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ARTICLE

Epstein–Barr virus reactivation after allogeneic hematopoietic stem cell transplantation: multifactorial impact on transplant outcomes

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Abstract

Epstein–Barr virus (EBV) reactivation after allogeneic hematopoietic cell transplantation (allo-HCT) is one of the major concerns that may lead to fatal EBV diseases. However, updated data are needed because of the remarkable evolution of the HCT protocol and donor selection. We conducted a retrospective study that enrolled 890 allo-HCT recipients. Independent risk factors for EBV reactivation were use of antithymocyte globulin, haploidentical donor, and the presence of chronic graft-versus-host disease. The cumulative incidence of EBV reactivation was 2.9%, 11.7%, 27.3%, and 41.9% for patients with 0, 1, 2, and 3 risk factors, respectively ($P < 0.001$). Posttransplant lymphoproliferative disorders (PTLDs) occurred in seven patients. EBV reactivation was associated with inferior survival in recipients who survived more than 2 years post-HCT ($P < 0.001$) but might time-dependently benefit those patients with malignancies by decreasing relapse incidence ($P = 0.046$). A decreased relapse incidence was observed 1 year after HCT for recipients at first or second remission ($P = 0.042$) and in the first year post-HCT for recipients with advanced diseases ($P = 0.032$). We concluded that with current management, PTLDs were efficiently controlled, but EBV reactivation still had a multifactorial impact on transplant outcomes. Multicenter prospective studies are warranted to validate these findings.

Introduction

Epstein–Barr virus (EBV) is a highly immunogenic latent γ-herpesvirus that has infected >90% of humans worldwide [1, 2]. Regulated by EBV-specific T cells, it can set up an asymptomatic infection for a lifetime in immunocompetent individuals [3]. However, under the immunocompromised circumstances created by hematopoietic cell transplantation (HCT), EBV reactivation is a frequent complication that may lead to uncontrolled B-cell proliferation and result in EBV-related posttransplant lymphoproliferative disorders (PTLDs) and other EBV diseases [1, 4–8]. The reported incidence of EBV reactivation post-HCT ranges from 0.1 to 63% depending on transplant type, antiviral agents, monitoring protocol, and assay sensitivity [9]. The overall incidence of EBV-PTLD varies from 1.2 to 12.9% among different studies [10–12], with a high mortality of up to 50–80% [13, 14] and over 90% for advanced patients [15, 16].

With the development of transplant protocols and post-HCT supportive care, the management of EBV reactivation and EBV diseases has markedly improved. In particular, preemptive treatment with rituximab has been widely adopted for the prophylaxis of PTLD [17, 18]. Meanwhile,

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the increasing number of transplants employing haploidentical donors and antithymocyte globulin (ATG) may potentially increase the risk of EBV reactivation [1]. However, few data have been reported recently describing the current prevalence and features of EBV reactivation post-HCT. Here, we conducted a retrospective study including 890 recipients who underwent allogeneic HCT (allo-HCT) to update the current prevalence, risk factors, and impact on outcomes of EBV reactivation.

Materials and methods

Patients

This was a retrospective study based on data derived from the transplant database in our center, which was established according to the European Society for Blood and Marrow Transplantation registry. The inclusion criteria were as follows: (1) patients who underwent allo-HCT in our center between July 2011 and July 2014 and (2) patients who received regular EBV management after HCT based on an institutional protocol. The study was approved by the Ethics Committee of our center and conducted in accordance with the Helsinki Declaration.

Donor selection, stem cell source, and transplant protocols

The algorithm of donor selection was based on HLA typing, age, donor sex, and ABO compatibility [19]. The preferred donor was an HLA-matched sibling. In the absence of a matched donor, a haploidentical donor could be the prioritized alternative option [20]. Donors were recommended to contribute a bone marrow graft, complemented with peripheral blood stem cells if the $CD34^+$ cell dose failed to achieve the target dose of 2×10^6/kg of recipient body weight. The majority of patients received myeloablative conditioning, including the modified Bu/Cy regimen and the modified TBI/Cy regimen. Patients who were intolerant to intensive chemotherapy received a reduced intensity conditioning (RIC) regimen based on fludarabine, low-dose busulfan, cytarabine or cyclophosphamide according to the primary disease [21].

