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Case Report

Multifocal lymphadenopathy due to cytomegalovirus and Epstein-Barr virus infection in lymphoma patients receiving chemotherapy: a report of two cases

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Abstract: We describe two cases of multifocal lymphadenopathy due to concurrent cytomegalovirus and Epstein-Barr virus infections after chemotherapy for diffuse large B-cell lymphoma. The patients presented with suspected local recurrence on computed tomography and positron emission tomography-computed tomography at 19 and 8 months after completion of chemotherapy, but neither had any signs of systemic involvement. The enlarged lymph nodes resolved spontaneously without treatment after 2 and 12 weeks, respectively. A compromised cellular immune system after chemotherapy may have allowed the viral infection. This report demonstrates that multifocal lymphadenopathy due to viral infection after chemotherapy in aggressive B-cell lymphoma can mimic tumor recurrence, and that spontaneous resolution without antiviral agents can be achieved.

Keywords: Cytomegalovirus, Epstein-Barr virus, lymphadenopathy, recurrence, lymphoma

Introduction

Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are members of the herpesvirus family that generally cause asymptomatic infection during childhood and can persist in a latent form after primary infection. Reactivation of CMV and/or EBV is common after solid organ or hematopoietic stem cell transplantation, and is linked to serious clinical disease [1]. CMV infection is documented as particularly prevalent in recipients of allogeneic stem cell transplants for lymphoma, but is being recognized with increasing frequency in patients undergoing chemotherapy [2].

In the present report, we describe two patients with diffuse large B-cell lymphoma (DLBCL) who underwent rituximab in combination with cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) chemotherapy. During the follow-up period, they presented with multifocal lymphadenopathy that by positron emission tomography (PET)-computed tomography (CT) mimicked tumor recurrence but which was subsequently revealed to be concurrent CMV lymphadenitis and EBV-associated reactive hyperplasia. To our knowledge, this is the first report to describe concurrent CMV and EBV infections appearing as multifocal lymphadenopathy in patients with DLBCL treated with chemotherapy.

Case presentation

Case 1

A 60-year-old man with a history of DLBCL in complete remission was referred with suspected recurrence in his left axillary and bilateral hilar lymph nodes. He was first diagnosed with DLBCL 2 years earlier and achieved complete remission following six cycles of R-CHOP chemotherapy. During regular follow-up, 19 months after completing chemotherapy, CT and PET-CT scans revealed enlargement of his left axillary and bilateral hilar lymph nodes, and increased 18F-fluorodeoxyglucose (FDG) uptake in the same areas (**Figure 1A**, **1B**). However, no other palpable lymph node or organomegaly was detected on physical examination and no recognizable symptoms were identified. The results

