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Central nervous system post-transplant lymphoproliferative disorder after allogeneic hematopoietic stem cell transplantation: The Nagasaki transplant group experience

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ABSTRACT

A 17-year-old male received allogeneic transplantation for acute lymphoblastic leukemia, and presented with generalized seizures due to a solitary brain lesion with massive necrosis on day +621. Epstein–Barr virus (EBV) DNA copies were below the cut-off value in plasma. Stereotactic biopsy of the cerebral lesion confirmed the diagnosis of post-transplant lymphoproliferative disorder (PTLD) with large atypical cells positive for CD20 and EBER. In order to diagnose primary central nervous system PTLD, the biopsy should be applied as early as possible when brain lesion with necrosis develops in post-transplant patients regardless of EBV-DNA in plasma.

1. Introduction

Post-transplant lymphoproliferative disorder (PTLD) is characterized by lymphoid or plasmacytic proliferation in a recipient after allogeneic hematopoietic stem cell transplantation (allo-HSCT) or solid organ transplantation. PTLD is regarded as one of the most serious post-transplant complications due to its high mortality [1]; therefore, an early diagnosis is important for the initiation of optimal interventions. In most cases, the outgrowth of donor-derived Epstein–Barr virus (EBV)-infected B cells results in the development of PTLD. EBV DNA monitoring using the quantitative polymerase chain reaction (qPCR) method was previously reported to be a sensitive modality for the early diagnosis of EBV-positive PTLD [1,2] because patients at an increased risk of overt PTLD development presented with EBV reactivation.

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Although different assays using whole blood, plasma, and peripheral blood mononuclear cells (PBMC) have been used to monitor EBV-positive PTLD, the qPCR method for EBV DNA in plasma is regarded as a more reliable assay than that in PBMC [3].

PTLD is a rare complication with an incidence of 1–3% among recipients after allo-HSCT [2,4] and represents a clinical heterogeneous manifestation. Due to the limited amount of information on primary central nervous system PTLD (CNS-PTLD) after allo-HSCT, the diagnostic value of EBV DNA copies in post-transplant patients with CNS-PTLD remains poorly understood.

We herein report a case of CNS-PTLD after allo-HSCT for which the qPCR method in PBMC, plasma, and cerebrospinal fluid (CSF) showed less evidence of EBV reactivation.

2. Case report

A 17-year-old male was diagnosed with acute T cell lymphoblastic leukemia (ALL) with the normal karyotype and SIL-TAL1 chimeric transcription. A CSF examination showed no evidence of CNS involvement. The patient achieved complete remission after induction therapy; however, due to allergies to methotrexate and L-asparaginase, he was unable to receive the standard consolidation program. Human leukocyte antigen 7/8 allele-matched (Cw-mismatched) unrelated bone marrow transplantation from a female donor without any T-cell depletion was performed using a myeloablative conditioning regimen (cyclophosphamide 120 mg/kg and total body irradiation 12 Gy/6 fr.). Tacrolimus and mycophenolate mofetil (MMF) were used as graft-versus-host disease (GVHD) prophylaxis. Neutrophil engraftment was achieved on day +12 and an XY-fluorescence *in situ* hybridization analysis revealed complete donor chimerism. He developed acute GVHD grade II on day +32, and the administration of prednisolone at 1.0 mg/kg was initiated.

The patient presented with the disturbance of consciousness due to generalized seizures during the GVHD treatment with tacrolimus 1.5 mg, prednisolone 7.5 mg, and MMF 2000 mg daily on day +621 after allo-HSCT. A peripheral blood count yielded a leukocyte count of 3.7×10^9/L, consisting of 26% neutrophils, 49% lymphocytes, and 25% monocytes; hemoglobin level, 13.6 g/dL; platelet count, 181×10^9/L. A lymphocyte subset analysis by flow cytometry showed that the percentages of CD22-positive cells, CD3-positive cells, and CD56-positive cells were 9.1, 81.3, and 12.1%, respectively. Magnetic resonance imaging (MRI) of the brain revealed a space-occupying lesion with ring enhancement and perifocal edema in the left front-parietal lobe (Fig. 1A, B), indicating several differential diagnoses, including opportunistic infections, PTLD, and the extramedullary relapse of ALL. Routine microbiological tests to detect bacteria, fungi, toxoplasma IgG, and interferon-gamma in blood samples were negative. The cell count in CSF was 4/mm^3 with small mononuclear cells. The EBV serostatus was as follows: anti-EA-DR IgG < × 10; anti-VCA IgM < × 10, anti-VCA IgG × 20, and anti-EBNA-IgG < × 10.

