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ORIGINAL ARTICLE

Incidence, risk factors, and clinical significance of Epstein–Barr virus reactivation in myelodysplastic syndrome after allogeneic haematopoietic stem cell transplantation

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Abstract

Epstein–Barr virus (EBV) reactivation is a life-threatening complication after allogeneic haematopoietic stem cell transplantation (allo-HSCT). In this study, we investigated the characteristics of EBV reactivation in 186 consecutive myelodysplastic (MDS) patients who underwent allo-HSCT in our centre. In 35 patients (18.8%) who experienced EBV reactivation after allo-HSCT, the median onset was 53 days (range 4–381 days). The cumulative incidence of EBV reactivation at the first, sixth, and twelfth month after allo-HSCT was 10.7%, 15.1%, and 17.9%, respectively. Twenty-five patients (71.4%) received pre-emptive rituximab therapy, and no patients developed post-transplant lymphoproliferative disorders. Stem cell source was proven to be a risk factor correlated with EBV reactivation. The cumulative incidence of relapse in the EBV-positive group was 11.4%, 25.2%, and 31.0% at the first, second, and third year after transplantation, respectively, being significantly higher than the corresponding 6.8%, 10.2%, and 10.2%, in the EBV-negative group ($P = 0.014$). Prognostic analysis showed that EBV reactivation was an independent risk factor for relapse-free survival (RFS). Patients in the EBV-positive group showed obviously shorter RFS than those in the EBV-negative group, with 3-year RFS of 62% and 85%, respectively ($P = 0.017$).

Keywords Epstein–Barr virus · Myelodysplastic syndrome · Stem cell transplantation · Relapse · Prognosis

Introduction

Allogeneic haematopoietic stem cell transplantation (allo-HSCT) is the only curative treatment option for selected patients with low-risk myelodysplastic syndrome (MDS) and most patients with high-risk MDS [1, 2]. Although advances in transplantation practices and supportive care have led to improvements in the prognosis of patients with MDS, post-transplant complications, including infection, relapse, and graft-versus-host disease (GVHD), remain a common cause of death and significantly affect patients' quality of life [3, 4].

Epstein–Barr virus (EBV) is a gammaherpesvirus that latently infects approximately 90% of human adults. The main targets of EBV infection are B cells, which are activated to proliferating blasts. EBV infection and spread are controlled by host antibodies and cytotoxic T lymphocytes (CTLs). Following allo-HSCT, immunocompromised recipients are vulnerable to EBV reactivation (EBV DNA-aemia) and EBV-related post-transplant lymphoproliferative disorders (PTLD). The incidence of EBV DNA-aemia varies among transplant centres, with reported incidences of 0.1–63% depending on transplant type, assay sensitivity, defined level of DNA-aemia, use of systematic screening, and timing [5]. The overall incidence of EBV-PTLD ranges from 1.2 to 12.9% after transplantation [6–8]. However, the mortality rates after development of an EBV-related PTLD can range from 50 to 80% [9, 10] and reach more than 90% without treatment in the

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early stage [11, 12]. The risk factors for EBV activation are predominantly related to the degree of T cell depletion or impairment, including mismatch between donor and recipient, T cell depletion from graft, degree and duration of immunosuppression, and use of anti-thymocyte globulin (ATG) or alemtuzumab (anti-CD52) [8, 13–15].

To date, most studies have focused on the impact of EBV-PTLD on outcomes of patients with haematological malignancies, and limited data have been reported on the incidence and features of EBV reactivation following allo-HSCT in MDS patients. The aim of this study was to define the incidence and potential risk factors of EBV reactivation in MDS patients after allo-HSCT. A further aim was to evaluate the correlation between EBV DNA-aemia and transplant-related complications and assess their impact on clinical outcomes.

Materials and methods

Patient characteristics

A total of 187 consecutive MDS patients who underwent allo-HSCT at the First Affiliated Hospital of Soochow University between December 2007 and June 2016 were included in this retrospective study. One patient who received allo-HSCT with unrelated cord blood cells was excluded from the analysis. The remaining 186 patients who received marrow or peripheral stem cell transplants were included in the final analysis. Informed consent was obtained from the patients before data collection. The study was performed in accordance with institutional guidelines and was approved by the Committees for the Ethical Review of Research at the First Affiliated Hospital of Suchow University. Among 186 patients included in our final analysis, 74 patients received HLA-matched sibling donor transplantation (MSDT), 48 patients received HLA-matched unrelated donor transplantation (MUDT) (14 patients with 8/10 matching HLA-A, B, C, DR, and DQ loci and 34 patients with 10/10 loci) and 64 patients received haplo-identical donor transplantation (HIDT) (39 patients with 5/10 loci, nine patients with 6/10 loci, seven patients with 7/10 loci, five patients with 8/10 loci, one patient with 9/10 loci, and three patients with 10/10 loci). All patients were followed until death or 08 February 2017. The patient characteristics are shown in Table 1.

