EBV-positive immunodeficiency lymphoma after alemtuzumab-CHOP therapy for peripheral T-cell lymphoma

BRIEF REPORT

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Short title for running head: EBV⁺ lymphoma after alemtuzumab-CHOP for T-NHL

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Abstract

Alemtuzumab-CHOP chemotherapy has become experimental trial therapy for aggressive T cell lymphoma. Several multicenter phase III trials will incorporate this scheme. As part of an ongoing phase II trial in which we recently treated 20 patients with 8 cycles of CHOP every 2 weeks with 3 additional gifts of 30 mg alemtuzumab per cycle, we observed the development of EBV+ lymphoproliferative disease, after completion of the immunochemotherapy in three patients with peripheral T cell lymphoma. As the occurrence of EBV+ lymphoproliferative disease is rare after alemtuzumab monotherapy, such as is given for chronic lymphocytic leukaemia, we feel that early reporting of this potential side effect is warranted. It may be caused by intrinsic T cell defects in patients with T cell lymphoma, or by the combination of alemtuzumab with CHOP chemotherapy.
Introduction

The poor prognosis of peripheral T-cell lymphoma has generated new trials with immunochemotherapy consisting of the anti-CD52 monoclonal antibody alemtuzumab combined with CHOP (cyclophosphamide, adriamycin, oncovin, prednisone) chemotherapy. Although increased infection rates have been reported in these alemtuzumab-CHOP trials, toxicity thus far has been manageable. Based on these preliminary data, two alemtuzumab- based phase III trials (ACT1 and ACT2) for patients with peripheral T-cell lymphoma have recently been launched by a large European Intergroup initiative.

We encountered secondary Epstein-Barr Virus (EBV)-related lymphoma in three patients shortly after completion of alemtuzumab-CHOP treatment. All patients participated in a phase II trial (HOVON 69 study) in which 20 patients participated, consisting of 8 CHOP cycles given at 14 days interval combined with three gifts of 30 mg alemtuzumab per cycle. The HOVON 69 phase II study was reviewed by the Central Dutch Medical Ethical Committee located at the University Medical Center Groningen, and by the Central Clinical Trial Office. Both gave permission for conducting the trial.

Patients

Case 1. In May 2006, a 41-year-old man presented with peripheral T-cell lymphoma, unspecified, stage IIIA, localized in retroperitoneal lymph nodes, spleen, and mediastinum. TCR clonality analysis showed clonal rearrangements of TCR beta and gamma genes. The lymphoma was EBV negative by in situ hybridization. Whole blood EBV-DNA copies were below detection level. He received 8 alemtuzumab-CHOP-14 cycles together with co-trimoxazol, valaciclovir and fluconazol prophylaxis until October 2006. Treatment was complicated by neutropenic fever and recurrent CMV-reactivation, treated with ganciclovir. In November 2006 CT scans showed a complete response. EBV-DNA viral load copies, which had remained below detection level until
then, subsequently increased up to levels of 3 to 10x10^3 copies/ml (normal <1x10^3). In December 2006 he developed ulcerative duodenitis. In January 2007 a CT scan showed a tumor mass obstructing the duodenal loop; EBV-DNA levels had increased (574x10^3). Rituximab was started, without improvement. Multiple biopsies from the duodenum and stomach in January and March 2007 revealed an EBV (EBER)-positive T-cell lymphoproliferative disorder with massive intraepithelial accumulation of EBER positive and CD2, CD3 and CD5 positive but CD4 and CD8 negative T-cells that were oligoclonal as analyzed by TCR beta and gamma PCR. In April 2007 a biopsy was performed from the tumoral mass around the duodenum. This showed an EBV negative T cell lymphoma that upon TCR beta and gamma gene analysis was clonally different from the first T cell lymphoma diagnosed in 2006. He was treated with salvage chemotherapy (DHAP - dexamethason, cytarabine, cisplatin). However, he developed massive hemophagocytic syndrome and died in July 2007. Autopsy was not performed.