Management of graft-versus-host disease (GVHD)

The prophylaxis of GVHD included cyclosporin A (CsA) and short-term methotrexate for HLA-matched sibling donor HCT, and mycophenolate mofetil (MMF) combined with ATG (Genzyme, MA, USA) [22] was added for patients receiving grafts from unrelated or haploidentical donors. The diagnosis of acute and chronic GVHD was made according to reference literature [23, 24]. Methylprednisolone at a dose of 1–2 mg/kg/day was given immediately as the first-line treatment in case of overt acute GVHD occurrence. The second-line drugs included tacrolimus, anti-CD25 monoclonal antibody, MMF, and ATG, etc. The first-line treatment of overt chronic GVHD was steroids and/or CsA.

Management of EBV reactivation

Q-PCR was applied to monitor EBV-DNA load in whole peripheral blood weekly from conditioning to +90 days post-HCT in all patients and once every 2 weeks from +90 days until +80 days. Additional detection was performed if symptoms of suspected virus infection were present. Ganciclovir at a dose of 10 mg/kg/day was used from −9 to −2 days to prevent virus infection and then replaced by acyclovir to avoid marrow toxicity. The treatment for EBV-reactivated recipients included tapering of immunosuppressive agents, ganciclovir, foscarnet sodium, and preemptive therapy with rituximab. Preemptive rituximab was prescribed if EBV-DNA reached 10^5 copies/mL or 10^4 copies/mL for 2 consecutive weeks.

Definition

EBV reactivation was defined as more than 10^2 copies/mL EBV-DNA in whole blood by Q-PCR. Person-years at risk were calculated from the date of transplantation to the date of death, last follow-up, or study end, whichever occurred first. The pattern of EBV reactivation occurrence by post-HCT intervals was evaluated by calculating EBV reactivation incidence rates, defined as the number of EBV reactivation cases divided by the number of person-years in each interval. The diagnosis of disease recurrence was based on clinical and pathological criteria. The survival time was calculated starting on the day of transplantation. Overall survival (OS) was calculated with the date of death, last follow-up, or study end, whichever occurred first, as the final date. If the patient was in remission, progression-free survival (PFS) was calculated with the date of death, recurrence, or last follow-up as the final date. Deaths unrelated to the underlying disease were recorded as treatment-related mortality (TRM).

Statistics

Differences in EBV-positive incidence rates among different donor–recipient relationships were compared with independent sample Kruskal–Wallis test. Risk analyses for EBV reactivation were conducted by the Cox regression model, and all risk factors whose P values were below 0.1 in univariate analyses were included in multivariate

Table 1 Characteristics of patients undergoing allogeneic HCT and univariate analysis of risk factors on EBV reactivation.

Factors	Cases	EBV+	Incidence	*P*	HR (95%CI)
Sex				0.835	1.016 (0.847–1.181)
F	362	73	20.2%		
M	528	102	19.3%		
Age				0.010	1.218 (1.049–1.413)
<30	410	97	23.7%		
≥30	480	78	16.3%		
Diagnosis				0.494	1.218 (0.692–2.143)
Other	835	162	19.4%		
Lymphoma	55	13	23.6%		
Conditioning regimen				0.750	1.049 (0.782–1.406)
MAC	821	163	19.9%		
RIC	69	12	17.4%		
Donor–recipient HLA compatibilities				<0.001	2.670 (1.984–3.594)
HLA-haploidentical	261	86	33.0%		
HLA-identical	629	89	14.1%		
Pretransplant status				0.604	1.047 (0.881–1.243)
1st or 2nd remission	671	132	19.7%		
Advanced status	219	43	19.6%		
ATG use				<0.001	5.125 (3.247–8.089)
No	338	21	6.2%		
Yes	552	154	27.9%		
TBI				0.786	1.037 (0.796–1.352)
No	803	160	19.9%		
Yes	87	15	17.2%		
Acute GVHD				0.078	1.336 (0.968–1.845)
None, grade I~II	666	122	18.3%		
Grade III~IV	224	53	23.7%		
GVHD				0.023	1.436 (1.051–1.96)
None	412	61	14.8%		
Chronic GVHD	478	114	23.8%		

analyses. OS and PFS were calculated using the Kaplan–Meier method and compared with the log-rank test. The cumulative incidence of relapse (CIR) was calculated by a competing risk model with TRM as a competing risk factor. All *P* values were two-sided and were defined as statistically significant if <0.05. Statistical analyses were performed using SPSS 19.0 software (SPSS, Chicago, IL, USA) and R 3.6.1 software package (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics

