RESEARCH ARTICLE

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Incidence of Epstein-Barr Virus Reactivation and Its Risk Factors in Allogeneic Hematopoietic Stem Cell Transplant Recipients

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Abstract

Purpose: Epstein-Barr virus (EBV) reactivation in allogeneic hematopoietic stem cell transplantation (allo-HCT) recipients can lead to significant complications including post-transplant lymphoproliferative disease. Despite progress in managing EBV reactivation in allo-HCT recipients, data on clinical characteristics and prognostic implications of EBV viral load remain limited. Here, we aim to evaluate the prevalence, identify risk factors, and assess the clinical implications of EBV-DNA positivity in allo-HCT recipients. **Methods:** A retrospective audit of laboratory records for EBV load monitoring in allo-HCT recipients during the period from 2021 to 2023 was performed. EBV viral load was assessed using quantitative PCR. The medical records were reviewed for clinical features, identifying risk factors, and prognostic impact of EBV-DNA positivity. **Results:** A total of 40 patients with a median age of 20.5 years were included. Patients were divided into two groups based on presence (>600 copies/mL) and absence of EBV-DNA. We observed EBV-DNA positivity in 16 (40%) patients with a median EBV viral load of 12,400 copies/ml. Patients with EBV-DNA positivity exhibited a higher incidence of acute graft versus host disease (p=0.039). Patients with EBV-DNA positivity tended to have poorer 1-year overall survival and disease-free survival, although the results did not reach statistical significance. **Conclusion:** Our data highlights the importance of monitoring EBV viral load in predicting the outcome in terms of overall survival in allo-HCT recipients. The development of GVHD has also surfaced as a significant element increasing the likelihood of EBV-DNA positivity. It is imperative to conduct further research and establish comprehensive protocols for the routine monitoring and management of EBV post-allo-HCT.

Keywords: EBV- HCT- viral load- risk factors- GVHD

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HCT) is a lifesaving therapeutic approach for both malignant and non-malignant conditions. However, recipients of allo-HCT face an increased risk of complications due to the immunosuppressive effects induced by the conditioning regimen. One of the most critical complications is the reactivation of latent viruses such as Epstein-Barr virus (EBV), a ubiquitous herpesvirus that infects over 90% of the global population. EBV primarily infects B cells where it establishes a life-long latent infection. In healthy individuals, EBV reactivation is kept in check by natural killer cells, cytotoxic T lymphocytes, and antibody-dependent cytotoxicity. However, in immunocompromised allo-HCT recipients, EBV reactivation can lead to the development of post-transplant lymphoproliferative disorders (EBV-PTLD), a severe complication characterized by uncontrolled B cell proliferation, which contributes to substantial morbidity and mortality in this patient population [1, 2].

The documented occurrence of EBV reactivation following HCT varies widely, ranging from 0.1% to 63%, influenced by factors such as the type of transplant, usage of antiviral medications, monitoring strategies, and the sensitivity of diagnostic assays [1]. Across various studies, the overall incidence of EBV-PTLD ranges from 1.2% to 12.9% [3-5]. EBV-PTLD is characterized by early onset, rapid progression, widespread dissemination, and a high mortality rate. With EBV's estimated doubling time of 2-3 days, early intervention is critical for managing PTLD.

To effectively treat PTLD, it is crucial to understand the risk factors associated with its development and the prognostic markers that influence patient outcomes [3]. Several pre-transplant factors including ABO mismatch,

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HLA mismatch, conditioning regimens such as ATG-based conditioning, as well as CMV/EBV serostatus disparities; and post-transplant risk factors such as acute and chronic graft versus host disease (GVHD) and intensive immunosuppressive therapy, increase the risk of EBV reactivation [6, 7].

While the definitive diagnosis of PTLD requires a biopsy, conducting this invasive procedure in these patients is challenging considering their poor health status post-allo-HCT. Detection of EBV-DNA in the peripheral blood by quantitative polymerase chain reaction (qPCR) has emerged as a valuable tool for predicting the development of EBV-PTLD [8]. Previous studies have demonstrated that EBV viral load is significantly higher in patients who have undergone HCT compared to healthy controls, with the onset of EBV-PTLD often preceded by a further increase in viral load [2,8-10]. The implementation of routine EBV-DNA monitoring has been shown to reduce the incidence of EBV-PTLD by enabling early detection and intervention [9].

