplastic anemia during the years 1988–1999 at the Helsinki University Central Hospital (HUCH). Four hundred and six patients were included in the present study (Table 1). Three patients were excluded due to lack of data on serum samples. Of the included patients 225 were at low risk (chronic myeloid leukemia in first chronic phase, acute myeloid leukemia, acute myeloid leukemia in first complete remission, severe

Table 1. The clinical and transplantation data of 406 patients with or without EBV-qPCR positivity in any sample.

	Total	*EBV-qPCR*				
		positive		*negative*		
	n = 406	*n=56*		*n=350*		*p*
Diagnosis						
AML	117	15	27%	102	29%	
CML	111	18	32%	93	27%	
ALL	63	6	11%	57	16%	
MDS	55	11	19%	44	12%	
MM	33	5	9%	28	8%	
CLL	4	1	2%	3	1%	
SAA	8	0		8	3%	
other	15	0		15	4%	
Risk group						
low risk	225	28	50%	197	56%	
high risk	181	28	50%	153	44%	*p*=0.35
Donor						
sibling	313	29	52%	284	81%	
unrelated	93	27	48%	66	19%	*p*<0.0001
Conditioning						
TBI + cyclophosph.	326	45	80%	281	80%	
cyclophosphamide	8	0		8	2%	
busulfan	67	9	16%	58	17%	
RIC	5	2	4%	3	1%	
TBI						
yes	326	45	80%	281	80%	
no	80	11	20%	69	20%	*p*=1.00
Graft						
bone marrow	374	53	95%	321	92%	
blood	32	3	5%	29	8%	*p*=0.69
Acute GVHD						
grade 0	240	21	38%	219	63%	
grade I	51	5	9%	46	13%	
grade II	60	3	5%	57	16%	
grade III	34	15	27%	19	5%	
grade IV	21	12	21%	9	3%	
Acute GVHD						
grade 0-II	351	29	52%	322	92%	
grade III-IV	55	27	48%	28	8%	*p*<0.0001
Methylprednisolone (maximal dose)						
<2 mg/kg/day	228	16	28%	212	60%	
≥2 mg/kg/day	178	38	68%	140	40%	*p*<0.0001
ATG (any reason)						
no	272	12	21%	260	74%	
yes	134	44	79%	90	26%	*p*<0.0001
Alive						
no	220	48	86%	172	49%	
yes	186	8	14%	178	51%	*p*<0.0001

AML: acute myeloid leukemia; ALL: acute lymphatic leukemia; CML: chronic myeloid leukemia; MM: multiple myeloma; CLL chronic lymphatic leukemia: SAA: severe aplastic anemia; TBI: total body irradiation; RIC: reduced intensity conditioning; GVHD: graft-versus-host disease: ATG: anti-thymocyte globulin. Low risk: CML in first chronic phase; AML and ALL in first complete remission; SAA: High risk: all others.

aplastic anemia) and 181 (all others) at high risk. Three hundred and ninety-three patients received myeloablative conditioning containing cyclophosphamide 60 mg/kg of body weight on 2 successive days together with either total body irradiation (TBI, 326 patients) or busulfan (67 patients). TBI was given fractionated in six 2-Gy doses over 5 days. Busulfan was given at a dose of 1 mg/kg four times per day on 4 consecutive days followed by cyclophosphamide for 2 days. Eight patients with severe aplastic anemia (SAA) were given only cyclophosphamide at a dose of 50 mg/kg/day on 4 consecutive days. Five patients were treated with varying reduced intensity conditioning regimens. Of the donors 313 were siblings and 93 were unrelated. As part of the conditioning antilymphocyte globulin (ATG, Atgam™, Upjohn, Kalamazoo, USA 55 patients; Thymoglobuline™, Merieux/Sangstat, Lyon, France 32 patients) was given to all except six patients with an unrelated donor. The graft source was bone marrow in 374 patients and blood stem cells in 32 patients. With the exception of two sibling donors, the graft was HLA-A, -B, and DR identical typed by the standard serologic tests or PCR-based methods in use at the time. The grafts were unmanipulated apart from red cell removal from ABO incompatible bone marrow grafts.

