

# An Epstein-Barr Virus-Associated Leukemic Lymphoma in a Patient Treated with Rabbit Antithymocyte Globulin and Cyclosporine for Hepatitis-Associated Aplastic Anemia

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**Title:**

**An Epstein-Barr virus-associated leukemic lymphoma in a patient treated with rabbit anti-thymocyte globulin and cyclosporine for hepatitis-associated aplastic anemia**

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## **Abstract**

Lymphoproliferative disorders (LPDs) are generally caused by uncontrolled B-cell proliferation induced by the Epstein-Barr virus (EBV) in the setting of impaired EBV-specific T-cell immunity, particularly when there is pharmacological immunosuppression including Anti-thymocyte globulin (ATG). We herein present an unusual case of EBV associated with LPD (EBV-LPD) in which LPD occurred 3 weeks after the use of rabbit ATG administered for severe hepatitis-associated AA, and he died of fulminant leukemic lymphoma five days after the onset. We also review the pertinent literature about EBV-LPD after immunosuppressive therapy, and document efficacy of EBV-viral load monitoring and the need for preemptive therapy.

## **Introduction**

Epstein-Barr virus (EBV) associated Lymphoproliferative disorders (LPD) is becoming a serious problem with a recent increase in the number of patients with immunodeficiency. In particular, patients who have undergone allogeneic hematopoietic stem cell transplantation (HSCT) are predisposed to EBV infection or reactivation and development of EBV-related diseases [1].

EBV monitoring is generally recommended for high risk patients such as HSCT recipients of HLA mismatched donors, and patients receiving Anti-thymocyte globulin (ATG) after HSCT. The early detection of EBV reactivation would make it possible to perform preemptive therapy with rituximab if necessary, thus preventing the proliferation of EBV infected B-cells and the evolution of B-cell lymphoma.

Acquired severe aplastic anemia (SAA) is a rare disease defined by peripheral blood pancytopenia associated with hypocellularity of bone marrow [2]. Because bone marrow failure is thought to result from an immune-mediated mechanism, immunosuppressive therapy (IST) is the treatment of choice in patients without a suitable donor for HSCT. IST including ATG and cyclosporine A (CsA) is the most effective treatment for

SAA [3]. Several studies have shown that the use of ATG increases the frequency of EBV reactivation and the risk of EBV-LPD [4]. However, this risk has not yet been sufficiently documented in patients treated with ATG for SAA. It is also unclear whether EBV-viral load monitoring during IST for SAA and subsequent preemptive therapy are beneficial [5-6].

We herein report an unusual case of fulminant EBV-LPD that occurred in a patient as a form of leukemic lymphoma three weeks after the first administration of rabbit ATG and CsA for hepatitis-associated AA.

## Case report

A 54-year-old male presented to the hospital with a 3 week history of general malaise and loss of appetite. He showed jaundice and had severely deranged liver function tests, with a total bilirubin of 5.5 mg/dL (0.3-1.2 mg/dL, direct fraction: 3.1 mg/dL), alkaline phosphatase (ALP) of 536 IU/L (115-359 IU/L), aspartate aminotransferase (AST) of 1021 IU/L (13-33 IU/L), alanine aminotransferase (ALT) of 2718 IU/L (8-42 IU/L) and gamma glutaryl transpeptidase (GGTP) of 288 IU/L (10-47 IU/L). His blood tests showed a platelet count of  $12 \times 10^9/L$ , hemoglobin (Hb) of 13.1 g/dL and a white blood cell count (WBC) of  $1.4 \times 10^9/L$ , with neutrophils of  $0.7 \times 10^9/L$ . The absolute counts of CD4 and CD8 positive T-cells were  $0.2 \times 10^9/L$  and  $0.1 \times 10^9/L$ . A blood film showed leukopenia and thrombocytopenia, with no abnormal morphology. There was no history of recent travel, blood transfusions, and the use of medications or excess alcohol consumption. Subsequent investigations showed no evidence of a viral etiology. Hepatitis A virus IgM, Hepatitis B virus (HBV) Ag, hepatitis B core IgM, HCV antibody, hepatitis C RNA PCR, hepatitis E virus IgM and IgG were all negative. The HBs antibody was positive, but HBV-PCR was negative.