Given the lack of direct anti-viral therapies for EBV, preventive measures, reduction in the dose of immunosuppressive therapy (ISS), and preemptive treatment are key strategies for mitigating reactivation risks. Preemptive rituximab therapy is effective when EBV-infected cells are B cells. However, rituximab may further compromise the immune system after transplantation, increasing susceptibility to infections and other complications. Although reducing ISS doses is a commonly adopted strategy for managing EBV reactivation, standard guidelines for ISS reduction, are limited and its long-term impact on patient outcomes remains unclear [11].

Despite advancements in post-transplant care, recent data regarding the prevalence and features of EBV reactivation post-HCT are limited. This retrospective study aims to investigate the incidence of EBV reactivation, risk factors for EBV reactivation, and prognostic impact of EBV viral load in allo-HCT recipients.

Materials and Methods

This is a retrospective observational single-center study. A total of 40 patients who underwent allo-HCT in the Department of Clinical Hematology and Medical Oncology within a 2-year span (2021-2023) were included. EBV viral testing was performed in the Department of Immunopathology. Weekly monitoring of EBV load was conducted post-allo-HCT until day +90. For patients encountering complications such as compromised T cell reconstitution or GVHD flare, extended monitoring was initiated. However, there was variability in the timing of EBV viral load monitoring, attributed to requests from treating consultants and resource availability. However, timing for EBV viral load monitoring showed variability which was attributed to treating consultant requests and resource availability. Pertinent clinical details of these 40 patients were obtained from medical records. Consequently, we obtained a waiver for ethical clearance from our institutional ethics committee.

EBV load monitoring

EBV-DNA was monitored using real-time quantitative PCR (RT-PCR) by targeting the non-glycosylated membrane protein BNRF1 (p143) of the EBV genome. Amplification conditions were 95°C for 10 min, 45 cycles at 95°C for 30 s, and 60°C for 30 s. Viral load was calculated via threshold cycle (CT) values against known standard concentrations, with a 600 copies/ml detection threshold.

Statistical Analysis

Patients were divided into two groups based on the presence and absence of EBV-DNA (>600 copies/ml). Overall Survival (OS) was assessed as the time interval between the date of allo-HCT to the date of death. Disease-Free Survival (DFS) was estimated as the time interval between the date of allo-HCT to the date of first progression, recurrence, or death without progression. Baseline clinical characteristics between study groups were compared using Fisher's exact test for categorical variables and the Mann–Whitney U test for continuous variables. Survival was analyzed using the Kaplan–Meier curve to evaluate OS and DFS. Statistical significance was set at $p < 0.05$. GraphPad Prism software 8.0.2 facilitated analyses.

Results

Clinical characteristics of patients based on the presence and absence of EBV-DNA

A total of 40 patients were enrolled in the study, with a median age of 20.5 years (range; 1-56). Among them, 28 (70%) were males and 12 (30%) were females. Patients were divided into two groups based on presence (>600 copies/mL) and absence of EBV-DNA. EBV-DNA was detected in 16 (40%) patients, while it was absent in 24 (60%) patients. The underlying diseases among our patients included acute leukemias, with acute lymphoblastic leukemia (n=14, 35%) and acute myeloid leukemia (n=10, 25%) being the most common followed by aplastic anemia (n=7, 17.5%) and Hodgkin lymphoma (n=4, 10%). Other patients who underwent allo-HCT had conditions such as chronic granulomatous disease (CGD), thalassemia major, osteopetrosis, myelodysplastic syndrome (MDS), and chronic myeloid leukemia (CML).

