Epstein-Barr virus-associated lymphoproliferative disease during imatinib mesylate treatment for chronic myeloid leukemia

Imatinib mesylate (IM) is a first generation ATP-competitive selective inhibitor for tyrosine kinases (TK) such as ABL, ARG, PDGFRα and β, and c-KIT.[1] While use of IM has markedly improved treatment outcomes of Philadelphia chromosome (Ph)-positive leukemias, including chronic myeloid leukemia (CML) and Ph-positive acute lymphoblastic leukemia,[2,3] it may be accompanied by early and late adverse effects (AF). Most AF with IM, such as skin eruption/rash, gastrointestinal tract symptoms, liver dysfunction, fluid retention, hematopoietic suppression, and myalgia/muscle cramps, are generally manageable, but potentially life-threatening AF such as cardiotoxicity or renal failure are also possible.[4] Although extremely rare, late onset of secondary neoplasms, such as non-Hodgkin lymphoma, have also been reported in IM treatment of Ph-positive leukemia.[5,6] Here, we report a case of Epstein-Barr virus-associated lymphoproliferative disease (EBV-LPD) that developed in a patient with CML during IM treatment.

A 79-year-old male with chronic phase CML had been continuously treated with IM since diagnosis in October 2014. The maximum tolerated dose was 300 mg in this patient. Complete cytogenetic response and complete molecular response (CMR) were achieved after 7 and 20 months of IM treatment, respectively. After 41 months of IM treatment, the patient became aware of severe malaise and swelling of multiple cervical lymph nodes. The Eastern Cooperative Oncology Group performance status (PS) worsened from 0 at baseline to 3. Blood tests revealed a sudden progression of anemia with a hemoglobin level of 9.8 g/dL (normal range: 13.7-16.8 g/dL) and marked thrombocytopenia of $9.0x10^9$/L (158.0-$348.0x10^9$/L), with an elevation of the peripheral leukocyte count to $16.8x10^9$/L ($3.3\text{-}8.6x10^9$/L), including 53% neutrophils, 21% lymphocytes, 4% monocytes, 1% myeloblasts, and 19% CD19-positive plasmacytoid atypical lymphocytes. While the number of peripheral CD3/CD4-positive T cells of 564.0 $x10^6$/L ($369.0x10^6$ - $1.44x10^9$/L) was within the normal range, the number of CD3/CD8-positive T cells of 245.0 $x10^6$/L ($255.0x10^6$ - $1.17x10^9$/L) decreased to below the lower limit. Further laboratory tests revealed an elevation of serum lactate dehydrogenase to 636 U/L (124-222 U/L) and acute elevation of serum creatinine to 4.77 mg/dL (0.65-1.07 mg/dL), which indicated the development of acute renal failure; elevation of serum total protein to 9.3 g/dL (6.6-8.0 g/dL) with hypoalbuminemia based on an albumin level of 2.3 g/dL (4.1-5.1 g/dL); polyclonal hypergammaglobulinemia with immunoglobulin (Ig) G 4,904 mg/dL (861-1747 mg/dL), IgA 1,102 mg/dL (93-393 mg/dL) and