Conditioning regimens and GVHD prophylaxis in Allo-HSCT

The majority of MDS patients ($n = 163$; 87.6%) received myeloablative conditioning (MAC) regimens; the remaining 23 patients received reduced intensive conditioning (RIC) regimens. For MSDT, MAC regimens comprised semustine (250 mg/m^2, day − 10), cytarabine (2 g/m^2/day, days − 9 to − 8), busulfan (3.2 mg/kg/day, days − 7 to − 5) and cyclophosphamide (1.8 g/m^2/day, days − 4 to − 3). For MUDT and HIDT, patients received a MAC regimen identical to that for MSDT but received higher doses of cytarabine (4 g/m^2/day, days − 9 to − 8). In addition, patients receiving MUDT also received hydroxycarbamide (80 mg/kg, day − 10). Rabbit ATG (Thymoglobulin; Imtix Sangstat, Lyon, France) was administered to patients receiving MUDT (10 mg/kg total dose, days − 5 to − 2) and HIDT (10 mg/kg total dose, days − 6 to − 3).

The RIC comprised semustine (250 mg/m^2, day − 10), fludarabine (30 mg/m^2/day, days − 10 to − 6), cytarabine (1.5 g/m^2/day, days − 10 to − 6), and busulfan (3.2 mg/kg/day, days − 5 to − 3). Additionally, ATG was used in patients receiving MUDT and HIDT (6 mg/kg total dose, days − 4 to − 1).

Patients who underwent MSDT received GVHD prophylaxis consisting of cyclosporine and methotrexate. GVHD prophylaxis in patients who underwent MUDT or HIDT consisted of cyclosporine, mycophenolate mofetil, and methotrexate. In addition, rabbit ATG (thymoglobulin) was administered to patients undergoing MUDT or HIDT and a few patients undergoing MSDT.

Monitoring, diagnosis, and treatment of EBV reactivation

EBV load was monitored by quantitative real-time PCR every week for 3 months after transplantation. From day 90 to day 180 post-transplantation, patients were monitored for EBV DNA load every 2 weeks. EBV monitoring was discontinued in patients at 6 months after allo-HSCT, except in patients with potential EBV reactivation. Patients with a peripheral blood EBV DNA load of ≥ 1000 copies/mL at least once were diagnosed with EBV reactivation (EBV DNA-aemia or EBV-positive). The EBV-negative group generally comprised patients whose peripheral blood EBV DNA load was undetectable (< 100 copies/mL). No patients in this study had low EBV DNA loads (100–1000 copies/mL).

In the present study, 25 of 35 (71.4%) patients with EBV reactivation were treated with rituximab (monoclonal anti-CD20 antibody; 375 mg/m^2 weekly) until EBV DNA-aemia negativity was achieved. No patients in the study developed EBV-PTLD.

Study end points, definitions, and statistical analysis

The study end points were overall survival (OS) and relapse-free survival (RFS). OS was defined as the time from allo-HSCT to death, regardless of cause, or last follow-up. RFS was defined as the time from allo-HSCT to relapse or death. Non-relapse mortality (NRM), incidence of relapse, and incidence of acute and chronic GVHD were also investigated. NRM was defined as death from any cause in the first 28 days after allo-HSCT or death without evidence of disease recurrence beyond day 28, with relapse as a competing event. Relapse was defined as presence of > 5% marrow blasts and/

Table 1 Univariate and multivariate analyses of risk factors for cumulative incidence of EBV reactivation