A total of 890 patients were included in this analysis according to the inclusion criteria. The characteristics of recipients with or without EBV reactivation are displayed in Table 1. The median age was 32 (range, 2–63) years old, and 528 patients were male. There were 212 cases of acute lymphoblastic leukemia, 378 of acute myeloid leukemia, 77 of chronic myeloid leukemia, 87 of severe aplastic anemia, 76 of myelodysplastic syndrome, 55 of lymphoma, and 5 of myelofibrosis. Most of the recipients received myeloablative conditioning, and 29.3% received a graft from a haploidentical donor.

Incidence of EBV reactivation and PTLD

One hundred and seventy-five recipients developed EBV reactivation (Fig. 1a), with a median time of 57 (range, 18–1006) days after HCT, and most of these patients (129) developed EBV reactivation within the first 100 days. The incidence of EBV reactivation peaked at 1–2 months after transplantation and then plummeted sharply, except for in haploidentical HCT recipients, in which the incidence of

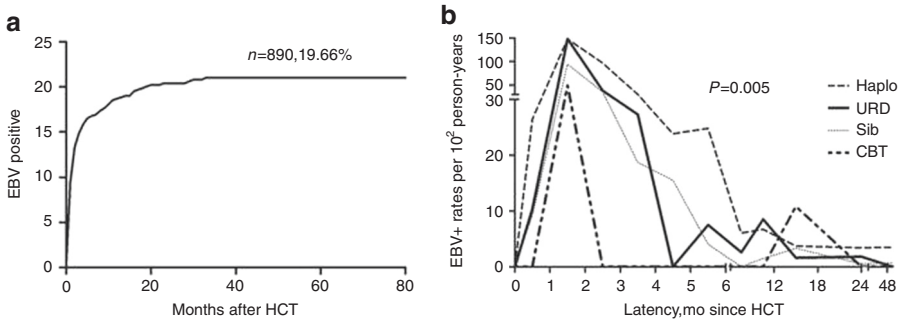

Fig. 1 Incidence of EBV reactivation after HCT. Comparison of: (**a**) incidence of EBV reactivation in 890 patients transplanted between July 2011 and July 2014; (**b**) variation tendency of EBV positivity incidence rate over time by donor–recipient relationships and histocompatibilities.

Table 2 Incidence rate and number of cases of EBV positive by latency.

Latency,month since HCT	No. of patients	Person-years at risk	No. of EBV+ cumulative cases	No. of EBV+ cases	EBV+ incidence rates per 10^2 person-years
0 to <1	890	73	10	10	13.7
1 to <2	866	71	91	81	113.8
2 to <3	843	69	121	30	43.7
3 to <4	804	66	133	12	18.2
4 to <5	776	64	141	8	12.6
5 to <6	753	62	146	5	8.1
6–	729	172	153	7	4.1
9–	649	151	162	9	5.9
12–	562	255	167	5	2.0
18–	458	368	173	6	1.6
30+	278	579	175	2	0.3

EBV+ indicates EBV reactivation.

EBV reactivation declined moderately (Fig. 1b). EBV reactivation remarkably decreased 1 year after transplantation and rarely occurred after 2 years (Table 2). The cumulative incidence of EBV reactivation was 18.2% for the first year, 19.3% for the first 2 years, and 19.6% for the first 3 years. There were statistically significant differences in the rate of EBV positivity over time among different donor–recipient relationships ($P = 0.005$) (Fig. 1b), and the highest incidence in the first year was observed in haploidentical HCT recipients. By the end of follow-up, seven patients developed PTLD.