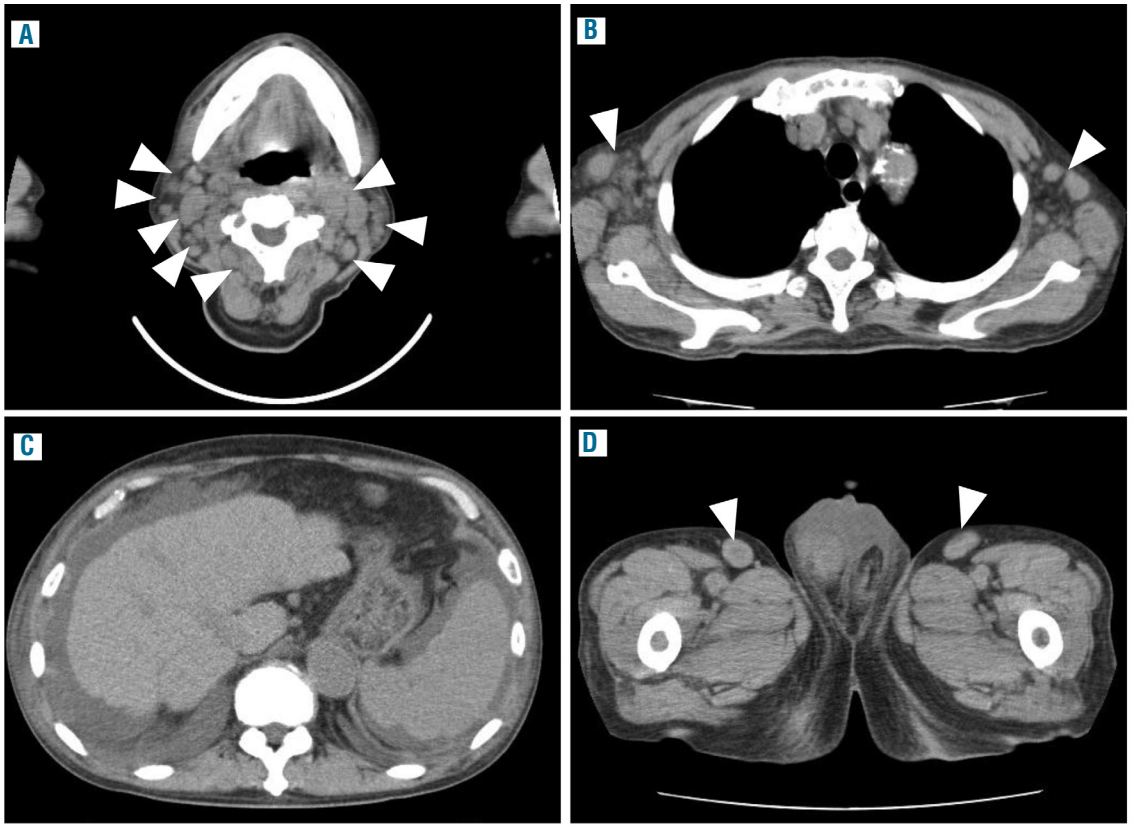

Figure 1. Computed tomography (CT). A CT scan showed generalized lymphadenopathy, shown by arrowheads, in the cervical lymph nodes (LNs) (A), axillary LNs (B), and inguinal LNs (D); and splenomegaly with moderate ascites (C).

IgM 497 mg/dL (33-183 mg/dL), which might associate with the emergence of various autoantibodies (AAb) including the positive tests for antinuclear antibody, anti-cardiolipin immunoglobulin G, Anti-SSA AAb and anti-SSB AAb, and the elevation of platelet associated-IgG to 116.0 ng/107Plt (0.0-27.5 ng/10^7Plt). Coombs tests were negative.; hyperuricemia based on uric acid of 17.3 mg/dL (3.7-7.8 mg/dL); elevation of soluble interleukin-2 receptor to 27,200 U/ml (145-519 U/ml); and minimal elevation of C-reactive protein to 1.01 mg/dL (0.00-0.14 mg/dL). The patient was also diagnosed with disseminated intravascular coagulation (DIC). Bone marrow (BM) aspiration showed an increase of small to medium-sized polyclonal plasmacytes up to 42.8% of all nucleated cells with normal cellularity and normal number of megakaryocytes in the background, while an invasion of abnormal neoplastic cells was excluded. The BCR-ABL transcript was below the detectable level in both peripheral blood and BM. Computed tomography (CT) showed the presence of generalized lymphadenopathy, splenomegaly, and ascites (Figure 1). Pathological examination of an axillary lymph node biopsy specimen revealed effacement of the nodal structure by extensive polymorphic infiltrate admixed with large cells and/or immunoblasts, which looked like Hodgkin and Reed-Sternberg cells. These cells were immunohistochemically positive for CD20, CD30, CD79a, BCL6, and MUM1 with EBV harboring by *in situ* hybridization (EBER-ISH), but not for CD10 or BCL2. The background showed mixed inflammatory cells of mainly CD3-positive small lymphocytes and plasma cells (Figure 2). The serum EBV-DNA copy number in a real-time polymerase chain reaction was 92,500 copies/μg DNA. These findings led to the diagnosis of EBV-associated B-cell LPD.