The cytomegalovirus (CMV)-IgG was positive and CMV-IgM was weakly positive. His anti-EBV antibody titers were VCA IgG positive, VCA IgM negative, EA negative, and EBNA positive. Parvovirus B19 IgM and IgG serology were negative. The patient was also negative for HIV antibodies. The bone marrow was severely hypocellular, which was consistent with AA. Immunophenotyping of bone marrow cells was normal, and there was no evidence of paroxysmal nocturnal hemoglobinuria. A liver biopsy was not performed due to the presence of severe thrombocytopenia. We diagnosed him with hepatitis-associated AA. ATG therapy was put on hold until his liver function tests improved, but his pancytopenia progressed without normalization of his jaundice. Rabbit ATG was started three weeks after admission at a dose of 3.75 mg/kg on days 1-5, and CsA at a dose of 3 mg/kg with methylprednisolone, which resulted in a rapid improvement in his liver function. He was further treated with prednisolone for prophylaxis of serum sickness, with normalization of liver function tests, but his hematological data still showed pancytopenia.

Three weeks after the administration of ATG, the patient developed a persistent high fever, which was refractory to antibiotics and antifungal

agents. Moreover, his liver function tests worsened, including a total bilirubin of 1.9 mg/dL, AST of 408 IU/L, ALT of 577 IU/L,  $\gamma$ GTP of 779 IU/L, and ALP of 848 IU/L. Blood tests showed a platelet count of  $15 \times 10^9/L$ , Hb of 9.2 g/dL and a WBC of  $7.5 \times 10^9/L$ . A peripheral blood smear revealed an increased number of lymphocytes ( $2.0 \times 10^9/L$ ), but no apparent hemophagocytic findings. The phenotype of the atypical lymphocytes was CD3-, CD10-, CD19+, CD20+ and IgG light chain lambda+. The serum ferritin (199,770 ng/mL) level was also markedly elevated. Based on these clinical signs, laboratory data, and the use of ATG, EBV-LPD was highly suspected. The administration of rituximab was considered, but severe metabolic acidosis and cardio-respiratory failure developed the evening after EBV-LPD was diagnosed and the patient died of the LPD on the following day.

The belated results of the peripheral blood EBV-DNA ( $3.3 \times 10^6$  copies/ $10^6$ WBC) and pathological examination from liver biopsy (demonstrating an increase in CD20 positive lymphocytes that were positive for EBV-encoded mRNA (EBER) by *in situ* hybridization) confirmed the diagnosis of EBV-associated diffuse large B-cell lymphoma (DLBCL). Infiltration of the



lymphoma was also detected in his bone marrow. We analyzed the patient's peripheral plasma EBV-DNA retrospectively. His plasma showed an elevation of the EBV-viral load to 700 copies/mL for the first time at 7 days before the onset of pyrexia, and the EBV-viral load rapidly increased within 5 days (Figure 1).

## **Discussion**

We herein describe the occurrence of fulminant EBV-LPD that was diagnosed following a sharp increase in the atypical B cell count in the peripheral blood three weeks after IST for hepatitis-associated AA. The patient died of cardio-respiratory failure associated with severe lactic acidosis due to rapidly progressive lymphoma. Lactic acidosis in association with hematologic malignancies normally shows an extremely poor prognosis [7].

It is well documented that EBV is an important complication of prolonged immunodeficiency. All patients who have a limited number of circulating T cells and retain B cells are at risk of developing EBV reactivation, as the interplay among EBV replication, latency, and immune control is not as balanced as in the healthy host. When there is pharmacological immunosuppression, EBV reactivation can lead to LPD because T cell function is severely impaired and B cells can evade T cell attack and expand. This is particularly common in patients undergoing HSCT with an ATG-containing conditioning regimen [4].

Treatment with ATG combined with CsA is the standard therapeutic

approach to SAA. Scheinberg et al. showed the risk of EBV disease in patients treated with ATG for AA to be low [5]. In their study of 78 patients with SAA who had received four different immunosuppressive regimens, including ATG, even though EBV reactivation occurred in most patients, none developed symptomatic EBV-LPD. To determine the incidence of clinically-significant EBV-LPD, we searched Medline for published articles about LPD after IST for AA. As shown in Table 1, a total of 8 cases of LPD that occurred after IST have been reported [6,8-14].