PBMC were separated after a Ficoll-Hypaque density gradient; and CD19-, CD3-, and CD56-positive cells were selected using immunomagnetic beads (Dynabeads M-450, Veritas, Tokyo, Japan.). DNA was extracted from PBMC, selected cells, whole blood, the plasma fraction, and CSF. A PCR assay was performed using the Taq-Man PCR kit (PE Applied Biosystems, Foster City, Calif.), as previously described [5]. EBV DNA copy numbers in plasma and CSF were below the cut-off value (1.0×10^2 copies/ml) (Table 1). The EBV DNA copy number was 1.1×10^2 copies/10^5 PBMC. The qPCR assay revealed that the EBV DNA copy number in the CD19-positive cell fraction was elevated (2.8×10^3 copies/10^5 cells), whereas those in the CD3- and CD56-positive cell fractions were not. A bone marrow examination showed complete donor chimerism and no evidence of ALL relapse due to the absence of SIL-TAL1 chimeric transcription. Stereotactic biopsy of the cerebral lesion confirmed the diagnosis of monomorphic PTLD with massive necrosis and large atypical cell proliferation. Immunohistochemical staining showed that large atypical cells were positive for CD20 and negative for CD3. A small number of EBV-encoded small RNA (EBER)-positive cells were detected (Fig. 1C–F). The biopsy sample was too small to evaluate the origin of PTLD cells by XY-fluorescence *in situ* hybridization.

To treat CNS-PTLD, tacrolimus was reduced, whereas difficulties were associated with the cessation of immune suppressants because of the progression of chronic GVHD. MRI of the brain showed an enlarged tumor on day +840, which indicated the progression of CNS-PTLD. He did not respond to three courses of the weekly administration of rituximab (375 mg/m^2). Local irradiation therapy (20 Gy/10 fr.) for CNS-PTLD was subsequently initiated on day +931, but was stopped after 5 fractions because of sepsis and progressive GVHD, and the patient died of multiorgan dysfunction on day +1018.

3. Discussion

The present case developed CNS-PTLD from day 620 after allo-HSCT, with the use of an unrelated bone marrow graft and the prolonged administration of immunosuppressive agents being risk factors for PTLD [1]. Among 580 patients who underwent their first allo-HSCT at the Nagasaki Transplant Group between January 1, 1990 and April 31, 2018, we encountered the first case of CNS-PTLD (0.17%), which was in line with its rarity after allo-HSCT, as previously reported [6]. In terms of a detailed analysis to detect EBV DNA and MRI findings, our results provided important insights into diagnostic modalities for CNS-PTLD.

The most interesting result of this case was that EBV DNA copy numbers in plasma and CSF remained below the cut-off value. This result was not consistent with the findings of a previous study on a large cohort showing that the EBV DNA copy number in plasma was a more sensitive marker to diagnose EBV-related diseases, including PTLD [1,3]. One possible reason for the present results was that EBV DNA in plasma was insufficient to reflect virus shedding from the CNS lesion. This has also been reported in cases of CNS-PTLD after solid organ transplantation [7]. Therefore, our results suggest that the careful interpretation of EBV DNA in plasma is needed when attempting to diagnose CNS-PTLD among post-transplant patients.

EBV DNA copy numbers in PBMC in the present case were lower than those in cases of EBV-positive PTLD without CNS lesions, although the early symptom of EBV-positive PTLD is frequently increasing levels of EBV DNA copies in PBMC [3]. This result of the present case was, at least in part, due to the lower percentage of the B-cell fraction in the lymphocyte subset during intensive immunosuppressive treatment for active GVHD. Based on these results in the present case, the monitoring assays for EBV DNA in plasma and PBMC using qPCR may be insufficient to establish a probable diagnosis of CNS-PTLD after allo-HSCT.

Ring enhancement on MRI was observed in between 4 and 11% of patients with primary CNS lymphoma, and in approximately 75% of PCNSL in immunocompromised patients, such as post-transplant and human immunodeficiency virus (HIV)-infected patients [8]. These MRI findings reflect pathological findings that CNS-PTLD may have necrotic lesions [9]. We also considered differential diagnoses, such as toxoplasmosis, abscess, tuberculosis, relapsed ALL, and PTLD. Based on the diagnostic value of EBV DNA in plasma and PBMC, it is important to note that early biopsy of brain lesions needs to be considered for post-transplant patients who developed brain mass lesions with ring enhancement in order to accurately diagnose CNS-PTLD. Since recent studies reported that the intrathecal administration of rituximab was effective for CNS-PTLD [10], early biopsy after MRI may be a promising diagnostic modality for the provision of specific therapy.