To relieve symptoms in a severely frail status, non-intensive chemotherapy of 250 mg/day intravenous (iv) methylprednisolone for the first 3 days plus 750 mg/m^2 cyclophosphamide iv on day 4 was selected as the initial therapeutic intervention. IM was terminated at the same time. Despite the palliative approach, complete response (CR) was achieved and serum *EBV* DNA turned to negative on day 35. However, since the patient had a poor PS due to development of congestive heart failure, *Staphylococcus aureus* bacteremia and repeated cytomegalovirus (CMV) antigenemia reactivation, which required antiviral therapy during the clinical course, chemotherapy was discontinued and the patient was switched to weekly 375 mg/m^2 rituximab monotherapy iv for 4 weeks (Figure 3). The number of CD3/CD4-positive T-cells (509.0 x10^6/L) was mostly unchanged from the onset of EBV-LPD after 2 months of treatment, while the number of peripheral CD3/CD8-positive T cells increased to 714.0 x10^6/L, indicating the recovery to the normal range. In addition, autoantibodies turned to be

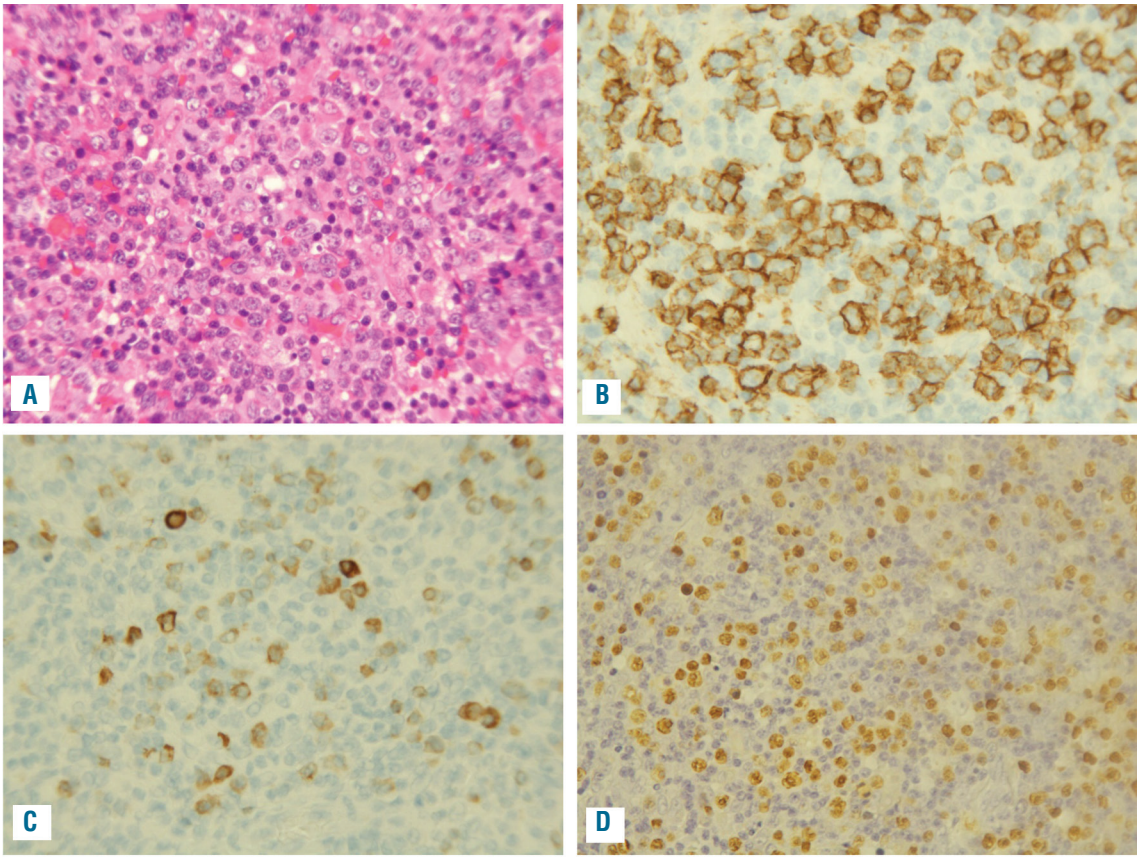

Figure 2. Histopathology of the left axillary lymph node. Histologic section of the left axillary lymph node stained with hematoxylin and eosin revealed complete effacement of the nodal structure by an extensive polymorphic infiltrate containing large abnormal cells and immunoblasts (A). These cells were immunohistochemically positive for CD20 (B) and CD79a (C), and had positive signals in the nuclei on EBER-ISH (D).

negative after treatment. At the time of writing, CR of EBV-LPD has been maintained without further chemotherapeutic intervention, and the BCR-ABL transcript has remained below the detectable level for 10 months.

EBV reactivation and EBV-LPD occasionally occur in a setting of immunosuppression, and the risks of their development increase with the degree of T-cell impairment.[7] For instance, patients are at markedly higher risks for EBV reactivation and development of EBV-LPD after allogenic hematopoietic stem cell transplantation (HSCT), especially in T-cell depleted transplantation.[8] Use of anti-thymocyte globulin (ATG) for prevention and/or treatment of graft-*versus*-host disease or treatment for aplastic anemia has also been shown to increase the risks for EBV reactivation and EBV-LPD significantly.[8,9]