The prophylaxis against GVHD consisted of cyclosporine A, a short course of methotrexate and, in addition, in 271 patients methylprednisolone as described previously.[23] Prophylactic methylprednisolone was given to 191 of the 313 patients with a sibling donor and to 80 of the 93 patients with an unrelated donor. High-dose methylprednisolone (>2 mg/kg/day) was the first-line treatment of acute GVHD of any grade, and ATG was the second-line treatment in the case of refractoriness to steroid treatment. Altogether 166 of the 406 patients (41%) suffered from grade I-IV acute GVHD[24,25] (Table 1). In 54 of the patients (37 with a sibling and 17 with an unrelated donor) the GVHD was grade III-IV. Sixty-five patients, 43 with a sibling and 22 with an unrelated donor, received ATG as second-line treatment for steroid-resistant acute GVHD.

At the time of the analysis 186 (46%) patients were alive with a median follow-up of 8 years (range, 1–16 years). One patient was lost from follow-up. The median survival of all the 406 patients was 3.3 years (range, 4 days–16 years). Two hundred and twenty patients had died between 4 days and 13 years (median, 210 days) after transplantation. The cause of death was relapse in 93 cases, transplant-related in 117 patients, and other in nine cases.

Quantitative EBV-PCR analyses

According to a predefined schedule, 5479 serum samples from 406 patients were collected and stored in a frozen state. The median number of samples per patient was 14 (range 1–26). The samples were collected over a period of 52 months, mainly in the first 3 months post-transplantation. One serum sample was stored before transplantation. During the post-transplant period of hospitalization, samples were collected once a week. After discharge from the hospital a serum sample was stored at every outpatient visit; in practice two to four serum samples per month up to 6 months after transplantation and once every 1–2 months thereafter. In the case of later hospitalization at HUCH, serum samples were stored once a week. Every patient with at least one post-transplant sample was included in the analyses.

EBV DNA in the serum samples was measured by the quantitative real-time polymerase chain reaction (qPCR) as described previously.[26] Patients with at least one positive serum sample were regarded as EBV-qPCR-positive and those without any positive sera as EBV-qPCR-negative. The threshold value for EBV-qPCR positivity was 500 genome equivalents/mL (geq/mL), with values of 50,000 geq/mL or higher being regarded as high.

Statistics

The χ^2 test with a continuity correction was used to compare the proportions between the groups. The differences in EBV DNA copy numbers between the groups were tested using the Mann-Whitney test. A multivariate Cox's proportional hazards model was used to simultaneously evaluate the time-dependent and time-independent risk factors for EBV-qPCR positivity. The time-dependent risk factors included in the model were the occurrence of acute GVHD, the use of high dose methylprednisolone, and the use of ATG. The time-independent risk factors included in the model were the type of donor, disease risk group based on the hematologic diagnosis, TBI-containing conditioning, the use of prophylactic methylprednisolone and, the type of the graft. The relative risk estimates with their 95% confidence intervals and respective *p*-values were reported from this analysis. Survival was analyzed with a multivariate Cox´s proportional hazards regression model. The occurrence of acute GVHD, the use of high dose methylprednisolone, the use of ATG, the type of donor, the hematologic risk group of the disease, the use of prophylactic methylprednisolone, and the type of the graft were included in the analysis. In addition, the overall survival of the groups of patients positive or not for EBV DNA and the groups positive before or after day 100 post-transplant were analyzed by Kaplan-Meier curves and compared by the log-rank-test. All statistics were performed with SPSS software for Windows.