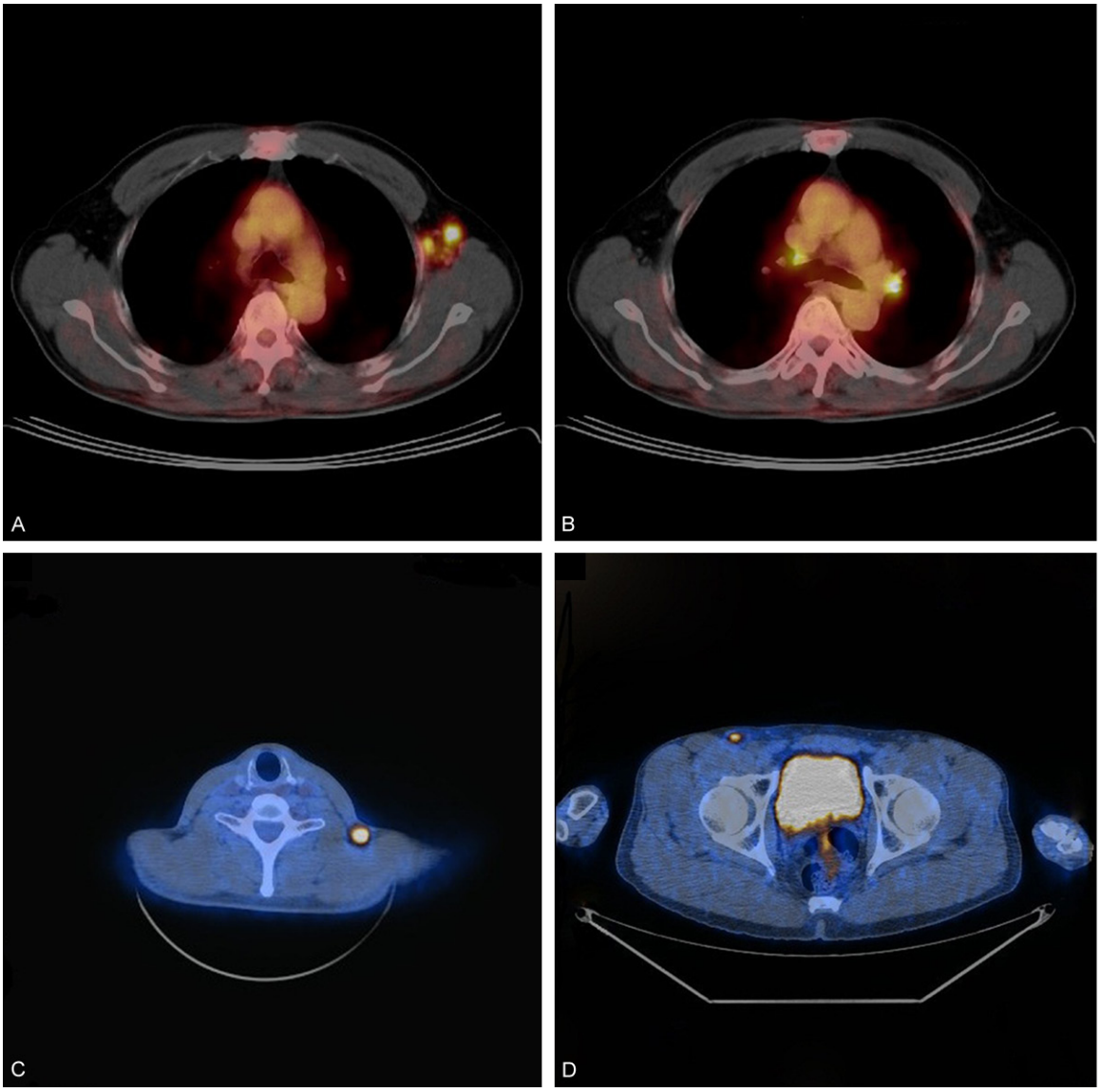

Figure 1. Positron emission tomography-computed tomography images showing increased 18F-fluorodeoxyglucose uptake in the axillary (A) and bilateral hilar (B) lymph nodes (case 1) and in the level Vb (C) and right inguinal (D) lymph nodes (case 2).

of a complete blood cell count and blood chemistry tests were normal. As local recurrence was suspected, an excisional biopsy of the left axillary lymph node (measuring 2.2 cm in diameter) was performed and immunohistochemical (IHC) investigations (the details of which are in the Pathological Findings section below) revealed the presence of CMV and EBV, which were not identified at the times of being diagnosing with DLBCL.

After revealing of CMV and EBV in the biopsy specimen, EBV quantitative PCR of whole blood was done additionally, and the result was below the limit of detection. Serologic tests for CMV and EBV were not performed. Because the patient presented with only multifocal lymphadenopathy, the administration of antiviral agents was not indicated. He was followed by physical examination of the enlarged lymph nodes and serial CT. Shrinkage of the enlarged lymph nodes was observed 2 weeks after the diagnosis. Currently, the patient remains in complete remission with no evidence of CMV and EBV reactivation.

Case 2

A 51-year-old man presented with swelling of the middle forehead that, first palpable 3 weeks

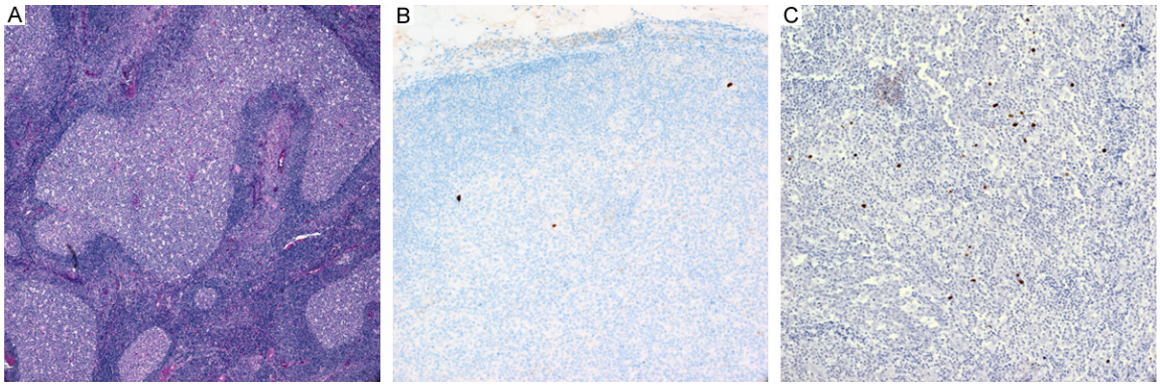

Figure 2. A. Histopathological examination of a lymph node biopsy specimen showed well-preserved lymphoid architecture with marked follicular hyperplasia (magnification, ×40). B. CMV immunoreactive cells were identified in the periphery of the lymphoid follicle and subcapsular sinus (magnification, ×400). C. EBER in situ hybridization recognized positive cells in the germinal center and interfollicular zone (magnification, ×400).

earlier, was increasing in size. Magnetic resonance imaging (MRI) and CT demonstrated a soft-tissue mass in the ethmoid and frontal sinus with bony destruction. Following biopsy of the lesion, DLBCL was diagnosed. The patient received six cycles of R-CHOP chemotherapy and achieved complete remission. Eight months after completion of chemotherapy, he complained of a palpable mass on the left lower side of his neck and in the right inguinal area. CT and PET-CT scans revealed several enlarged lymph nodes at left neck level Vb and in the right inguinal area, with increased 18F-FDG uptake (**Figure 1C**, **1D**). However, no other findings on physical examination or symptoms were apparent. The results of a complete blood cell count and blood chemistry tests were normal. As local recurrence was suspected, an excisional biopsy of a level Vb lymph node (measuring 1.5 cm in diameter) was performed and IHC investigations (detailed in the Pathological Findings section below) revealed the presence of CMV and EBV, which were not identified at the times of being diagnosing with DLBCL.