In contrast to the response to ATG, the development of EBV-LPD during IM therapy for CML in a non-transplant setting is extremely rare, with only two cases reported based on our search of the English literature. The first case showed rapid progression of ulcerating cutaneous EBV-positive B-cell LPD on the head. Interestingly, the tumor showed spontaneous regression after reducing the dose of IM from 500 to 400 mg/day.[10] In the second case, EBV-LPD occurred after 30 months of IM treatment for CML in CMR. Initial clinical manifestations of EBV-LPD included pancytopenia, fever, night sweats, and DIC. The tumor did not resolve after the discontinuation of IM therapy and was refractory to rituximab monotherapy, but CR was obtained with conventional CHOP (cyclophosphamide, vincristine, doxorubicin and prednisolone) therapy in combination with subcutaneous injection of interferon-alpha.[11] The appropriate therapeutic strategy for EBV-LPD during IM therapy is unknown, including the effect of IM discontinuation, although IM was discontinued in all three cases, including our case. Fortunately, our case was in CMR at the onset of EBV-LPD; therefore, IM discontinuation was possible. Management of CML would be more problematic in a case of EBV-LPD complicated with active CML.

The mechanism underlying IM-induced immunosuppression is not completely clear; however, the most likely explanation is that IM inactivates key kinase targets in immune cells. Activation of T lymphocytes in response to antigen is controlled by the activation of T-cell receptors (TCRs) in physiologic condition.[12] TKs play prominent roles in TCR signal transduction, and IM may interfere with this signaling. A previous *in vitro* study showed that IM inhibits proximal signal transduction components of the CD3-TCR complex and decreases ZAP70 and LAT phosphorylation.[13] The same *in vitro* study also showed that IM has dose-dependent inhibitory effects on proliferation and activation of T cells, and significantly reduces antigen-triggered expansion of $CD8^+$ T cells in response to immunodominant cytomegalovirus and EBV peptides.[13] Importantly, T cell proliferation is substantially blocked by 1 μM IM, which is a clinically achievable concentration in daily dosing of 400 mg IM, and almost completely suppressed by 10 μM IM.[13] Another *in vivo* study showed that a quarter of patients with CML treated with 400 mg/day IM for 3 to 12 months developed mild lymphopenia.[14] Expansion of specific CD8-positive cytotoxic T cell (CTL) is important in clearance and control of viral infection, thus suppressed EBV-specific CD8-positive CTL responses by IM might provoke EBV-LPD. Indeed, CD3/CD8-positive T cells were significantly decreased at the onset of EBV-LPD in our case. We speculate that the scenario described above could be the