Results

Fifty-six (14 %) of the 406 patients had at least one EBV-qPCR-positive serum sample after allogeneic stem cell transplantation (Table 2). The median number of positive sera per patient was two (range, 1–7). The first positive sample was collected on the median of day 63 post-transplant (range, from day 10 pre-transplant to day 537 post-transplant). In 12 of the 56 patients (21%) the EBV-qPCR

Table 2. The time of the first EBV-qPCR positive serum sample and the first and highest EBV-DNA copy numbers (median, range) in 56 allogeneic stem cell transplantation recipients, and the outcome of EBV-qPCR positivity.

EBV-qPCR posititivity	Number of patients	First positive sample: Day post-transplant Median	Day post-transplant Range	EBV-DNA copies/mL of serum Median	EBV-DNA copies/mL of serum Range	Highest copy number/mL of serum Median	Highest copy number/mL of serum Range	Survived
All patients	56	63	- 10–537	4500	650–660×10^6	9900	800–1090×10^6	8
Converted to negative	19	63	- 10–273	4000	700–36200	6000	1380–36200[1]	7
Increased progressively or high in the last serum	21	66	24–102	12670	650–660×10^6	1950000	50100–1090×10^6	0
Low in the last serum	16	60	12–537	3200	800–15000	4700	800–17700	1

1.In four patients >10,000 copies/mL.

Table 3. Risk factors for serum EBV DNAemia after allogeneic stem cell transplantation (multivariate Cox's proportional hazards model).

Variable	p	Relative Risk	95% CI
Risk of disease[1]	0.87	1.04	0.60–1.81
Donor (sibling, unrelated)	0.93	0.96	0.41–2.26
Graft (BM, PBSC)[2]	0.57	1.42	0.43–4.68
TBI containing condition	0.57	0.79	0.36–1.75
High dose steroid (≥2 mg/kg/day)	0.55	0.73	0.25–2.08
GVHD grade III–IV	0.015	1.70	1.11–2.62
ATG	< 0.001	5.78	2.47–13.50

[1]Low risk: chronic myeloid leukemia in first chronic phase, acute lymphatic leukemia and acute myeloid leukemia in first complete remission, severe aplastic anemia. High risk: all others. [2]BM bone marrow; PBSC peripheral blood stem cells.

positivity first appeared at least 100 days post-transplant. The hematologic diagnosis had no impact on the risk of EBV DNAemia with the exception that none of the eight patients transplanted for severe aplastic anemia was found to be EBV-qPCR-positive at any time (Table 1).

Timing and outcome of EBV reactivation

In 19 patients EBV-qPCR positivity converted to negativity (*converted to negative* group), in 21 patients the EBV copy numbers increased progressively or the number of copies was high (>50,000 geq/mL) in the last available serum sample (*high in the last serum* group), and in 16 patients the EBV copy numbers remained <50 000 geq/mL in all positive samples including the last one (*low in the last serum* group) (Table 2).

The median time from transplantation to the first EBV DNA-positive serum sample was similar in the three groups of patients as shown in Table 2. In the whole group of EBV-positive patients the median number of EBV DNA copies/mL in the first positive sample was 4500 (range, 650–660×10^6), and the median of the highest copy number was 9900 (range, 800–1090×10^6). The median of the copy numbers in the first positive serum sample was significantly higher in the *high in the last serum* group than in the *low in the last serum* and *converted to negative* groups (p=0.003 and p=0.016, respectively). However, the range of EBV DNA copies in the first positive samples was wide in all groups, and in all groups the lowest positive result was just above 500 geq/mL. Table 2 also shows the medians and ranges of the highest copy numbers in the three subgroups.

Of the 19 patients in the *converted to negative* group one patient with altogether four positive serum samples experienced EBV DNAemia twice with copy numbers of 36,200 geq/mL on day 39 and of 16,500 geq/mL on day 168 post-transplant. One patient with three positive samples had an EBV-DNA level of 21,500 geq/mL on day 10 post-transplant. Of the remaining 17 patients whose EBV positivity resolved, two patients showed DNA copy numbers slightly above 10,000 geq/mL in one sample each, while in 15 patients the copy numbers were < 10,000 geq/mL in all positive sera. None of the patients in the *converted to negative* group received any treatment for EBV.