Variables	No.	EBV positive	CI of 1-year EBV reactivation (%)	Univariate analysis *P* value	Multivariate analysis HR	*P* value
Sex				0.268		
Male	117	25 (21.4%)	20.7			
Female	69	10 (14.5%)	14.7			
Age (years)				0.121		
< 40	96	22 (22.9%)	22.0			
≥ 40	90	13 (14.4%)	13.5			
WHO				0.244		
RAEB-1	46	7 (15.2%)	15.3			
RAEB-2	58	15 (25.9%)	24.3			
Other	82	13 (15.9%)	14.9			
Blast				0.222		
≥ 5%	100	22 (22.0%)	14.1			
< 5%	86	13 (15.1%)	21.1			
Karyotype				0.233		
Good	102	23 (22.5%)	20.8			
Int	56	7 (12.5%)	11.9			
Poor	25	5 (20.0%)	20.2			
IPSS risk				0.147		
Low/Int-1 risk	89	13 (14.6%)	13.7			
Int-2/High risk	94	22 (23.4%)	21.8			
Disease progression				0.526		
No	155	28 (18.1%)	16.9			
Yes	31	7 (22.6%)	23.0			
AML transformation				0.918		
No	169	32 (18.9%)	17.9			
Yes	17	3 (17.6%)	17.6			
Therapies				*0.057*		
Supportive care	58	5 (8.6%)	8.6		1	–
DAC	60	14 (23.3%)	22.2		2.28	0.160
CT	17	2 (11.8%)	11.8		1.31	0.760
DAC + CT	51	14 (27.5%)	25.6		2.24	0.160
Transplant type				*< 0.001*		
MSDT	74	2 (2.7%)	1.4		1	–
MUDT	48	7 (14.6%)	14.6		12.62	0.100
HIDT	64	26 (40.6%)	40.0		3.34	0.240
Conditioning				*0.069*		
RIC	23	1 (4.3%)	4.3		1	–
MAC	163	34 (20.9%)	19.8		2.92	0.400
ATG				*< 0.001*		
No	60	34 (27.0%)	0.0		1	–
Yes	126	1 (1.7%)	26.4		5.47	0.240
Stem cells				*0.001*		
PB	83	7 (8.4%)	8.5		1	–
PB + BM	77	18 (23.4%)	22.2		7.89	*0.003*
BM	26	10 (38.5%)	35.2		18.69	*< 0.001*
Acute GVHD				0.958		
None or I	102	20 (19.6%)	17.9			
II-IV	72	14 (19.4%)	19.5			

Table 1 (continued)

Variables	No.	EBV positive	CI of 1-year EBV reactivation (%)	Univariate analysis *P* value	Multivariate analysis HR *P* value
Severe chronic GVHD					
No	142	29 (20.4%)	19.2	0.680	
Yes	24	4 (16.7%)	16.7		

CI cumulative incidence, Disease progression: MDS that progressed to advanced stage or AML before transplantation, *DAC* decitabine, *CT* chemotherapy, *MSDT* matched sibling donor transplantation, *MUDT* matched unrelated donor transplantation, *HIDT* haplo-identical donor transplantation, *RIC* reduced intensive conditioning, *MAC* myeloablative conditioning, *ATG* rabbit anti-thymocyte globulin. In univariate analysis, a *P* value of less than 0.10 is of clinical significance and indicated in italics; In multivariate analysis, a *P* value of less than 0.05 is of statistical significance and indicated in italics

or reappearance of the underlying disease. Relapse incidence was estimated by considering relapse as the event of interest and death without relapse as a competing event. Acute GVHD was graded according to previously published criteria [16], and chronic GVHD was graded as limited or extensive. The cumulative incidence of acute and chronic GVHD was estimated by considering the corresponding type of GVHD as an event of interest and death without GVHD as a competing event. The cumulative incidence of EBV reactivation was estimated by considering EBV reactivation as an event of interest and death without EBV reactivation as a competing event.

OS and RFS were computed using the Kaplan–Meier method, with the log-rank test used for univariate comparisons. Prognostic factors with values of $P < 0.05$ in the univariate analyses were entered into a Cox proportional hazards model to determine their effects on survival. The cumulative incidence method was applied to compute the incidence of NRM, relapse, acute GVHD, chronic GVHD, and EBV reactivation in a competing risks setting, with the Gray test used for comparisons of different groups [17]. For risk factors of cumulative incidence of EBV reactivation, only factors with values of $P < 0.10$ in the univariate analyses were chosen for further evaluation by multivariate regression analysis proposed by Fine and Gray [18]. All analyses were performed using the SPSS software package (SPSS, Chicago, IL, USA) and R software package (R version 3.3.3; The R Foundation for Statistical Computing, www.R-project.org).

Results

Patient characteristics

A total of 186 consecutive MDS patients who received marrow or peripheral stem cell transplants from December 2007 to June 2016 were included in the final analysis. The median age of the included patients was 39 years (range 7–62 years). The clinical characteristics of the 186 MDS patients with or without EBV reactivation are shown in Table 1.

Clinically significant grade II–IV acute GVHD occurred in 72 patients (41.4%) at day 100 after transplantation, with median onset of 28 days (range 4–85 days). The cumulative incidence of grade II–IV acute GVHD at 30, 60, and 90 days after transplantation was 21.1%, 41.7%, and 44.5%, respectively. Among 166 patients who survived for at least 100 days, chronic GVHD was diagnosed in 82 (49.4%), with the median onset of 5.3 months (range 3.4–34.5 months). Twenty-four (14.5%) patients showed extensive chronic GVHD, and the cumulative incidence of extensive chronic GVHD at the first, second, and third year was 13.8%, 19.8%, and 21.2%, respectively. At the last follow-up, 73 (39.2%) patients had died, including 57 patients (30.6%) who died from NRM. The cumulative incidence of NRM at the first, second, and third year was 24.4%, 28.4%, and 30.1%, respectively. Among the 186 patients, 25 (13.4%) had relapsed by the final follow-up, and the cumulative incidence of relapse at the first, second, and third year was 7.7%, 13.1%, and 14.1%, respectively.