There were no significant differences in ABO and HLA match versus mismatch, conditioning regimen (specifically ATG-based, with rabbit-ATG being predominantly used, and equine-ATG for aplastic anemia cases), chronic GVHD presence, and donor-recipient CMV/EBV serostatus match versus mismatch, CMV activation and other virus activation after allo-HCT between patients with EBV-DNA positivity and those without it. However, patients with EBV-DNA positivity had a higher frequency of acute GVHD (p=0.039). The characteristics of patients are detailed in Table 1.

Clinical characteristics of patients with EBV-DNA positivity

We conducted additional analysis on 16 patients who tested positive for EBV-DNA positivity (>600 copies/

Table 1. The Patients's Characteristics

S.No.	Characteristics	Patients with EBV positivity (N=16)	EBV negative patients (N= 24)	Total patients (N=40)	P value
1	Gender, no. (%)				
	Male	12 (75%)	16 (66.7%)	28 (70%)	
	Female	4 (25%)	8 (33.3%)	12 (30%)	
2	Median age (range)	14.7 (1-56) years	22.5 (8-53)	20.5 (1-56) years	
3	Underlying disease, no. (%)				
	ALL	5 (31.2%)	9 (37.5%)	14 (35%)	
	AML	6 (37.5%)	4 (16.6%)	10 (25%)	
	Aplastic anemia	3 (18.7%)	4 (16.6%)	7 (17.5%)	
	Hodgkin lymphoma	1 (6.2%)	3 (12.5%)	4 (10%)	
	Other	1 (6.2%)	4 (16.6%)	5 (5%)	
4	HLA match status, no. (%)				0.999
	Full match family donor	7 (43.7%)	14 (58.3%)	21 (52.5%)	
	Haploidentical match	7 (43.7%)	10 (41.7%)	17 (42.5%)	
	Unrelated matched donor	2 (12.5%)	-	2 (12.5%)	
5	Blood group, no. (%)				0.5255
	Matched	6 (37.5%)	12 (50%)	18 (45%)	
	Mis-matched	9 (56.2%)	11 (45.8%)	20 (50%)	
	Rh mismatched	1 (6.25%)	1 (4.2%)	2 (5%)	
6	ATG regimen, no. (%)	5 (31.3%)	5 (20.8%)	10 (25%)	0.4824
7	Donor-recipient relationship, no. (%)				0.324
	Father	2 (12.5%)	6 (25%)	8 (20%)	
	Sibling	11 (68.7%)	18 (75%)	29 (72.5%)	
	Unrelated	3 (18.7%)	-	3 (7.5%)	
8	Donor Gender, no. (%)				0.812
	Male	10 (62.5%)	13 (54.2%)	23 (57.5%)	
	Female	6 (37.5%)	11 (45.8%)	17 (42.5%)	
9	GVHD, no. (%)				
	Acute GvHD	8 (50%)	4 (16.6%)	12 (30%)	0.0367
	Chronic GVHD	1 (6.25%)	4 (16.6%)	5 (12.5%)	0.6309

mL) post-allo-HCT. Among these patients, eleven (69%) reached peak EBV-DNA levels before post-transplant day 100, while 5 (31%) reached peak levels after 100 days following transplantation. Further, we categorized patients based on the viral load which was 2-3log (> 600 copies/mL) in 5 (31%) patients, 4log (> 10,000 copies/mL) in 8 (50%) patients, 5log (> 100,000 copies/mL) in 2 (13%) patients, and 6log (> 1,000,000 copies/mL) in 1 (6%) patient. Among these 16 patients, 9 experienced two or more episodes of EBV-DNA positivity after transplantation. The median duration of EBV viremia was 11 days (range: 7–41 days), with a median onset at 67.5 days (range: 3–365 days). The median EBV-DNA copy numbers in the first positive blood sample were 4.8×10^3 (range: 6.36×10^2 to 4.8×10^7). Rituximab was administered to patients (5/16) whose viral load was consistently high up to 40,000 copies/mL, however, the rest of the patients with low EBV-DNA positivity resolved

Table 2. Impact of EBV Reactivation on Clinical Outcome

No.	Clinical outcomes	Patients with EBV positivity (N=16)	EBV negative patients (N= 24)	Total patients (N=40)	P value
1	EBV Viremia				
	Time of first EBV viremia, median (range)	67.5 (3-365) days			
	Duration of EBV viremia	11 (7-41) days	-	-	-
2	Overall survival at 1-years				0.4414
	Alive	11 (68.75%)	19 (79.2%)	30 (75%)	
	Death	5 (31.2%)	5 (20.8%)	10 (25%)	
3	Disease free survival at 1-years	9 (56%)	17 (71%)	26 (65%)	0.4124

spontaneously.