Risk factors for EBV DNAemia

In the univariate analyses, statistically significant risk factors for EBV-qPCR positivity were unrelated donor, acute GVHD, methylprednisolone at a maximal daily dose of ≥2 mg/ kg, and ATG given for any reason. The hematologic diagnosis or risk group, conditioning, graft, or the use of prophylactic methylprednisolone had no correlation in the univariate analysis with the occurrence of EBV-DNA positivity. In the multivariate analysis only ATG and grade III – IV acute GVHD remained as statistically significant independent risk factors (Table 3). Of the 313 patients transplanted from a sibling donor 29 (9 %) had at least one EBV-qPCR-positive serum sample.

Of these 29 cases of EBV DNAemia, 18 (62%) occurred in patients with grade III-IV acute GVHD. Grade III-IV acute GVHD complicated 37 transplants (12%) from a sibling donor. Eighteen of these 37 patients (49%), but only 11 of the 276 patients (4%) with grade 0–II acute GVHD, showed EBV-DNA positivity (p<0.0001).

Of the 93 patients transplanted from an unrelated donor, 27 (29%) had at least one EBV-qPCR-positive serum sam-

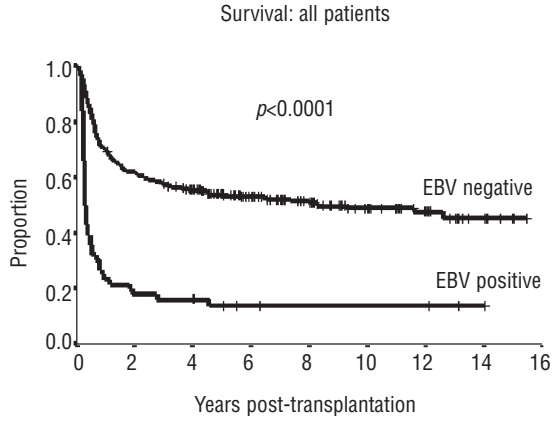

Figure 1. The survival of all patients.

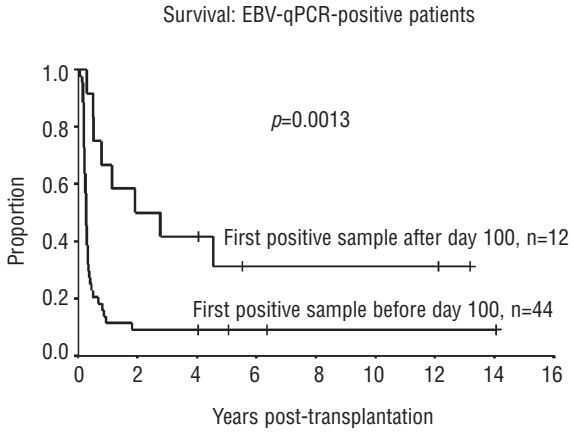

Figure 2. The survival of EBV-qPCR-positive patients according to the time of the first positive serum sample.

ple. Seventeen of the 93 patients (18%) suffered from grade III-IV acute GVHD and eight of them (47%) showed EBV-qPCR positivity. For comparison, among the 76 patients with grade 0 – II acute GVHD, 19 (25%) had at least one EBV-DNA-positive serum sample. The difference did not reach statistical significance (p=0.13).

Effect of EBV-qPCR positivity on survival

Eight of the patients with EBV-qPCR-positive serum samples were alive at the time of the present analysis, seven in the *converted to negative* group and one in the *low in the last serum* group. The cause of death in the 48 patients with EBV-qPCR positivity who died was relapse in eight cases and transplant-related in 40 cases. One of the eight relapsed patients from the *low in the last serum* group also had donor lymphocyte infusion-induced GVHD and PTLD 8 months after the last positive serum sample.