In 35 patients (18.8%) who experienced EBV reactivation after allo-HSCT, the median onset was 53 days (range 4–381 days). The cumulative incidence of EBV DNA-aemia at the first, third, sixth, and twelfth month after allo-HSCT was 10.7%, 14.0%, 15.1%, and 17.9%, respectively. Among the 35 patients with EBV DNA-aemia, 13 experienced EBV reactivation at only a single time point after allo-HSCT, while the remaining 22 patients had EBV DNA-aemia on two or more occasions. Twenty-five patients (71.4%) received pre-emptive rituximab therapy, and no patients developed PTLD. All patients were followed until death from any cause or the last follow-up date (08 February 2017).

Risk factors for EBV reactivation

The univariate analyses showed that patients who received non-supportive treatment (decitabine or chemotherapy or decitabine plus chemotherapy) had a higher cumulative incidence of EBV reactivation. In addition, patients who received HIDT, MAC conditioning, marrow-derived stem cells, and ATG for GVHD prevention had a higher cumulative incidence of EBV reactivation. The results of the univariate analyses are shown in Table 1. We then performed multivariate regression analysis using the risk factors with values of $P < 0.1$ in the univariate analyses and found that stem cell source (BM vs. PB $P < 0.001$, HR = 18.69; BM + PB vs. PB $P = 0.003$, HR = 7.89) was an independent risk factor for cumulative incidence of EBV reactivation. Transplant type,

especially HIDT, although demonstrating higher cumulative incidence of EBV reactivation, did not reach statistical significance after multivariate analysis ($P = 0.10$, HR = 12.62).

Correlations between EBV reactivation and transplant-related complications

As shown in Fig. 1a, the cumulative incidence of EBV reactivation in patients with grade II–IV acute GVHD was 19.5% at 1 year after transplantation, similar to17.9% in patients with grade I or without acute GVHD (Fig. 1a; $P = 0.958$). There was also no significant difference in the cumulative incidence of EBV reactivation in patients with and without severe chronic GVHD (Fig. 1b; $P = 0.680$). Furthermore, there was no significant difference in NRM between the patients who experienced EBV reactivation after allo-HSCT and those who did not (Fig. 1c). However, the cumulative incidence of relapse in the EBV-positive group was 11.4%, 25.2%, and 31.0% at the first, second, and third year after transplantation, respectively, being significantly higher than the corresponding 6.8%, 10.2%, and 10.2% in the EBV-negative group (Fig. 1d; $P = 0.014$).

EBV reactivation and prognosis

To evaluate the prognostic significance of EBV reactivation, we compared OS and RFS with regard to EBV reactivation status.