Risk factor analyses for EBV positivity after HCT

In the univariate analysis, five factors significantly associated with EBV reactivation after HCT were identified, including haploidentical HLA match ($P < 0.001$), ATG as GVHD prophylaxis ($P < 0.001$), age younger than 30 years ($P = 0.010$), and the development of chronic GVHD ($P = 0.023$). Moreover, grade II–IV acute GVHD had marginal significance ($P = 0.078$, HR = 1.336, 95% CI: 0.968–1.845) (Table 1), which met the inclusion criteria for the multivariate analysis. The multivariate analysis revealed three independent risk factors for EBV reactivation after HCT (Fig. 2), including ATG as GVHD prophylaxis ($P < 0.001$), HLA-mismatched donor ($P = 0.001$) and appearance of chronic GVHD ($P = 0.042$). The cumulative incidence of EBV reactivation was low (2.9%) among patients with no risk factor but increased to 11.7%, 27.3%, and

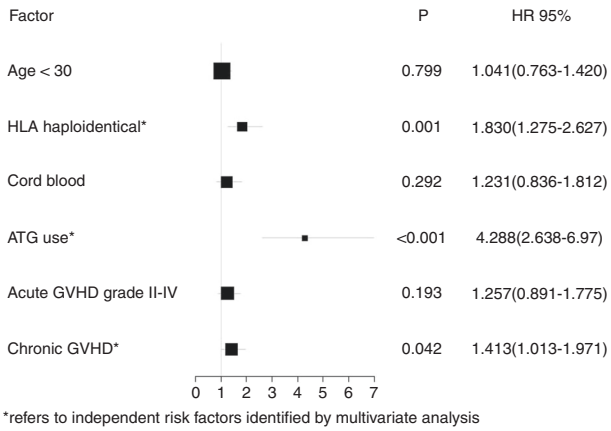

Fig. 2 Multivariate analysis of risk factors for EBV reactivation after HCT. Haploidentical donor, ATG use and the presence of chronic GVHD were identified as independent risk factors for EBV reactivations.

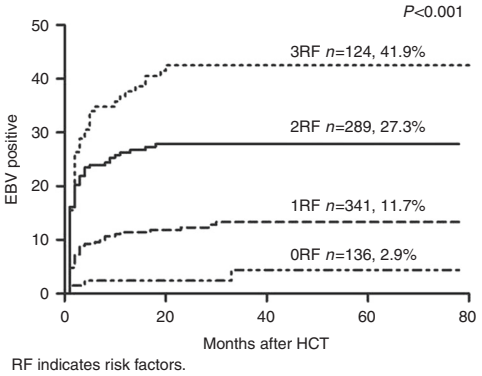

Fig. 3 Accumulative effect of risk factors on EBV reactivation. A stepwise increment of EBV reactivation incidence was observed with increasing number of independent risk factors identified in multivariate analysis.

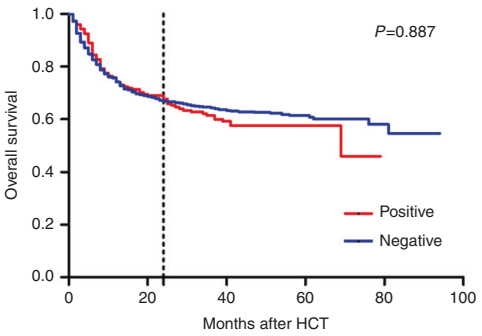

Fig. 4 Overall survival of recipients between EBV-positive and negative group. Adverse impact of EBV reactivation on survival presented in recipients survived more than 24 months (dotted line) post-HCT ($P < 0.001$).

41.9% for those with 1, 2, and 3 risk factors, respectively ($P < 0.001$) (Fig. 3).