The survival of the EBV-qPCR-positive patients was inferior to that of the EBV-qPCR-negative patients (Figure 2). In multivariate analysis independent risk factors for inferior survival were high-risk hematologic disease (p<0.0001, CI 1.77–2.06), EBV-qPCR positivity (p<0.0001, CI 1.68–3.49), acute GVHD grade II–IV (p=0.01, CI 1.11–2.36), and acute GVHD grade III–IV (p=0.002, CI 1.30–3.28). The use of ATG did not reach statistical significance (p=0.053, CI 1.00–1.85). Among the patients with EBV-qPCR positive samples, the cumulative survival was significantly better in those with the first positive serum sample later than 100 days post-transplantation (Figure 3).

Post-transplant lymphoproliferative disorder

PTLD was confirmed either *post-mortem* or during life in 22 of the 406 (5.2%) patients. Nineteen of these 22 patients had had one or more EBV-qPCR-positive sera in the present analysis. In addition, PTLD was diagnosed in three patients whose serum samples had always been negative in the present analysis. They all had steroid refractory GVHD after donor lymphocyte infusions given for relapse. Only two of the patients in this retrospective study had received treatment for PTLD, in both cases with no effect. An autopsy was performed on 31 of the 48 patients with positive EBV-qPCR who died. In the *high in the last serum* group, PTLD was confirmed in 14/21 patients. In one patient the re-evaluation did not confirm PTLD, in three patients autopsy was not performed, and in three patients no autopsy material was available for re-evaluation. Of the *low in the last serum* group, PTLD was confirmed in five of nine autopsied patients, while in the *converted to negative* group none of the four patients autopsied showed any signs of PTLD.

Discussion

The present study showed, in a large number of patients, that EBV DNAemia in serum is a common finding after allogeneic stem cell transplantation also in patients transplanted with an unmanipulated graft from an HLA-identical donor. Overall, 14% of the patients in this series showed EBV DNA in their serum. The rate of EBV-DNA positivity after transplantation from a sibling donor was about 10% whereas that after transplantation from an unrelated donor was 20-30% depending on the intensity of the immunosuppressive treatment, as shown in our previous study.[5] EBV-qPCR positivity resolved without any EBV-directed treatment in one third of the present cases, while in another third the EBV DNA copy number increased progressively or was high in the last serum sample available. In the last third the copy numbers were low in all positive sera including the last sample. Eight of the 56 patients with EBV positivity were alive at the time of the present analysis. The EBV copy numbers or the collection time of the first positive serum

sample did not predict the outcome of EBV viremia.

Today a prospective *non-intervention* study of EBV DNAemia would not be possible. Currently in many transplant centers the policy for patients with a rising EBV DNA load is to give rituximab infusions or donor lymphocytes.[27,28] The systematic storage of one pre-transplant sample of serum and several post-transplant samples taken at frequent intervals from all consecutive patients allowed us to study retrospectively the incidence, timing, and course of EBV DNAemia in the absence of EBV-specific treatment. There were some differences in the sampling intervals between the patients, although these differences were compensated for by the large number of patients together with the abundant number of serum samples collected.