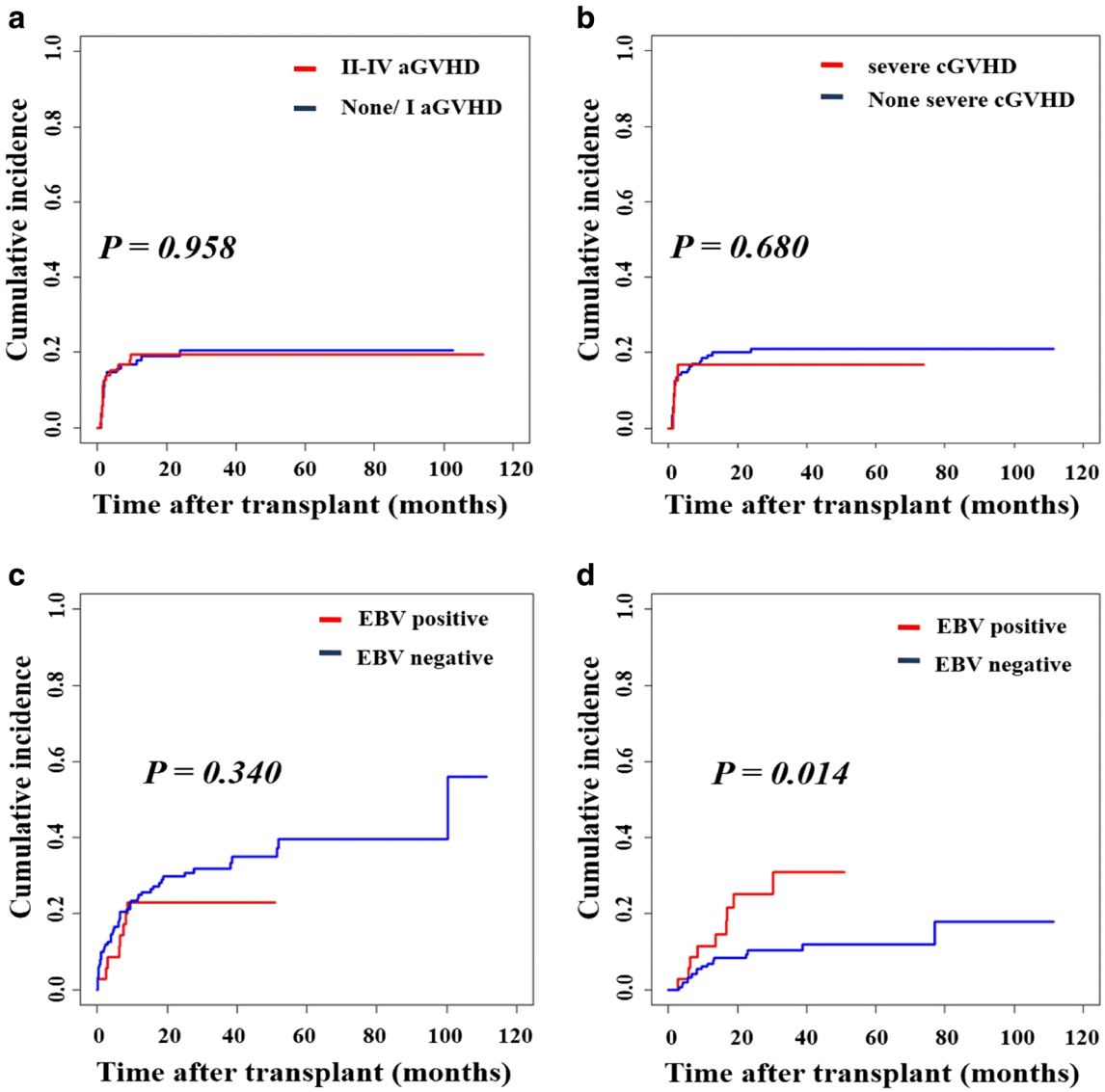

Fig. 1 Cumulative incidence of Epstein–Barr virus (EBV) reactivation in myelodysplastic syndrome (MDS) patients with and without grade II–IV acute graft-versus-host disease (GVHD) after allogeneic haematopoietic stem cell transplantation (allo-HSCT) (**a**), cumulative incidence of EBV reactivation in MDS patients with and without severe chronic GVHD (**b**), cumulative incidence of non-relapse mortality (NRM) based on EBV reactivation in MDS patients after allo-HSCT (**c**), cumulative incidence of relapse based on EBV reactivation in MDS patients after allo-HSCT (**d**)

Overall, there was no significant difference in OS between the patients who experienced EBV reactivation after allo-HSCT and those who did not (Fig. 2a; $P = 0.584$). However, the patients in the EBV-positive group showed obviously shorter RFS than those in the EBV-negative group, with 3-year RFS of 62% and 85%, respectively (Fig. 2b; $P = 0.017$). The results of univariate analyses on risk factors for DFS are listed in Table 2. After multivariate analysis, apart from progression of AML before transplantation and lack of grade II–IV acute GVHD after transplantation, EBV reactivation was proven to be a significant risk factor for RFS (Table 3).

EBV load or duration and prognosis

We further evaluated the prognostic significance of different levels of virus load and different durations of EBV DNA-aemia. The median duration of EBV DNA-aemia was 14 days (range 5–115 days). The median number of EBV copies was 231 × 103 copies/mL (range 4.59×10^3–8450×10^3 copies/mL). Based on the number of EBV copies, we divided the patients into a high EBV-positive load group and a low EBV-positive load group, using the median value of 231×10^3 copies/mL as the cut-off. The results showed that different EBV loads did not significantly impact OS and RFS in patients with EBV reactivation (Fig. 3a, b). Similarly, transient or persistent EBV DNA-aemia did not significantly impact OS and RFS in patients with EBV reactivation (Fig. 3c, d).