Impact of EBV reactivation on transplant outcomes

With a median follow-up of 36 months (range, 0–94 months), the estimated 2-year OS was comparable between groups with or without EBV reactivation (60.5% ± 1.9% versus 72.6% ± 3.4%, $P = 0.887$) (Fig. 4). Meanwhile, there was no statistical difference in PFS ($P = 0.905$), TRM ($P = 0.385$), or CIR ($P = 0.399$) between the two groups. However, it seemed that EBV reactivation had a late-onset impact for recipients who survived more than 2 years. The TRM of EBV-reactivated recipients was significantly higher than that of the EBV-negative group (5-year TRM: 8.9% ± 0.8% versus 3.7% ± 0.1%, $P = 0.003$), resulting in decreased PFS (5-year PFS: 72.0% ± 6.1% versus 84.4% ± 2.0%, $P = 0.035$) and OS (5-year OS: 75.9% ± 6.0% versus 91.7% ± 1.6%, $P < 0.001$) despite a similar CIR ($P = 0.818$) (Fig. 5). The main causes of death in the EBV-reactivated cohort included relapse ($n = 38$), severe infection ($n = 21$), GVHD ($n = 11$), hemorrhage events ($n = 2$), and PTLD ($n = 1$). In EBV-negative recipients, the main causes of death consisted of relapse ($n = 151$), severe infection ($n = 62$), GVHD ($n = 40$), hemorrhage events ($n = 5$), disseminated intravascular coagulation ($n = 1$), thrombotic microangiopathy ($n = 3$), thrombotic thrombocytopenic purpura ($n = 1$), acute pancreatitis ($n = 1$), and pulmonary fibrosis ($n = 1$). There was no statistical difference in causes between the two groups of patients ($P = 0.102$).

For patients with malignant diseases consisting of acute lymphoblastic leukemia, acute myeloid leukemia, myelodysplastic syndrome, and lymphoma, we found that CIR was significantly reduced in the EBV-reactivated cohort compared with the EBV-negative group (5-year CIR: 16.3% ± 0.1% versus 24.8% ± 0.04%, $P = 0.046$). In particular, none of the recipients in the EBV-reactivated group relapsed beyond 2 years after HCT, while 4.12% of patients in the EBV-negative group relapsed beyond 2 years after HCT ($P = 0.041$). For recipients with stable disease (within the 1st or 2nd remission), the CIR dramatically decreased in the EBV-reactivated cohort after 1 year post-HCT (2.7% ± 0.04% versus 11.2% ± 0.04%, $P = 0.042$) (Fig. 6a), although OS and PFS were not improved. Conversely, for recipients with advanced disease (in progression or at or beyond the 3rd remission), the benefit of EBV reactivation on CIR was observed in the first year post-HCT (14.2% ± 0.4% versus 31.1% ± 0.1%, $P = 0.032$) (Fig. 6b), leading to superior 1-year OS (68.4% ± 7.5% versus 51.5% ± 4.0%, $P = 0.042$) as well as PFS (60.5% ± 7.9% versus 45.0% ± 4.0%, $P = 0.047$) within 1 year after HCT compared with the respective values seen in EBV-negative patients.

Discussion

As a result of the immunocompromised circumstances created under HCT, EBV reactivation is a frequently reported complication posttransplantation that inhibits B-cell apoptosis and induces viral oncogene expression and genetic and epigenetic alterations that lead to B-lymphocyte transformation [8, 25–30]. EBV reactivation may be asymptomatic initially but could lead to a series of EBV-related diseases, including pneumonitis, enteritis, and ophthalmitis, without intervention. In addition, EBV reactivation presents in 60–80% of PTLD patients [27, 28], which is usually a fatal malignant complication [5].

Given the inferior outcome caused by EBV reactivation, a large number of studies have explored its risk factors [1, 5, 7, 13, 16, 31] and management strategies, particularly Q-PCR monitoring and preemptive treatment with rituximab [9, 16]. Pooled results from published studies in HSCT