None of the patients in our series had been transplanted with an HLA-mismatched or T-cell-depleted graft, or had received alemtuzumab-containing conditioning which have all been shown to increase the risk of EBV viremia.[7,11,19,29] Severe acute GVHD and the use of intensive immunosuppression, especially ATG, increased the risk of EBV viremia in the present study. The overall incidence of EBV-DNAemia was in accordance with results of previous studies. About 10% of recipients of a graft from an HLA-identical sibling donor, and from 20 up to 50% of recipients of a graft from an unrelated donor have been reported to have at least one EBV-qPCR-positive serum after transplantation. In the study by van Esser *et al.*[29] including 152 consecutive patients, EBV reactivation was seen in 28% of 67 patients transplanted with an unmanipulated graft (the numbers of sibling and unrelated donors were approximately equal) and in 54% of 85 patients transplanted with a partially T-cell-depleted graft. Independent risk factors for EBV reactivation in van Esser's study were partial T-cell-depletion together with administration of ATG, and a $CD34^+$ cell count of greater than 1.35×10^6/kg in the graft. Neither acute GVHD nor T-cell depletion alone was associated with EBV reactivation. PTLD was observed only after T-cell-depleted stem cell transplants. In the study by Torre-Cisneros *et al.*,[30] which included 100 consecutive bone marrow transplant patients, the only independent risk factor for EBV reactivation was the use of a $CD4^+$ lymphocyte-depleted graft. Of patients transplanted with a $CD4^+$ lymphocyte depleted graft, 26/40 (65%) showed EBV reactivation whereas among those transplanted with an unmanipulated bone marrow graft the corresponding figure was 4/60 (7%). In the study by Cesaro *et al.*,[28] with 79 pediatric allogeneic transplant patients, 53% developed EBV reactivation at a median of 45 days post-transplantation. Of the patients in Cesaro's study, 72% were transplanted from an unrelated donor and, in addition, in 22% of the cases the graft was HLA-mismatched. In the multivariate analysis ATG was the only factor associated with EBV reactivation.

The majority of the patients in our series had been transplanted before any reliable diagnostic methods or effective treatment for EBV viremia or PTLD were available. Therefore, with few exceptions, no EBV-targeted treatment was given. The prevalence of PTLD cannot be verified in this retrospective analysis; only half of the patients underwent a *post mortem* examination. For some of the patients no samples remain to be reanalyzed. Because the clinical entity of PTLD was not known at the time the remaining samples were collected, these were not obtained uniformly and cannot be used for studies on this condition. However, in a third of the EBV-qPCR-positive patients the progression of EBV viremia to PTLD could be confirmed retrospectively. There were no differences in the median number of the EBV-DNA copies in the first positive serum sample or in the time of the onset of EBV viremia between the PTLD patients and the other EBV-positive patients. Two patients became EBV-negative without any EBV-targeted intervention, despite relatively high copy numbers (from 20,000 to 35,000 copies/mL).

The current treatment options for EBV-induced PTLD are rituximab, to which the response rate ranges from 60–100%, reduction of immunosuppression, donor lymphocyte infusions, and local treatments such as irradiation. It is most important in diagnosing PTLD to recognize the possibility of EBV reactivation in highly immunosuppressed patients as well as to confirm or exclude the suspicion by EBV-qPCR. The clinical features of PTLD are, however, diverse including adenopathy and unexplained fever; alternatively, the disease may manifest as an aggressive disseminated lymphoid proliferation.[3,5,8] The diagnostic work-up is further complicated by simultaneous problems such as severe GVHD and other infections. In addition to conventional risk groups i.e. those transplanted with a mismatched or T-cell depleted graft, patients with steroid-refractory acute GVHD receiving second line immunosuppressive treatment should be regarded as being at high risk of PTLD. As indicated in the present and previous studies,[27-30] surveillance of EBV DNAemia should be considered during profound immunosuppression in selected high-risk patients. Reduction of immunosuppression, if possible, and pre-emptive or prompt treatment with rituximab are indicated for patients with high or progressively rising EBV-DNA copy numbers.

Authors' Contributions

EJ: head investigator; SA: analysis; KH: EBV-qPCR analysis; LV: transplant physician and clinical expert; JT: pathologist, post mortem analysis;TR: supervisor.

Conflict of Interest

The authors reported no potential conflicts of interest.