No viremia was detected by CMV antigenemia assay; however, serological screening indicated the presence of CMV-specific IgG (53 AU/mL; cutoff level: <4 AU/mL) but not IgM (enzyme-linked fluorescent assay index 0.04; cutoff level: <0.7). Serological tests for anti-EBV antibodies produced the following results: positive for viral capsid antigen (VCA) IgG and EBV nuclear antigen; negative for VCA IgM and early antigen. EBV DNA was undetectable by real-time quantitative PCR of whole blood. The patient was followed without specific treatment for viral infection. Shrinkage of the enlarged lymph nodes was observed 3 months after the diagnosis. Currently, the patient remains in a state of complete remission with no evidence of CMV and EBV reactivation.

Pathological finding

Histopathological findings were similar in the lymph node biopsies from both cases. Staining with hematoxylin and eosin revealed a relatively preserved nodal architecture with reactive follicular hyperplasia and paracortical expansion (**Figure 2A**). The large germinal centers contained some apoptotic bodies, and the paracortical areas contained variably sized lymphoid cells, including immunoblasts. There was no evidence of recurrent lymphoma. Immunostaining for CD3, CD10, CD20, and BCL-2 revealed no abnormal immunoarchitecture. With suspicion of viral lymphadenopathy, immunostaining for CMV and *in situ* hybridization (ISH) for EBV-encoded RNA (EBER ISH) were performed. CMV immunoreactive cells were identified in the periphery of the lymphoid follicle and subcapsular sinus (**Figure 2B**). EBER ISH-positive cells were present in the germinal center and interfollicular zone (**Figure 2C**). A diagnosis of CMV lymphadenitis with EBV-associated reactive hyperplasia was made.

Discussion

The gold standard for diagnosis of most viral diseases is the detection of virus in the patient's specimen by laboratory assay [3]. In immunocompromised hosts, the viral serology may not

be diagnostic; therefore, a combination of various laboratory tests or tissue biopsy is necessary for unequivocal diagnosis [4]. In this study, one patient was negative for a CMV antigenemia assay and had a non-diagnostic serology test result for CMV and EBV; however, the lymph node biopsy demonstrated the existence of CMV and EBV in the host cells.

More than 90% of the Korean adult population has antibodies against CMV or EBV [5, 6]. Compromised cellular immune responses are associated with reactivation of herpesviruses. Decreased T-cell immunity in DLBCL patients treated with R-CHOP chemotherapy persists for up to 2 years after therapy, and CD3+, CD8+, and CD56+ cell numbers are at their lowest level after three cycles of the regimen, but continue to increase for a year after its completion, remaining stable thereafter. CD4+ T-cell numbers are at their lowest level after six cycles and recover slowly for 2 years after the therapy; however, at that point there are still fewer than at diagnosis [7]. In our report, the two patients presented with multifocal lymphadenopathy at 8 and 19 months after completion of chemotherapy; therefore, incomplete recovery of T cells could have allowed a reactivation of the viruses.

Concurrent infection with CMV and EBV has been observed in patients who have undergone solid organ transplantation and bone marrow transplantation, and in an infant with hemophagocytic lymphohistiocytosis [8-11]. CMV itself represents an indirect marker of immunosuppression and contributes to the development of EBV-associated post-transplantation lymphoproliferative disease in heart transplant recipients [12]; it is also an important variable predictive of EBV reactivation in allogeneic stem cell transplantation [13]. In toddlers with hemophagocytic lymphohistiocytosis, such a co-infection worsens the clinical course [14].

The histological features of CMV lymphadenitis and EBV-associated reactive hyperplasia are indistinguishable in that follicular hyperplasia and monocytoid B-cell infiltration with epithelioid histiocytes are observed in both conditions. CMV- and EBV-infected cells were not visible in the hematoxylin and eosin stained sections of our patients' biopsies. However, CMV IHC and EBER ISH revealed the virally infected cells in the periphery of the lymphoid follicle and subcapsular sinus, and in the germinal center and interfollicular zone, respectively. Neither patient's earlier, DLBCL diagnostic lymph node biopsy contained CMV IHC- or EBER ISH-positive cells, which suggests that the infections described here were not a reactivation of a previous infection. Therefore, the diagnoses of CMV lymphadenitis and EBV-associated reactive hyperplasia were made.

The initial imaging findings of enlarged lymph nodes in both cases strongly raised the suspicion of recurrent lymphoma. Nevertheless, the patients had no signs of systemic involvement. We recommend that the possibility of virus-associated lymphadenitis be considered in the differential diagnosis of asymptomatic patients with multifocal lymphadenopathy and that in such cases, based on our experience, a treatment plan of cautious follow-up without antiviral agents is a reasonable option.

Disclosureof conflict of interest

None.

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