In conclusion, CNS-PTLD needs to be considered in post-transplant patients who present with brain mass lesions with ring enhancement as well as the early biopsy of cerebral lesions regardless of the EBV DNA copy number. Further clinical and experimental investigations are

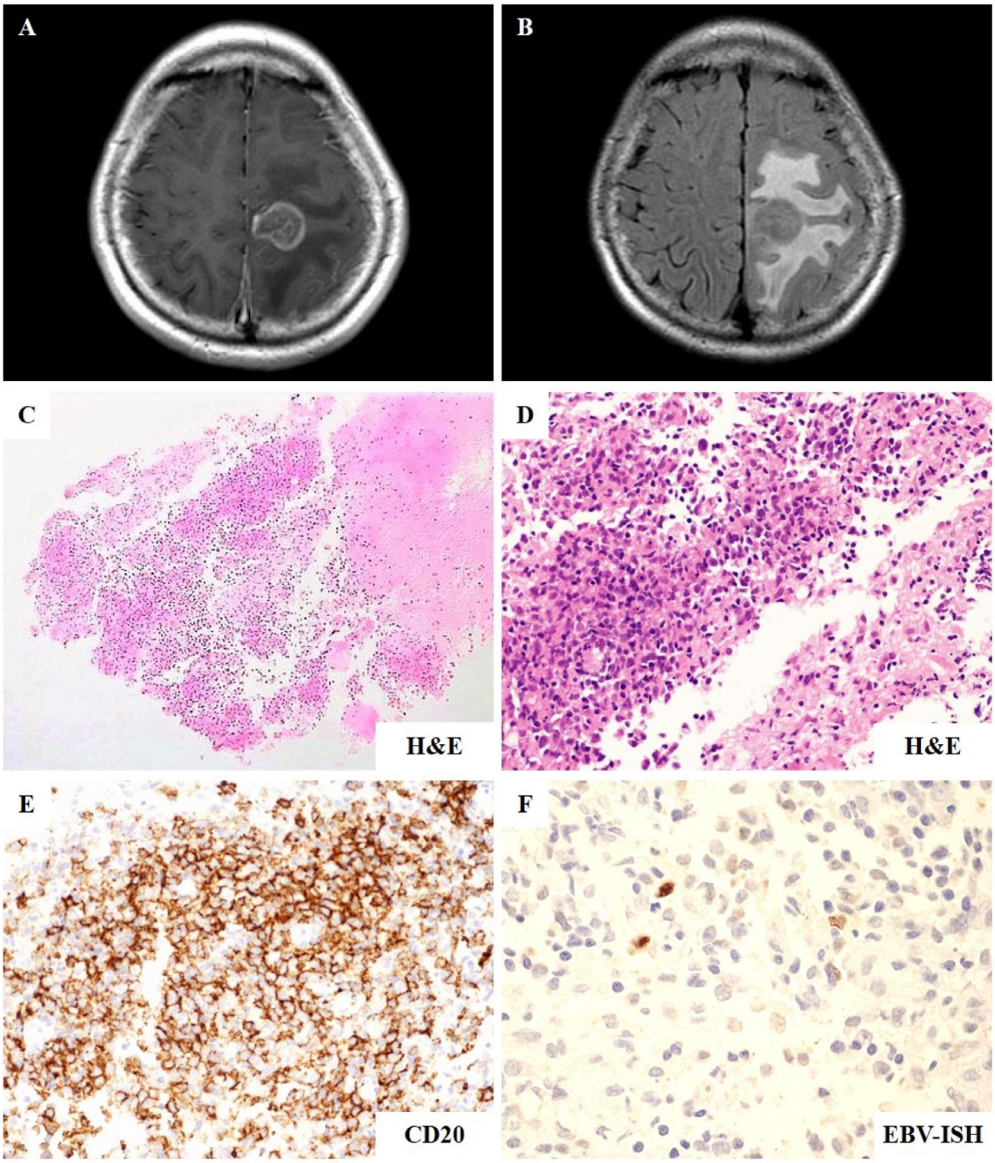

Fig. 1. MRI findings of CNS-PTLD and histopathological features of CNS-PTLD.
Axial gadolinium-enhanced T1-weighted imaging (A), and fluid-attenuated inversion recovery (FLAIR) on magnetic resonance images (MRI) (B). MRI showed an approximately 20-mm ring-enhanced lesion in the left front-parietal lobe with perifocal edema.
Cerebral biopsy showed extensive necrosis (C; H&E stain, ×100) and the infiltration of large atypical lymphocytes (D; H&E stain, ×400). Atypical cells were positive for CD20 (E; ×400). A small number of Epstein–Barr virus (EBV)-encoded small RNA-positive cells were detected (F: ×600).

Table 1
Results of the qPCR assay for EBV DNA.

Specimen	Results	
Whole blood	5.0×10^3	copies/ml
Plasma	$<1.0 \times 10^2$	copies/ml
PBMC	1.1×10^2	copies/10^5 cells
CD3+ cells	9.2	copies/10^5 cells
CD19+ cells	2.8×10^3	copies/10^5 cells
CD56+ cells	8.2	copies/10^5 cells
CSF	$<1.0 \times 10^2$	copies/ml

Abbreviations; EBV, Epstein–Barr virus; qPCR, quantitative polymerase chain reaction; PBMC, peripheral blood mononuclear cells; CSF, cerebrospinal fluid.

required to develop optimal monitoring methods and diagnostic modalities for CNS-PTLD.

Conflict of interest

The authors declare no conflicts of interest associated with this manuscript.

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