References

1. Munz C, Bickham KL, Subklewe M, Tsang ML, Chahroudi A, Kurilla MG, et al. Human CD4(+) T lymphocytes consistently respond to the latent Epstein-Barr virus nuclear antigen EBNA1. J Exp Med 2000; 191:1649-60.
2. Callan MF. The immune response to Epstein-Barr virus. Microbes Infect 2004;6:937-45.
3. Faye A, Vilmer E. Post-transplant lymphoproliferative disorder in children: incidence, prognosis, and treatment options. Paediatr Drugs 2005;7:55-65.
4. Sundin M, Le Blanc K, Ringden O, Barkholt L, Omazic B, Lergin C, et al. The role of HLA mismatch, splenectomy and recipient Epstein-Barr virus seronegativity as risk factors in post-transplant lymphoproliferative disorder following allogeneic hematopoietic stem cell transplantation. Haematologica 2006; 91:1059-67.
5. Juvonen E, Aalto SM, Tarkkanen J, Volin L, Mattila PS, Knuutila S, et al. High incidence of PTLD after non-T-cell-depleted allogeneic haematopoietic stem cell transplantation as a consequence of intensive immunosuppressive treatment. Bone Marrow Transplant 2003; 32:97-102.
6. Gross TG, Steinbuch M, DeFor T, Shapiro RS, McGlave P, Ramsay NK, et al. B cell lymphoproliferative disorders following hematopoietic stem cell transplantation: risk factors, treatment and outcome. Bone Marrow Transplant 1999;23:251-8.
7. Curtis RE, Travis LB, Rowlings PA, Socie G, Kingma DW, Banks PM, et al. Risk of lymphoproliferative disorders after bone marrow transplantation: a multi-institutional study. Blood 1999;94:2208-16.
8. Loren AW, Porter DL, Stadtmauer EA, Tsai DE. Post-transplant lymphoproliferative disorder: a review. Bone Marrow Transplant 2003; 31: 145-55.
9. Kruger WH, Schuler F, Lotze C, Schwesinger G, Mentel R, Busemann C, et al. Epstein-Barr virus reactivation after allogeneic stem cell transplantation without lymph node enlargement. Ann Hematol 2005;84:477-8.
10. Cohen J, Gandhi M, Naik P, Cubitt D, Rao K, Thaker U, et al. Increased incidence of EBV-related disease following paediatric stem cell transplantation with reduced-intensity conditioning. Br J Haematol 2005; 129:229-39.
11. Snyder MJ, Stenzel TT, Buckley PJ, Lagoo AS, Rizzieri DA, Gasparetto C, et al. Posttransplant lymphoproliferative disorder following nonmyeloablative allogeneic stem cell transplantation. Am J Surg Pathol 2004;28:794-800.
12. Brunstein CG, Weisdorf DJ, Defor T, Barker JN, Tolar J, van Burik JA, et al. Marked increased risk of Epstein-barr virus-related complications with the addition of anti-thymocyte globulin to a non-myeloablative conditioning prior to unrelated umbilical cord blood transplantation. Blood 2006;108:2874-80.
13. Barker JN, Martin PL, Coad JE, DeFor T, Trigg ME, Kurtzberg J, et al. Low incidence of Epstein-Barr virus-associated posttransplantation lymphoproliferative disorders in 272 unrelated-donor umbilical cord blood transplant recipients. Biol Blood Marrow Transplant 2001; 7:395-9.
14. Peres E, Savasan S, Klein J, Abidi M, Dansey R, Abella E. High fatality rate of Epstein-Barr virus-associated lymphoproliferative disorder occurring after bone marrow transplantation with rabbit antithymocyte globulin conditioning regimens. J Clin Microbiol 2005;43:3540-3.
15. Park S, Noguera ME, Briere J, Feuillard J, Cayuela JM, Sigaux F, et al. Successful rituximab treatment of an EBV-related lymphoproliferative disease arising after autologous transplantation for angioimmunoblastic T-cell lymphoma. Hematol J 2002;3:317-20.
