Case Report

Epstein-Barr Virus Encephalitis and Disseminated Adenovirus Infection after Haploidentical Allogeneic Hematopoietic Stem Cell Transplantation for a Patient with Ph-Like Acute Lymphoblastic Leukemia

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Abstract
Viral reactivation or infections are common complications after allogeneic hematopoietic stem cell transplantation, especially in haploidentical transplantation. Here, we presented a young patient with Ph-like acute lymphoblastic leukemia who suffered Epstein-Barr virus (EBV) encephalitis and disseminated adenovirus (ADV) infection after haploidentical peripheral blood (PB) stem cell transplantation. The patient was a 16-year-old boy and received PB stem cells from his HLA-haploidentical matched father. On day +44 after transplantation, he had viremia with cytomegalovirus, and EBV was diagnosed as EBV encephalitis after 2 weeks. On day +117, he had disseminated ADV infection and fulminant ADV hepatitis. It is very rare that successive EBV encephalitis and fulminant ADV hepatitis are present in the same patient. We summarized risk factors, clinical manifestations, diagnostic criteria, and effective treatments about EBV encephalitis and disseminated ADV infection. We try to enhance our understanding of the prevention, diagnosis, and potential treatment of EBV and ADV disease by reviewing the entire procedure.

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**Introduction**

Ph-like acute lymphoblastic leukemia (Ph-like ALL) as a high-risk subgroup of B-ALL was identified in children, adolescents, and young adults. The leukemic-cell gene expression profile of Ph-like ALL is similar to that of Ph + ALL; however, instead of BCR-ABL, such patients harbor a highly diverse range of genetic alterations, activating tyrosine kinase signaling. Ph-like ALL comprises up to 15% of childhood B-ALL and 20%–25% in adolescents and young adults. These patients have a very high rate of disease relapse and poor overall survival. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) might improve the prognosis of patients with Ph-like ALL.

**Case Report**

A 16-year-old boy was admitted to our center in June 2018 with an 8-month history of Ph-like ALL. Originally, initial complete blood count revealed anemia (77 g/L), leukocytosis (53 $\times$ 10^9/L), and normal platelet count. Bone marrow smear showed 87% leukemic blasts, which were positive for CD10, CD19, CD22, CD34, and TdT and negative for myeloid markers with immunophenotyping as common B subtype of ALL. There was no any detectable abnormality by conventional cytogenetic and genetic studies. Deletions of exon 1–8 of IKZF1 gene, the fusion of P2RY8 and CRLF2 gene, and the mutation of CRLF2 gene were found with bone marrow cells by next-generation sequencing (NGS). He was diagnosed with Ph-like ALL.

The patient was referred to our center for allo-HSCT when he got the second complete remission in June 2018. The boy was 1.88 m in height, 118 kg in weight, and 33.4 in body mass index. His body surface area was 2.57 m^2. He received myeloablative conditioning with etoposide 30 mg/kg, cyclophosphamide 120 mg/kg, and total body irradiation 10 Gy. Cyclosporine A, short-term methotrexate, and antithymocyte globulin (ATG, Thymoglobulin) 10 mg/kg were administered to prevent graft versus host disease (GVHD). On July 2, 2018, he received peripheral blood (PB) stem cell graft with 2.6 $\times$ 10^6/kg CD3+T and 9.7 $\times$ 10^6/kg CD34+ cells from his HLA-haploidentical-matched father. The time of neutrophil engraftment was on day +14, and platelet engraftment on day +15. Complete chimerism was achieved on day +14. The patient developed grade I acute GVHD with skin rash on day +17 but resolved quickly after low-dose (0.5 mg/kg/day) methylprednisolone treatment.