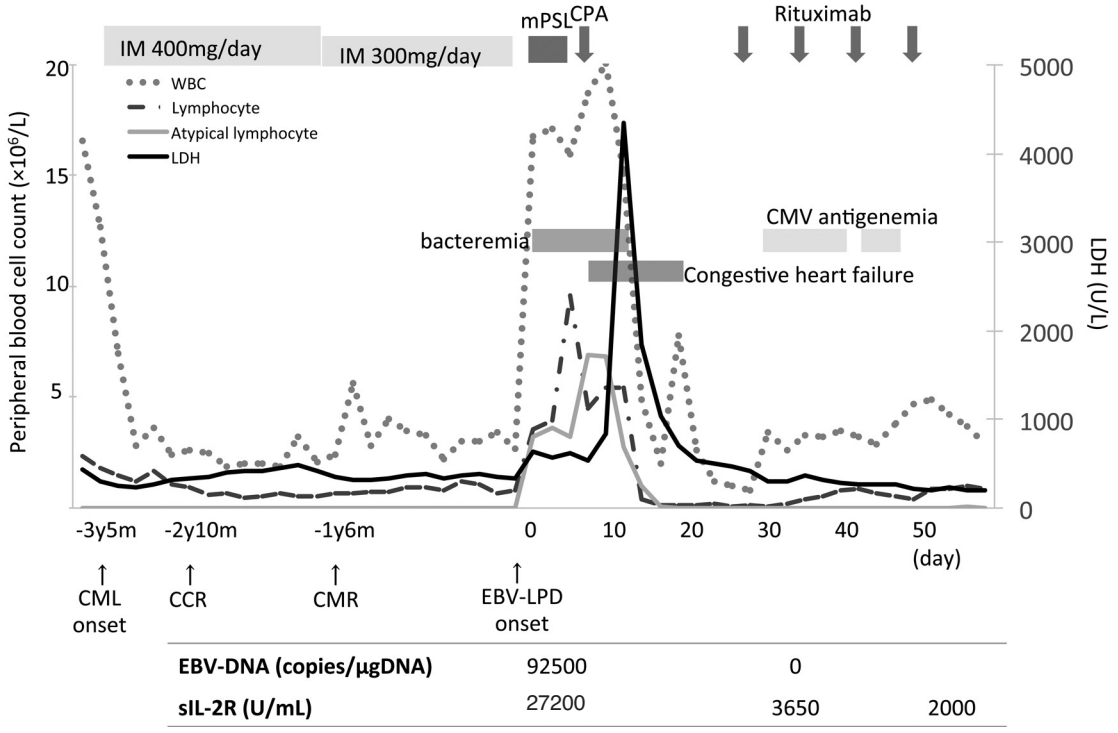

Figure 3. Clinical course. mPSL: methylprednisolone, CPA: cyclophosphamide, CCR: complete cytogenetic remission, CMR: complete molecular response, CMV: cytomegalovirus, EBV: Epstein Barr virus, LDH: lactate dehydrogenase, IM: imatinib mesylate, sIL-2R: soluble interleukin 2 receptor 2.

underlying mechanisms for the EBV reactivation and the subsequent development of EBV-LPD under IM treatment in our case. These immunosuppressive effects also occurred with dasatinib, a second-generation BCR-ABL TK inhibitor for CML that has profound inhibitory effects on functionally important key kinases such as Hck, Lck and Lyn in B cells, T cells, mast cells, and basophils.[15] The emergence of EBV-associated mucosal leukoplakia of the tongue has also been reported in a patient with CML during dasatinib treatment.[15]

In conclusion, we have reported a rare case of a patient with CML who developed EBV-LPD during treatment with IM. This case indicates that particular attention is needed for patients who develop lymphocytopenia during treatment with TK inhibitors, including IM, for Ph-positive leukemias.

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