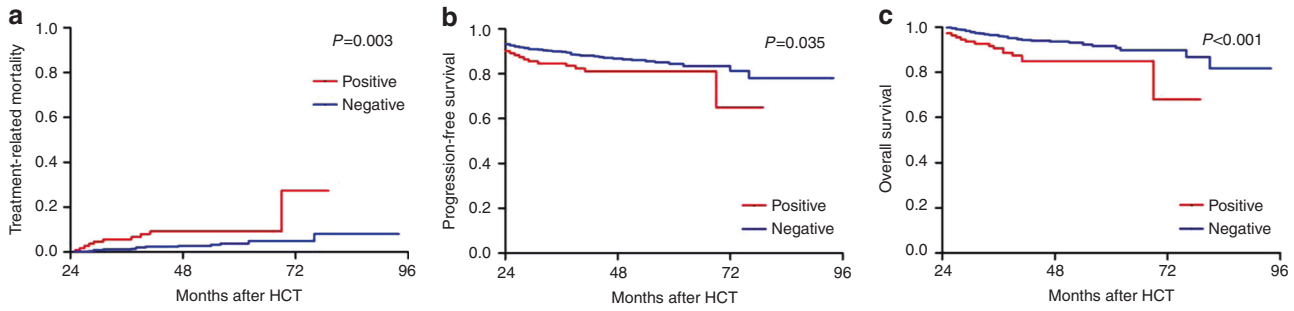

Fig. 5 Transplantation outcome of recipients between EBV-positive and negative group beyond 2 years. Comparison of: (**a**) treatment-related mortality; (**b**) progression-free survival; (**c**) overall survival.

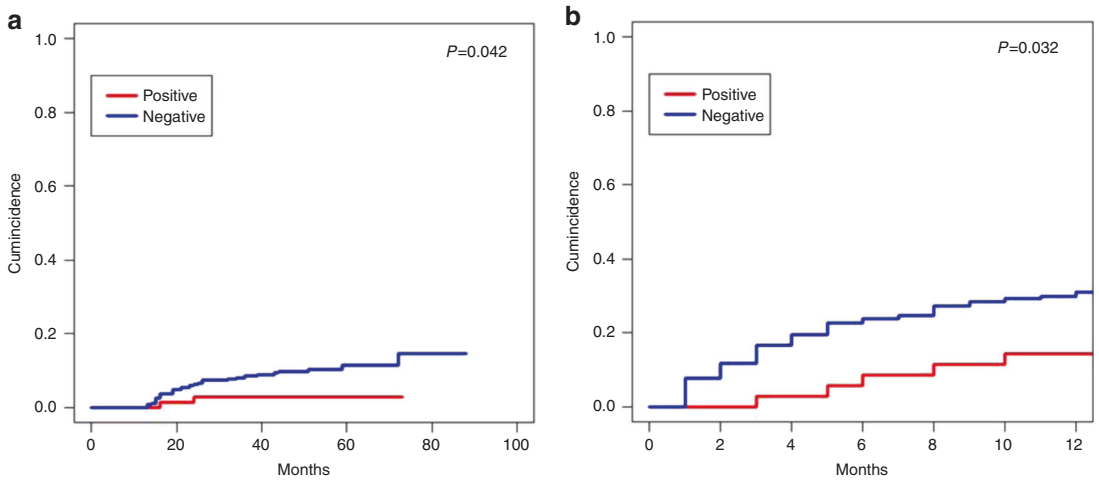

Fig. 6 Cumulative incidence of relapse between EBV-positive and negative group by time since transplantation. Comparison of: (**a**) recipients with malignancies in remission stage; (**b**) recipients with malignancies in advanced stage.

recipients suggest that administration of rituximab results in a positive outcome for ~90% of patients treated preemptively and 65% of patients with EBV-PTLD [10, 11, 31–37]. However, as a validated risk factor, haploidentical HCT has become the preferred alternative option for patients who lack a matched donor and currently accounts for >50% of allogeneic transplants in China [38]. In recent decades, the increasing use of haploidentical transplantation procedures [38–40], particularly the Beijing protocol, which employs ATG as GVHD prophylaxis [38, 41], has greatly increased the risk of EBV reactivation. Given the unknown data on EBV reactivation post-HCT in the current treatment environment, studies to update these data are warranted.