On day +44 posttransplant, the young patient was readmitted because of persistent high fever for 2 days, without cough, diarrhea, or other clinical symptoms. EBV-DNA and CMV-DNA were 6.6 $\times$ 10^5 copies/mL (cutoff value: 1,000 copies/mL) and 2.8 $\times$ 10^4 copies/mL (cutoff value: 1,000 copies/mL) in PB samples, respectively, by quantitative polymerase chain reaction (PCR). He was seriously in lack of functional T lymphocytes (CD3+7, CD4+1, and CD8+6 cells/UL). Blood culture for *Staphylococcus epidermidis* was positive. Computed tomography (CT) of the chest, paranasal sinus, and magnetic resonance imaging (MRI) of the epigastrium were all negative. Imipenem 500 mg q6h and linezolid 300 mg q12h were prescribed intravenously for bacterial infection. Ganciclovir 5 mg/kg/12 h and two doses of rituximab 375 mg/m^2 per week were administered for EBV infection. His body temperature declined to around 38°C, and EBV-DNA and CMV-DNA loads in PB decreased to 2.5 $\times$ 10^5 and 4.5 $\times$ 10^4 copies/mL, respectively, after 2 weeks. However, on day +61, he suffered sudden grand mal epileptic seizure with high fever of 40.3°C and was in immediate coma. The cranial CT scan was normal. The EBV-DNA load declined to 1.1 $\times$ 10^4 copies/mL, and CMV-DNA was negative in PB at that time. Cyclosporine A was discontinued for suspicion of a central nervous system (CNS) adverse effect. Lumbar puncture was performed with high cerebrospinal fluid (CSF) pressure of 280 mm H$_2$O, white blood cell count of 101 $\times$ 10^6/L, and protein 2.1 g/L.
Flow cytometry analysis of the CSF showed no leukemia blasts. CSF-related laboratory examinations were shown in Table 1. EBV-DNA was detected at the level of $3.5 \times 10^4$ copies/mL in the CSF by PCR and 14 gene sequences by NGS. Based on these findings, EBV encephalitis was diagnosed [1]. Ganciclovir 5 mg/kg/12 h, foscarnet 40 mg/kg/8 h, dexamethasone 30 mg/day, and high-dose gamma globulin 0.4 g/kg/day for 5 days were given for the treatment of EBV encephalitis. The 3rd dose of rituximab of 375 mg/m² was given intravenously and then, rituximab 30 mg and dexamethasone 5 mg intrathecally. He regained consciousness without any abnormality 3 days later. Intrathecal injection of rituximab 30 mg was repeated every the other day (a total of 3 times) until EBV-DNA was negative in the CSF by PCR on day +69. The patient was discharged from the hospital soon. The results of complete blood count, viral load in PB, liver function, and body temperature were shown in Table 2.

Table 1. CSF-related laboratory examinations

|               | d61 | d63 | d65 | d69 | d118 |
|---------------|-----|-----|-----|-----|------|
| CSFP, mm H₂O  | 280 | 350 | 295 | 200 | 310  |
| Leukocyte, ×10⁹/L | 101 | 80  | 51  | 6   | 1    |
| Protein assay, g/L | 2.1 | 1.2 | –   | 0.6 | 0.5  |
| LDH, U/L      | 33  | 38  | –   | 26  | 24   |
| Chloride, mmol/L | 116 | 113 | –   | 118 | 115  |
| Glucose, mmol/L | 2.6 | 3.2 | –   | 2.3 | 3.2  |
| Blast         | Negative | Rare | Negative | Negative | Negative |
| NGS           | –   | Negative | EBV+ | –   | ADV+  |

CSFP, cerebrospinal fluid pressure; –, not done.

Table 2. The dynamic changes of blood count, viral load, liver function test and body temperature