Case 2. In November 2005, a 32-year-old woman, with a history of stable multiple sclerosis, was diagnosed with a panniculitis-like T-cell lymphoma, T cell receptor alpha/beta phenotype, stage IV with extensive localizations in the skin and lymph nodes on both sides of the diaphragm. EBV antibodies or viral EBV DNA load were not measured. T-cell clonality analysis and in situ hybridization for EBV were not performed. She received 8 cycles of alemtuzumab-CHOP-14 until March 2006. Prophylaxis was given with co-trimoxazol, valacyclovir and fluconazole during and after completion of therapy because of persistent severe T-cell lymphocytopenia. A PET-CT scan after completion of therapy showed a complete remission. In August 2006 she had an epileptic seizure. A large intracerebral mass was biopsied, which yielded CD20^+ necrotic tumor cells. EBV serology was strongly positive with IgG and IgM-antibodies for EBV-early antigen, and IgG antibodies for EBV-nuclear antigen; whole blood EBV viral load showed less than 2000 copies/ml. EBER in situ hybridization was negative, but possibly false-negative due to necrosis. She received radiotherapy, followed by intravenous Rituximab
maintenance. In November 2006 she developed a pharyngeal diffuse large B-cell lymphoma that was CD20-negative (directly after rituximab), and EBV strongly positive. B cell clonality was not assessed. Whole blood EBV viral load was not measured. Because of poor performance, involved-field radiotherapy was administered, and she received palliative treatment with prednisone. In April 2007 prednisone was tapered because of gradual improvement. In August 2007, however, she developed subcutaneous lesions that showed both a relapse of the original panniculitis-like T-cell lymphoma and an EBV-positive B-cell plasmacytoid lymphoproliferative disease with clonal expression of cytoplasmic kappa light chains. PCR clonality analysis showed clonal rearrangement of TCR beta and gamma genes. She refused further treatment. Presently, January 2008, the patient is still alive and even improving. All subcutaneous lesions have disappeared spontaneously. Her CD4 and CD8 counts remain severely depressed.

Case 3. In January 2006, a 59-year-old man developed stage III angioimmunoblastic T-cell lymphoma (AILT) with involvement of multiple lymph nodes and the spleen. At that time, no atypical B cell proliferation was seen and only scattered EBV+ (presumably B) cells were detected in the lesional tissue. Clonality analysis was not performed. Whole blood EBV-DNA load was negative. He received 6 alemtuzumab-CHOP-14 cycles together with co-trimoxazol, valaciclovir and fluconazol prophylaxis until April 2006. Because of recurrent CMV re-activations, the last 2 cycles were not given. A PET and CT scan disclosed a complete remission. In April 2007 a CMV retinitis was treated by foscarnet, EBV-DNA was still below detection level. In December 2007, he developed fever, dyspnea, generalized lymphadenopathy, splenomegaly, multiple skin nodules, pleural fluid and ascites. A lymph node biopsy disclosed a relapse of the original AILT associated with an EBV+ CD20+ B-cell lymphoproliferative disease from which he died soon afterwards. EBV-DNA load measured 991x10^3 copies/ml. The EBV positive B-cell lymphoproliferation was monoclonal by PCR analysis.
**Pathology**

*EBV assessment:* the detection of Epstein-Barr encoded RNAs (EBER1-2) was performed by in situ hybridization on paraffin-embedded tissue sections using a fluorescein-conjugated EBER peptide nucleic acid probe (DAKO, Glostrup, Denmark). The reaction was visualized with alkaline phosphatase conjugated anti-FITC sheep IgG Fab fragments (Roche, Mannheim, Germany) followed by 5-bromo-4-chloro-3-indolyl-phosphatase, 4-nitrobluetetrazolium (Roche) and MgCl$_2$ incubation. Clonality of the EBV episomes was not investigated.