Discussion

This study assessed the incidence and risk factors of EBV reactivation in a consecutive series of 186 MDS patients who underwent allo-HSCT. We found a cumulative incidence of 15.1% and 17.9% for EBV DNA-aemia at 6 and 12 months after allo-HSCT in MDS patients, similar to the findings of previous studies [19–21]. In terms of risk factors for EBV reactivation, previous studies found correlations between EBV reactivation and several factors, including degree of HLA mismatch between donor and recipient, manipulation of graft to deplete T cells, degree and duration of immunosuppression used to prevent and treat GVHD, and use of ATG [8, 22]. Consistent with these reports, our study showed that transplant type (HIDT), MAC conditioning, marrow-derived stem cells, and use of ATG were correlated with EBV reactivation in univariate analyses. However, after multivariate analysis, stem cell source, especially marrow-derived stem cells, was proven to be the most significant factor, and HIDT did not reach statistical significance. The differences in findings between our study and previous reports may be attributed to the different criteria applied for patient selection, conditioning regimens, and constitution of transplant type among the studies.

EBV reactivation, especially EBV-related PTLD, is an uncommon, but life-threatening, complication after allo-HSCT. Therefore, prompt diagnosis and early treatment are necessary, because of the rapidly progressive nature of the disease [9, 23, 24]. Current treatment approaches for EBV reactivation according to the sixth European Conference on Infections in Leukaemia (ECIL-6) guidelines include administration of rituximab, reduction of immunosuppression, administration of EBV-CTLs, donor lymphocyte infusion, and chemotherapy [5]. In the present study that included 35 patients with EBV DNA-aemia, no patients had progressed to PTLD at the final follow-up. The low incidence of PTLD in our study may be attributed to the following two reasons. First, early detection strategies involving measurement of EBV-DNA loads in peripheral blood samples by quantitative real-time PCR can help to identify high-risk patients [25–27]. Second, pre-emptive anti-B cell therapies, such as rituximab, were used in 71.4% of patients in our study and were proven to be successful in preventing PTLD [10, 28–30].

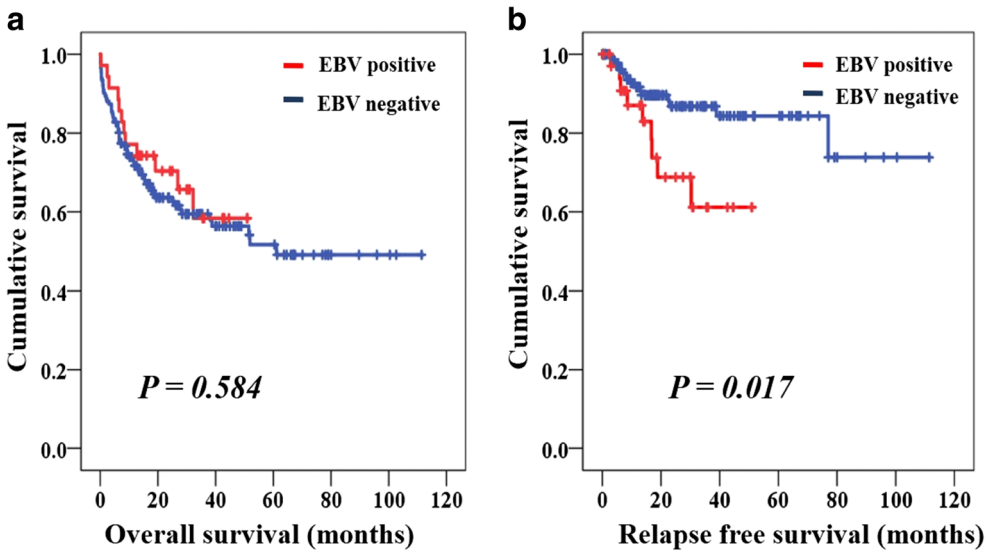

Fig. 2 Overall survival (**a**) and relapse-free survival (**b**) according to Epstein–Barr virus (EBV) reactivation

Table 2 Univariate analyses of risk factors for relapse-free survival

Variables	No. of patients	3-year RFS (%)	*P* value
Sex			0.186
Male	117	74	
Female	69	88	
Age (years)			0.104
< 40	96	84	
≥ 40	90	76	
WBC(× 10^9/L)			0.433
≥ 4	37	77	
< 4	149	81	
HB(g/L)			0.600
≥ 100	31	77	
< 100	154	81	
PLT(× 10^9/L)			*0.042*
≥ 50	70	70	
< 50	116	86	
WHO			0.124
RAEB-1	46	73	
RAEB-2	58	73	
Other	82	90	
Blast			*0.022*
≥ 5%	100	71	
< 5%	86	90	
Karyotype			0.142
Good	102	84	
Int	56	73	
Poor	25	84	
IPSS risk			*0.037*
Low/Int-1 risk	89	86	
Int-2/High risk	94	74	
Disease progression			0.071
No	155	81	
Yes	31	71	
AML transformation			*0.001*
No	169	83	
Yes	17	53	
Therapies			*0.019*
Supportive care	58	82	
DAC	60	91	
CT	17	84	
DAC + CT	51	66	
Transplant type			0.597
MSDT	74	86	
MUDT	48	78	
HIDT	64	75	
Conditioning			0.722
RIC	23	89	
MAC	163	79	
ATG			0.322
Yes	126	77	
No	60	86	
Stem cells			0.095
BM	26	66	
PB	83	80	
BM + PB	77	85	
Acute GVHD			*0.027*
II-IV	72	91	
Other	102	72	
Chronic GVHD			
No	84	70	*0.007*
Yes	82	89	
CMV			0.637
Positive	58	85	
Negative	128	77	
EBV			*0.017*
Positive	35	62	
Negative	151	85	