|               | d44 | d48 | d51 | d55 | d58 | d62 | d65 | d69 | d72 | d117 | d120 | d121 |
|---------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|
| T-max, °C     | 38.9| 38.7| 38.9| 38.6| 38.4| 40.2| 37.6| 37.5| 37.8| 38.9 | 38.9 | 37.5 |
| Leukocyte, ×10⁹/L | 5.1 | 4   | 3.8 | 2.3 | 4.6 | 7.4 | 4.5 | 1.5 | 3   | 2.85 | 0.5  | 0.4  |
| Hemoglobin, g/L | 106 | 106 | 90  | 88  | 95  | 76  | 70  | 81  | 127 | 107  | 115  |      |
| Platelet, ×10⁹/L | 76  | 61  | 40  | 35  | 45  | 44  | 33  | 53  | 26  | 43   | 14   | 14   |
| Lymphocyte, ×10⁹/L | 0.2 | 0.6 | 0.6 | 1.9 | 1.8 | 3.9 | 5.1 | 1.6 | 1.3 | 1.8  | 0.2  | 0.2  |
| CMV-DNA, copies/mL | –  | 1,250| 28,800| 0 | 4,500| 0 | 0   | 0   | 0    | 0    | 0    | 0    |
| EBV-DNA, copies/mL | –  | 159,800| 664,587| 529,821| 24,850| 10,783| 5,300| 0   | 0    | 0    | 0    | 0    |
| ALT, U/L       | 85  | 51  | 38  | 52  | 51  | 65  | 46  | 172 | 143 | 146  | 618  | 2,370 |
| AST, U/L       | 43  | 30  | 37  | 52  | 60  | 61  | 46  | 93  | 66  | 136  | 1,312 | 6,485 |
| LDH, U/L       | 459 | 387 | 384 | 372 | 405 | 548 | 408 | 361 | 333 | 475  | 1,812 | 6,873 |
| γ-GT, U/L      | 70  | 81  | 71  | 192 | 297 | 192 | 153 | 148 | 137 | 320  | 417  | 546  |
| AKP, U/L       | 67  | 58  | 54  | 51  | 69  | 58  | 40  | 39  | 62  | 74   | 77   | 128  |
| TB, µmol/L     | 23  | 16  | 18  | 18  | 21  | 24  | 16  | 25  | 35  | 68   | 60   | 87   |
| DB, µmol/L     | 10  | 6   | 6   | 6   | 7   | 10  | 5   | 7   | 10  | 35   | 25   | 34   |

d44, the 44th day after allo-HSCT; T-max, the highest temperature of the day; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactic dehydrogenase; γ-GT, gamma glutamyl transpeptidase; AKP, alkaline phosphatase; TB, total bilirubin; DB, direct bilirubin
On day +117 after transplantation, he was admitted to our center again because of diarrhea, nausea, vomiting, and mild fever after eating steak 1 day ago. Blood counts showed that hemoglobin was 127 g/L, leukocyte $2.8 \times 10^9$/L, and platelet $43 \times 10^9$/L. Functional T-lymphocyte counts of CD3+, CD4+, and CD8+ were 2,884, 121, and 2,659 cells/μL in PB, respectively. Biochemical tests showed elevated aminotransferases (ALT 146 U/L and AST 136 U/L), lactate dehydrogenase (475 U/L), and total bilirubin (68 μmol/L). EBV-DNA and CMV-DNA were negative in PB, but ADV-DNA was positive at $5.4 \times 10^7$ copies/mL in PB and $9.8 \times 10^7$ copies/mL in his stool. After receiving broad-spectrum antibiotics with meropenem and linezolid, the patient's symptom resolved within 2 days. On day +120, blood routine examination showed blood cells sharply decreased with neutrophil of $0.5 \times 10^9$/L, platelet of $14 \times 10^9$/L, and hemoglobin of 107 g/L. Liver function tests showed his liver enzyme increased remarkably. The next day, the patient had a shock with massive gastrointestinal hemorrhage and extensive spontaneous skin hemorrhage. Liver enzymes increased dramatically with the peak value of AST of over 6,000 U/L and lactate dehydrogenase of 6,873 U/L, along with significant prolongation of coagulation time. Detailed liver function tests were shown in Table 2. ADV-DNA was detected in blood and CSF samples by NGS with 6,742 and 450 gene sequences, respectively. The patient was finally diagnosed with disseminated adenovirus infection and fulminant hepatic function failure [2]. Unfortunately, the young patient passed away on day +121.