Despite the evolution of HCT protocols and antiviral therapies, independent risk factors for EBV reactivation seem to have remained constant, as identified in our study, including use of ATG, grafts from haploidentical donors, and appearance of chronic GVHD [12, 42–45]. To determine the cumulative impact of these independent risk factors, a risk factor evaluation model was created to compare the incidence of EBV reactivation according to the number of risk factors patients had. As a result, the cumulative incidence of EBV reactivation depended on the number of risk factors involved, which was in line with the research of Uhlin et al. [7]. ATG use is a well-recognized risk factor [12, 43, 44], which was confirmed in our study by both univariate and multivariate analyses. As GVHD prophylaxis, ATG could immunosuppress recipients to pave the way for EBV reactivation by removing T cells from both recipients and donors, which impairs the cellular immune function and/or prolongs the immunosuppressive periods after HCT. The impact of haploidentical donors is usually attributed to unavoidable T-cell depletion in vivo (mainly caused by ATG) or in vitro in various protocols. However, we found that a haploidentical graft was a risk factor independent of ATG, which potentially hinted an alternative pathway that increases the risk of EBV reactivation [46]. GVHD is a profound risk factor resulting from the impairment in specific immune responses due to cytokine storms [42]. Chronic stimulation as well as long-term immunosuppression for treatment may also lead to an increased risk of EBV reactivation [7, 45]. Age is another

reported risk factor, and previous studies [1, 47–49] indicated that both younger and older (age 50 years or older) patients could have an increased risk of EBV diseases and PTLDs. In our study, age was a risk factor identified in the univariate analysis rather than the multivariate analysis, presumably due to the higher proportion of haploidentical grafts in younger recipients than in older recipients.

With the development of effective virus management strategies and supportive care for HCT in recent years, the outcome of recipients with post-HCT EBV reactivation has obviously improved. Generally, no statistical differences in OS, TRM, PFS, or CIR were found between the groups with or without EBV reactivation, partially in accordance with the findings of Peric et al. [44]. However, controversial data about the impact of EBV reactivation on HCT outcome have also been published. Auger et al. indicated that controlled EBV reactivation in the setting of HCT was associated with superior OS, probably related to a significant increase in circulating NK cells [50]. Intriguingly, a different opinion presented by Li et al. showed that patients with high or very low levels of cell-bound EBV-DNA had a shorter OS than those with moderate EBV load, potentially attributed to the phenomenon of "sneaking through" [51]. According to our data, the impact of EBV reactivation was time-dependent and disease-dependent. We further found that the EBV-reactivated group showed inferior outcomes beyond 2 years after HCT, but CIR decreased in patients with malignancies, starting after 1 year post-HCT for recipients at stable status and immediately after HCT for recipients with progressive disease. A study from Hoegh-Petersen et al. [52] also suggested an improved CIR for EBV-reactivated recipients because of the quick reconstitution of EBV-associated T cells.

It should be noted that the tapering of immunosuppressive agents for EBV-positive patients might also contribute to an enhanced graft-versus-tumor (GVT) effect, although accompanied by an increased risk of GVHD. There are other recognized factors affecting the CIR and the outcomes, particularly the genetic abnormalities in malignancies. Because various entities were included in this study, no stratification system was applicable for all the enrolled patients. Moreover, the risk for patients with or without EBV reactivation was comparable ($P = 0.559$) when stratified by the NCCN guidelines and had no impact on EBV reactivation in the cohort of our study ($P = 0.594$).

Nevertheless, the conclusion of our study was restricted by several limitations, including the inherited drawbacks of a single-center retrospective study, the diversity of underlying diseases, deviations in treatments both pre- and post-HCT, disproportionate conditioning regimens, and insufficiency of representativeness. In addition, the impact of biological parameters such as EBV microRNA and immune reconstitution in EBV-reactivated recipients should also be further explored.

In conclusion, our study revealed that with current EBV management, PTLDs were efficiently controlled, although the incidence of EBV reactivation post-HCT remained high in patients with existing risk factors. The majority of EBV reactivation occurred in the first 2 months after transplantation, but haploidentical HCT recipients had longer exposure durations than recipients who received non-haploidentical grafts. The impact of EBV reactivation was multifactorial, depending on the underlying disease and time post-HCT. Our results need to be validated by multicenter prospective studies and further explored to facilitate an optimized EBV management strategy.

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Compliance with ethical standards

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

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