16. Paya CV, Fung JJ, Nalesnik MA, Kieff E, Green M, Gores G, et al. Epstein-Barr virus-induced post-transplant lymphoproliferative disorders. ASTS/ASTP EBV-PTLD Task Force and The Mayo Clinic Organized International Consensus Development Meeting. Transplantation 1999;68:1517-25.
17. Svoboda J, Kotloff R, Tsai DE. Management of patients with post-transplant lymphoproliferative disorder: the role of rituximab. Transpl Int 2006;19:259-69.
18. Gottschalk S, Heslop HE, Roon CM. Treatment of Epstein-Barr virus-associated malignancies with specific T cells. Adv Cancer Res 2002; 84: 175-201.
19. Greenfield HM, Gharib MI, Turner AJ, Guiver M, Carr T, Will AM, et al. The impact of monitoring Epstein-Barr virus PCR in paediatric bone marrow transplant patients: can it successfully predict outcome and guide intervention? Pediatr Blood Cancer 2006;47:200-5.
20. Wagner HJ, Cheng YC, Huls MH, Gee AP, Kuehnle I, Krance RA, et al. Prompt versus preemptive intervention for EBV lymphoproliferative disease. Blood 2004;103:3979-81.
21. Weinstock DM, Ambrossi GG, Brennan C, Kiehn TE, Jakubowski A. Preemptive diagnosis and treatment of Epstein-Barr virus-associated post transplant lymphoproliferative disorder after hematopoietic stem cell transplant: an approach in development. Bone Marrow Transplant 2006;37:539-46.
22. Van Esser JW, Niesters HG, Van Der Holt B, Meijer E, Osterhaus AD, Gratama JW, et al. Prevention of Epstein-Barr virus-lymphoproliferative disease by molecular monitoring and preemptive rituximab in high-risk patients after allogeneic stem cell transplantation. Blood 2002;99:4364-9.
23. Ruutu T, Volin L, Parkkali T, Juvonen E, Elonen E. Cyclosporine, methotrexate, and methylprednisolone compared with cyclosporine and methotrexate for the prevention of graft-versus-host disease in bone marrow transplantation from HLA-identical sibling donor: a prospective randomized study. Blood 2000;96: 2391-8.
24. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA-A matched sibling donors. Transplantation 1974;18:295-304.
25. Thomas ED, Storb R, Clift RA, Fefer A, Johnson L, Neiman PE, et al. Bone-marrow transplantation (second of two parts). N Engl J Med 1975;292:895-902.
26. Aalto SM, Juvonen E, Tarkkanen J, Volin L, Ruutu T, Mattila PS, et al. Lymphoproliferative disease after allogeneic stem cell transplantation--pre-emptive diagnosis by quantification of Epstein-Barr virus DNA in serum. J Clin Virol 2003;28:275-83.
27. Annels NE, Kalpoe JS, Bredius RG, Claas EC, Kroes AC, Hislop AD, et al. Management of Epstein-Barr virus (EBV) reactivation after allogeneic stem cell transplantation by simultaneous analysis of EBV DNA load and EBV-specific T cell reconstitution. Clin Infect Dis 2006; 42: 1743-8.
28. Cesaro S, Murrone A, Mengoli C, Pillon M, Biasolo MA, Calore E, et al. The real-time polymerase chain reaction-guided modulation of immunosuppression enables the pre-emptive management of Epstein-Barr virus reactivation after allogeneic haematopoietic stem cell transplantation. Br J Haematol 2005; 128:224-33.
29. van Esser JW, van der Holt B, Meijer E, Niesters HG, Trenschel R, Thijsen SF, et al. Epstein-Barr virus (EBV) reactivation is a frequent event after allogeneic stem cell transplantation (SCT) and quantitatively predicts EBV-lymphoproliferative disease following T-cell-depleted SCT. Blood 2001;98:972-8.
30. Torre-Cisneros J, Roman J, Torres A, Herrera C, Caston JJ, Rivero A, et al. Control of Epstein-Barr virus load and lymphoproliferative disease by maintenance of CD8+ T lymphocytes in the T lymphocyte-depleted graft after bone marrow transplantation. J Infect Dis 2004;190:1596-9.