Viola et al. recently reported a patient who developed LPD 1 month after the use of horse ATG for the treatment of SAA [14]. Although the interval between ATG therapy and the onset of LPD in this case was short, similar to our case, he had received chemotherapy and autologous HSCT for the treatment of NHL 3 years prior to the ATG therapy, both of which may have predisposed the patient to develop LPD.

Wondergem et al. described a patient who received a higher dose rabbit ATG for SAA after failing to respond to horse ATG. The patient then developed life-threatening EBV-related lymphoma [6]. In our patient, LPD occurred after his first ATG therapy. To the best of our knowledge, this is

the first report of an EBV-related DLBCL in a patient treated with a single course of rabbit ATG.

Recently, the viral load has been shown to be a significant predictor of EBV related post-transplant lymphoproliferative disorders [15-16], and early treatment with an anti-CD20 antibody is recommended as preemptive therapy in patients undergoing an alternative donor transplant [1].

Because of the rapid clinical course of EBV-LPD, immediate treatment is crucial to reduce mortality [6]. No lymphocytosis or pyrexia was observed when the EBV-DNA level began to increase in our patient, (Figure 1).

Preemptive therapy with rituximab may improve the treatment outcome of EBV-LPD, not only after HSCT [17], but also after IST for SAA. However, the EBV copy number in the plasma of the current patient at day 19 of ATG therapy, 1 week before the onset of pyrexia, was 700 copies/mL, but it increased by more than 20 fold on day 24 (16,000 copies/mL). Therefore, once a week screening of EBV would have been useless for this patient. The prompt examination of blood for EBV copy number in response to clinical signs such as pyrexia may be more practical than surveillance for appropriately starting rituximab. The preventive administration of

rituximab would be a possible option for AA patients with a high risk of developing EBV-LPD.

Several risk factors for susceptibility to EBV-related lymphoma have been identified. Dierksheide et al. showed that an IFN-gamma polymorphism affects a likelihood of EBV reactivation [18]. Genotyping of the IFN-gamma gene may be useful for identifying patients at greater risk of developing EBV-LPD. The percentage of CD4<sup>+</sup> T cells in hepatitis-associated AA patients is reported to be significantly lower than that in non-hepatitis-associated AA patients [19]. Therefore, the presence of hepatitis-associated AA may have predisposed our patient to developing EBV-LPD.

The short time interval between ATG treatment and diagnosis and the fulminant course of EBV-LPD in our case and in the case reported by Viola et al. may be related to the profound immunosuppressive state associated with hepatitis and precedent chemotherapy in the cases.

The present case indicates that fulminant EBV-associated lymphoma can occur in patients with AA even after a single course of rabbit ATG therapy. Close monitoring of the EBV-viral load is therefore prerequisite for rabbit

ATG therapy of AA.

## Tables

Table 1. Reports of Lymphoproliferative disorders (LPD) after immunosuppressive therapy for aplastic anemia (AA)

References	Case	Type of LPD	Immunosuppression
Dorr, 1996	17 F	Lymphoma	ATG, CsA
Sarangi, 1999	22 M	T-ALL	CsA
Takeuchi, 2000	54 F	B-ALL	CsA, PSL etc
Hirose, 2001	24 M	T-ALL	CsA
Calistri, 2006	38 M	Infectious mononucleosis	rATG, CsA, M-PSL → hATG
Wongergem, 2008	42 F	EBV(+)DLBCL	hATG, CsA → rATG, CsA
Suzuki, 2009	63 F	EBV(-)DLBCL	ATG, CsA
Viola, 2010	55 M	EBV(+) plasma cell hyperplasia	hATG
Our case	54 M	EBV(+)DLBCL	rATG, CsA, PSL

ALL = acute lymphoblastic leukemia, DLBCL = diffuse large B-cell

lymphoma, EBV = Epstein-Barr virus, ATG = anti-thymocyte globulin, hATG

= horse ATG, rATG = rabbit ATG, CsA = cyclosporine, PSL = prednisolone

## Figure legends

**Figure 1.** The changes in plasma Epstein-Barr virus (EBV)-DNA and his body temperature. Open and closed circles represent EBV-negative and positive states. Increase in EBV-DNA was observed two weeks after administration of Anti-thymocyte globulin (ATG).



## Acknowledgements

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EBV-DNA  
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