Survival of allo-HCT patients with positive EBV-DNA

The median follow-up time for all the patients was 13.5 months (range: 6-24 months). Thus, we set the OS time-point at 12 months (1-year). The OS rate of EBV-DNA positive patients at 1 year was 68.7% (11/16), slightly lower than 79% (19/24) in the EBV-DNA negative patients, though these differences lacked statistical significance. Median OS at 1-year post-allo-HCT for the five EBV-DNA positive patients (who died) was 9 ± 4 months.

The DFS rate for EBV-DNA positive patients was 56%, (9/16) while the EBV-DNA negative patients exhibited a 71% DFS rate (17/24) (Table 2).

Discussion

This retrospective study aimed to explore the incidence, risk factors, and prognostic impact of EBV-DNA positivity in allo-HCT patients. Our findings reveal a notable occurrence (40%) of EBV-DNA positivity in allo-HCT recipients. This trend aligns with reported incidences in pediatric and adult allo-HCT studies [10, 11]. Further, no PTLD development was observed among our patient cohort with a median follow-up of 13.5 months.

Upon analyzing patient characteristics of EBV positivity, we identified acute GVHD as a significant risk factor associated with EBV-DNA positivity. Acute GVHD, a condition triggered by immune cells attacking recipient tissues, creates an environment conducive to EBV reactivation due to immune dysregulation. EBV reactivation, in turn, exacerbates GVHD by promoting tissue damage. The intricate interplay between EBV reactivation and GVHD underscores the bidirectional nature of their relationship, consistent with previous research [12, 13]. Although we explored other potential risk factors such as recipient age, donor-recipient relationship, HLA match status, and conditioning regimens, our small study cohort limited the detection of significant differences.

Upon analyzing EBV-positive patients, we observed distinct outcomes based on viral load levels. Patients with low EBV positivity (≤ 40,000 copies/ml of blood) resolved spontaneously without requiring B-cell depletion therapy. However, individuals with progressive or high viral loads (≥ 40,000 copies/ml) received rituximab, leading to the absence of EBV viremia upon subsequent analysis. The threshold values for initiating rituximab vary widely in clinical practice, highlighting the necessity for serial EBV viral load monitoring [7, 14, 15]. Establishing precise thresholds for initiating EBV-specific therapy warrants comprehensive investigation through large prospective studies.

Additionally, our study observed decreased overall one-year survival and disease-free survival in allo-HCT patients with EBV-DNA positivity, though statistical significance was not reached. As EBV-positivity remains a significant concern among allo-HCT recipients, implementing monitoring guidelines is crucial for early detection and intervention.

In conclusion, our study underscores the complexities surrounding EBV reactivation in allo-HCT recipients. It highlights the need for vigilant monitoring and the development of tailored guidelines to manage EBV-related risks effectively. While limitations persist due to its small sample size and absence of EBV-specific IgM and IgG antibody data, our findings contribute to the broader knowledge of EBV epidemiology in allo-HCT and its implications for patient care.

Author Contribution Statement

L.R: Data generation and interpretation and manuscript preparation, A.K: provided clinical data and valuable clinical insights, J.S. and S.A helped in data generation, M.K: helped in statistical analysis and tables formation, P.M: clinical data and inputs, R.W.M: Study design, data generation, interpretation, and manuscript preparation.

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Ethical declaration

This study is retrospective in nature. As a result, ethical clearance was waived by the Institutional Ethics Committee (intramural) of PGIMER, Chandigarh, India (IEC No. INT/IEC/2023/SPL-649)."

Availability of data

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare that they have no competing interests.

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