*B-cell and T-cell clonality assessment:* Immunoglobulin- and T-cell receptor clonality analysis was performed on DNA extracted from paraffin-embedded tissue by multiplex IgH and TCR beta and gamma PCR according to the BIOMED-2 protocol. The PCR products were detected by heteroduplex analysis or GeneScanning (TCR beta and gamma analysis courtesy of Prof. J.J. van Dongen, Erasmus Medical Center, Rotterdam).

**Discussion**

EBV-lymphoproliferative disease is a familiar complication after organ transplantation and related to the severity of immunosuppression. Most cases are of B-cell type, but T-cell EBV-lymphoproliferative disease can also occur. A comparable lymphoproliferative disorder may occur in the context of autoimmune disorders treated with immunosuppressive or immunomodulating agents. Here we report the occurrence of EBV-lymphoproliferative disease after strong suppressive immunochemotherapy consisting of alemtuzumab-CHOP for peripheral T-cell lymphoma.

Alemtuzumab (Campath-1H) targets CD52 that is present on virtually all B- and T-lymphocytes, monocytes and NK-cells, and is therefore highly immunosuppressive. Particularly the prolonged T-cell deficiency can lead to opportunistic infections. Alemtuzumab has been widely used in patients with chronic lymphocytic leukemia (CLL), T-cell prolymphocytic leukemia (PLL), and more recently T-cell lymphoma.
Despite the large numbers of patients with CLL or T-PLL treated, the occurrence of EBV lymphoproliferative disease after therapy with alemtuzumab seems very rare\textsuperscript{15-17}. A patient with refractory Sézary syndrome, treated with alemtuzumab, who developed fatal adenoviral and enteroviral infections and EBV-positive large B-cell lymphoma was recently published\textsuperscript{18}.

The fact that we observed several cases with EBV-lymphoproliferative disease after the completion of alemtuzumab-CHOP therapy for T-cell lymphoma is remarkable. Case 3 suffered from AILT, which is more often accompanied by secondary EBV\textsuperscript{+} B-cell transformations\textsuperscript{19,20}, however, both other cases had non-AILT T-cell lymphoma. Our observation suggest a relationship between this complication, the alemtuzumab-CHOP regimen, and T-cell lymphoma.

A relationship between EBV\textsuperscript{+} B-cell lymphoma and T-cell lymphoma has been described, not only in AILT, but also in peripheral T-cell lymphoma\textsuperscript{21}. Out of 600 cases with nodal T-cell lymphoma, 17 cases with secondary EBV-associated B-cell lymphoma were reported; 13 with AILT, one peripheral T-cell lymphoma, and three cases with co-existing EBV-related B-NHL as part of a composite lymphoma. These published cases were all treated before alemtuzumab became part of the therapy. The HOVON 69 alemtuzumab-CHOP scheme contained a very intensive alemtuzumab dose (90 mg per CHOP-14 cycle) compared with other trials in T-cell lymphoma. Within the forthcoming ACT1 and ACT2 trials, Alemtuzumab-CHOP-14, be it with less alemtuzumab (60 mg per CHOP-14 cycle) will be the experimental therapy for patients with peripheral T-NHL. It needs to be seen whether we will more often encounter EBV-driven B-cell and/or T-cell lymphomas if alemtuzumab-CHOP becomes standard therapy for patients with T-cell lymphoma. Although the poor outcome of T-cell lymphoma justifies high-risk therapy modalities, awareness of this complication is important. In addition, EBV-DNA monitoring could lead to early detection. However, whether early treatment, e.g. anti-CD20 monoclonal antibody in case of a secondary EBV+ B-cell lymphoma, will be effective after alemtuzumab-CHOP, remains to be determined.
Authors Contribution Statement

- HC K-N is the principal investigator of the HOVON 69 study, analyzed the data and wrote the article
- JLC was responsible for case 2 and corrected the article
- JEB was responsible for case 2
- GW v I is the co-investigator of the HOVON 69 study, analyzed the data and corrected the article
- SR revised all pathology and corrected the article

There are no conflicts of interest to disclose.

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References


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