**Discussion**

EBV encephalitis is one of the common CNS complications, which accounts for about 17% of total viral encephalitis after allo-HSCT, with a fatality rate as high as 33% [3]. Risk factors for EBV encephalitis are T-cell depletion of the graft, use of ATG, unrelated or HLA-mismatched transplant, and acute and chronic GVHD [4]. The present case had high risk of EBV reactivation after HLA-haploidentical transplantation and GVHD prophylaxis with ATG. Cranial CT often showed no significant abnormalities, and MRI is more capable of detecting abnormalities than CT. The patient was unable to have cranial MRI due to coma, whereas the MRI was negative when he became awake. The current therapy for EBV infection of CNS includes rituximab, reduction of immunosuppressant, and EBV-specific cytotoxic T-lymphocyte infusion [5]. In this case, the patient ceased cyclosporine A, was treated by preemptive rituximab until the clearance of EBV-DNA in PB, and intrathecal injection of rituximab until the removal of EBV-DNA in the CSF. Preemptive rituximab was an effective treatment for EBV infection despite inducing a delayed B-cell reconstitution [6]. Of note, the efficacy of rituximab by intravenous infusion was poor for posttransplant lymphoproliferative disorder in the CNS because of the blood-brain barrier [7]. A study demonstrated that intrathecal administration of rituximab was effective and safe for pediatric patients, and the response rate was 88.9% [8].

The incidence of ADV viremia was 18% in children and only up to 6% in adults [9]. Disseminated adenovirus infection is uncommon in adult transplant recipients but can prove fatal at the rate of 26% [9]. Cord blood transplantation, T-cell depletion of graft, severe lymphopenia (CD3+ T <300 cells/μL in PB), treatment with ATG, and grade II-IV acute GVHD are highly associated with ADV infection. Our patient was at high risk due to GVHD prophylaxis with ATG and lymphopenia. Diagnosis of ADV disease is typically made by combining clinical features with detection of viral DNA using PCR or identification of viral inclusions on histopathology [10]. The clinical features of fatal hepatic function failure caused by ADV include high fever, explosive rise of transaminases, hypodense lesions in the...
hepatic by a CT scan, hemodynamic lability, and a bleeding diathesis [11]. Our case is consistent with these clinical characteristics as defined above. Detection of ADV in the stool, blood, and other body fluids by quantitative PCR or NGS was used for the diagnosis of ADV infection in immunocompromised patients [12]. Liver biopsy is considered the gold standard but limited for the diagnosis due to the invasive procedure. Our patient was at high risk of developing disseminated adenoviral disease due to lymphopenia and viral copies above 1,000/μL, which increases the risk of mortality to as high as 70% [2]. Effective therapy of ADV infection in immunocompromized patients included reduction of immunosuppression agents and valid antiviral treatment [13]. Antiviral drugs like CMX001 (brincidofovir) is an unlicensed orally bioavailable lipid conjugate of cidofovir, which has been extensively used as a preemptive anti-adenoviral therapy [14]. It has activity against adenovirus in immunosuppressed animal models and is currently under investigation for adenovirus infection in phase III trials (ClinicalTrials.gov identifier: NCT02087306). Other treatments like infusion of ADV specific T-cells expanded from the donor or a third party may be of some benefits.

**Conclusion**

In conclusion, successive EBV encephalitis and disseminated ADV infection in the same patient were very rare. Frequent monitoring of virus loads and prompt immune reconstitution after transplant might reduce virus infections.

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**Statement of Ethics**

This study was approved by Clinical Research Ethics Committee of Shanghai General Hospital, China. All procedures in this study were conducted in accordance with the Shanghai Municipal Clinical Research Ethics Committee approved protocols (reference number: 2021SQ324). Written informed consent was obtained from patient’s father for publication of this case report and any accompanying images.

**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

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**Author Contributions**

The first author Xiao Zhou was responsible for data collection and analysis and article writing. The coauthor Xianmin Song conceptualized the idea of the article. The corresponding author Liping Wan gave guidance on the idea and language of the article. The authors report no conflict of interest.

**Data Availability Statement**

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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