Disease progression: MDS that progressed to advanced stage or AML before transplantation, *DAC* decitabine, *CT* chemotherapy, *MSDT* matched sibling donor transplantation, *MUDT* matched unrelated donor transplantation, *HIDT* haplo-identical donor transplantation, *RIC* reduced intensive conditioning, *MAC* myeloablative conditioning, *ATG* rabbit anti-thymocyte globulin, *GVHD* graft-versus-host disease, *CMV* cytomegalovirus, *EBV* Epstein–Barr virus. A *P* value of less than 0.05 is indicated in italics

In our study, EBV reactivation was found to be correlated with a high risk of disease relapse. After multivariate analysis, EBV reactivation was proven to be an independent risk factor for RFS. T cells are believed to play a major role in the graft-versus-leukaemia reaction and disease relapse [31]. One previous study showed that leukaemia antigen-specific T cells can be detected after transplantation and are associated with decreased malignancy relapse [32]. Another study from Hoegh-Petersen et al. suggested that high EBV-specific T cell counts were associated with decreased relapse in AML [33]. In that study, high EBV-lysate-specific CD4 T cells producing IFNγ, EBV-lysate-specific

Table 3 Multivariate analysis of relapse-free survival

	Univariate *P*	Multivariate *P*	HR (95% CI)
PLT ≥ 50 × 10^9/L	0.042	0.073	0.47 (0.21–1.07)
Blast ≥ 5%	0.022	0.107	3.29 (0.77–13.99)
Higher risk IPSS	0.037	0.784	1.21 (0.31–4.78)
AML transformation	0.001	*< 0.001*	7.32 (2.53–21.17)
Therapies	0.019	0.498	0.82 (0.45–1.47)
II-IV acute GVHD	0.027	*0.045*	0.34 (0.12–0.98)
Chronic GVHD	0.007	0.112	0.46 (0.18–1.20)
EBV positive	0.017	*0.016*	2.92 (1.22–6.99)

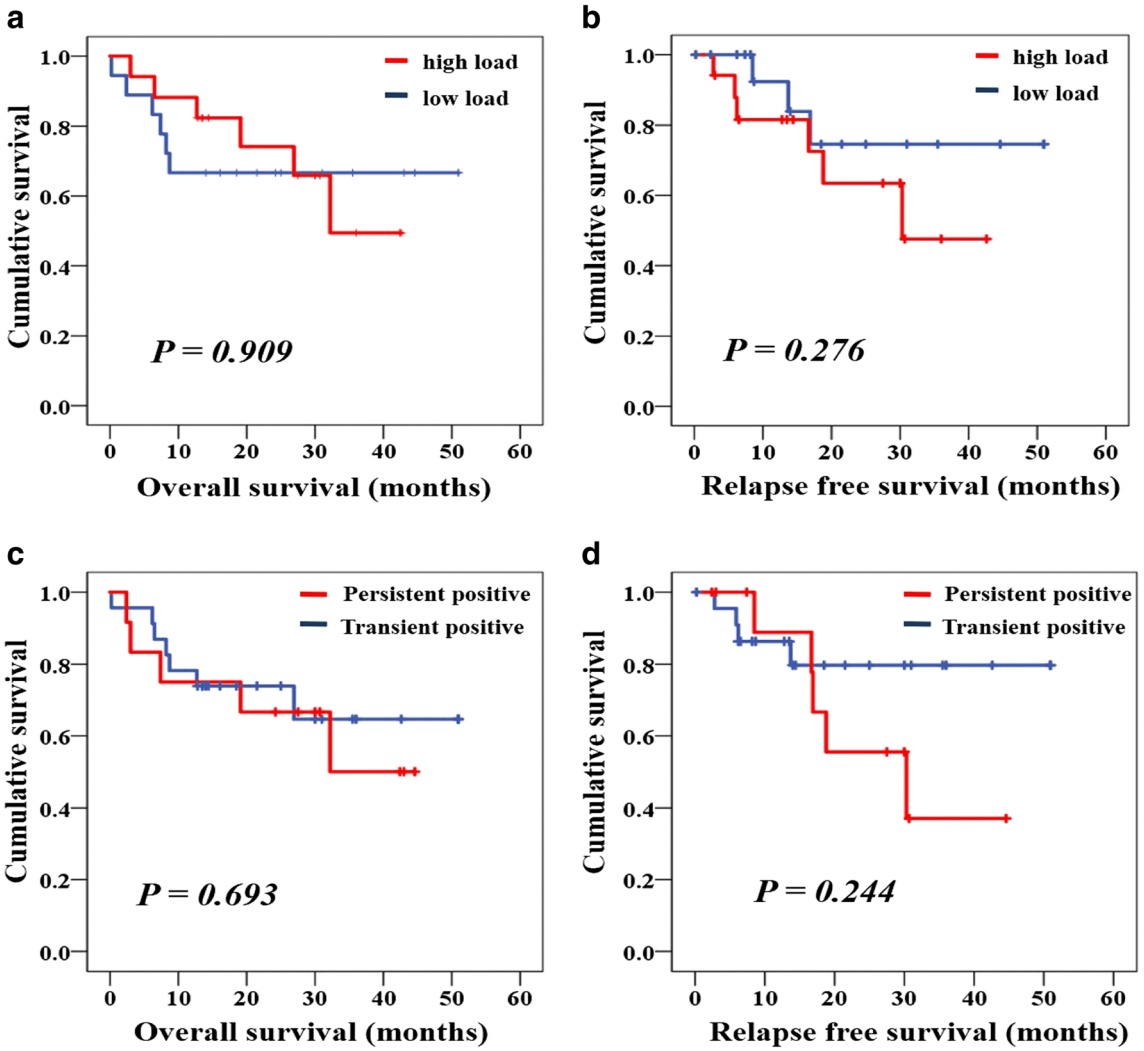

Fig. 3 Overall survival according to Epstein–Barr virus (EBV) load (**a**). Relapse-free survival according to EBV load (**b**). Overall survival according to EBV duration (**c**). Relapse-free survival according to EBV duration (**d**)

CD4+ T cells producing IL-2, EBV-lysate-specific CD8+ T cells producing IFNγ and TNFα, EBNA3-specific CD8+ T cells producing IFNγ, and EBNA3-specific CD8+ T cells producing IFNγ and TNFα were shown to be correlated with decreased relapse in AML. In patients after haplo-HSCT, delayed recovery of CD4/CD8 double-negative T cells was proven to be correlated with increased EBV reactivation in patients after haplo-HSCT [19]. This study also showed that the EBV-positive group had higher relapse in patients with haematological malignancy after haplo-HSCT, which was in consistent with our findings. In addition, other factors, including the growth potential/burden of leukaemia and the NK cell response to leukaemic cells, may also play a role in disease relapse [34]. Because this is a retrospective study, we did not have data on immune reconstitution and therefore could not define which T cell subset was involved in the EBV reactivation and disease relapse.

Given the important role of EBV in the pathogenesis of PTLD, many researchers have investigated the role of viral load, as determined by quantitative PCR during the diagnosis. Measurement of EBV viral load has emerged as a useful tool to initiate pre-emptive treatment. This strategy, in which immune suppression is reduced and/or rituximab is administered under guidance of the EBV viral load, has shown promising results in both solid organ transplantation and HSCT [24, 35, 36]. Impressive results with very favourable toxicity profiles have also been obtained for prophylactic and pre-emptive administration of EBV-specific CTLs [37]. Two previous studies suggested a clear link between EBV proliferation and development of EBV-PTLD in patients after liver transplantation [38, 39]. Another recent study showed a correlation between the cumulative burden of multiple viruses (including EBV) and increased mortality of patients after transplantation [40]. In our study, no patients progressed to EBV-PTLD, regardless of virus load. We also did not observe any significant correlations

between virus load or duration and patient OS or RFS. Considering the different criteria for patient inclusion (we only included MDS patients) and the relatively small number of patients, such differences may be acceptable.

In conclusion, our study reports the incidence and risk factors of EBV reactivation in MDS after allo-HSCT. We also show the correlation between EBV reactivation and disease relapse. Monitoring of EBV viral load by quantitative PCR and early systematic pre-emptive rituximab therapy can allow for a significant reduction in the risk of EBV-related PTLD. Our findings could be of benefit to protect against EBV reactivation and improve the prognosis of MDS patients after allo-HSCT.

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Compliance with ethical standards

The study was performed in accordance with institutional guidelines and was approved by the Committees for the Ethical Review of Research at the First Affiliated Hospital of Suchow University. Informed consent was obtained from the patients before data collection.

Conflict of interest The authors declare that they have no conflict of interest.

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