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EBV Induced CD30 Positive Diffuse Large B-Cell Lymphoma in a Mixed Phenotypic Leukemia patient treated with Clofarabine

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Keywords

Diffuse large cell lymphoma; clofarabine; EBV; mixed phenotype leukemia

Introduction

Lymphoproliferative disorders (LPD) have been reported in several clinical settings associated with immunosuppression such as primary immune deficiency, HIV infection, bone marrow or solid organ transplantation, methotrexate or tumor necrosis factor-therapy, and aging.^{1,2} A significant number of these cases are associated with EBV. While EBV-positive LPD have been documented in patients with low grade lymphoma's treated with purine analogues including cladribine^{3,4} or fludarabine,^{5,6} we could not identify any reports of EBV-positive LPD after purine analogue (including clofarabine) therapy for AML or MPAL. As clofarabine is being used with increasing frequency in AML therapy^{7,8} such phenomenon may be noted with increasing frequency in the future. In our patient R-CHOP (rituximab, cyclophosphamide, his-adriamycin, vincristine, prednisone) therapy^{9,10} effectively treated the EBV-positive LPD and this may help guide treatment in future occurrences of a similar nature.

Case Report

A 50 year old Caucasian male presented to M. D. Anderson Cancer Center leukemia clinic with a 30-day history of pancytopenia and fatigue. Significant past medical history included hypertension and coronary atherosclerosis. Physical examination revealed fever and splenomegaly. Complete blood count showed a white cell count of $1 \times 10^9/L$ (normal range, $4-11 \times 10^9/L$), hemoglobin of 72 g/L (normal range, 140–180 g/L), platelet count of $31 \times 10^9/L$ (normal range, $140-440 \times 10^9/L$), and absolute neutrophil count of $0.06 \times 10^9/L$ (normal range, $1.7-7.3 \times 10^9/L$). Bone marrow biopsy revealed cellular marrow with 65% blasts. Flow cytometry immunophenotype analysis of bone marrow aspirate material detected a distinct

population of immature cells with mixed myeloid/T-lymphocyte phenotype. The neoplastic cells were positive for CD7 (partial), CD34, CD117, HLA-DR (partial), myeloperoxidase (small subset), CD13, CD64, CD33 (partial), CD71, CD123 (partial), TdT, CD10, CD38, and CD33 (partial), and cytoplasmic CD3. The neoplastic cells were negative for CD1a, CD2, surface CD3, CD4, CD5, CD8, CD14, CD19, CD41, TCR alpha/beta, and TCR gamma/delta. The immunophenotype of the blasts fulfilled diagnostic criteria for mixed phenotype acute leukemia” (MPAL), T/Myeloid as defined by the European Group for the Immunological Characterization of Leukemias (ELGL)¹¹ and by the 2008 WHO classification.¹² Conventional cytogenetic studies revealed a complex karyotype including -3, add (5) (q35), -7, -11, -15, -16, -18, and -20 chromosomal aberrations. He was seen by the stem cell transplant service at initial diagnosis of biphenotypic leukemia with complex karyotype and was thought to be a good candidate for consolidation with allogeneic stem cell transplantation. The patient received induction therapy with clofarabine (15mg/m² × 3days), cytarabine (0.75 g/m² × 3days), idarubicin (8 mg/m² × 3days) and solumedrol (40mg IV × 3days). Day 21 bone marrow aspirate showed complete morphologic and cytogenetic remission.

Post chemotherapy, the patient became pancytopenic. Twenty-eight days after completion of induction therapy he presented to the emergency room with a sore throat and progressive swelling in the neck. Computerized tomography (CT) of the neck showed necrotic lymphadenopathy in bilateral submandibular regions and enhancement of the palatine tonsils. Throat swab and blood cultures grew vancomycin-resistant enterococci. Initial antibiotics included meropenem and linezolid. Subsequently, fine-needle aspiration of a submandibular lymph node revealed inflammatory infiltrate with no neoplastic cells and no microbial growth on cultures.

The patient went on to receive two cycles of consolidation therapy at 4-weekly intervals per protocol with clofarabine, cytarabine and idarubicin. Four weeks after completion of the second consolidation cycle he presented with worsening neck swelling. Repeat CT neck showed resolving submandibular lymphadenitis. CT of the chest showed multiple lung nodules. Biopsy of a lung nodule showed diffuse infiltration by neoplastic cells with perivascular infiltration and necrosis. The neoplastic cells were large, with irregular shaped nuclei and inconspicuous nucleoli (Figure 1C). They were positive for CD20 (Figure 1D), CD30 (Figure 1E), PAX-5 and were negative for CD3, CD33, CD34, and TdT. In situ hybridization studies for Epstein-Barr virus small encoded RNA (EBER) demonstrated that the neoplastic cells were strongly positive for EBER (Figure 1F). Peripheral blood EBV quantitative PCR identified a viral load of 16,626 EBV copies/ml of plasma (normally undetectable). These results led to a diagnosis of EBV-positive diffuse large B-cell lymphoma. We retrospectively performed EBER on initial bone marrow specimen to see if EBV was present at time of initial diagnosis and may have caused the MPAL. EBER was negative on the initial bone marrow. We were unable to perform FISH for c-myc or BCL2/IGH as extensive necrosis in the tissue sample precluded FISH analysis.

Whole body PET-CT showed increased FDG-uptake in bilateral pulmonary nodules, bilateral cervical lymph nodes, stomach, spleen, and liver (Figure 1A). Bone marrow biopsy showed 0% abnormal blasts and no morphologic evidence of DLBCL nor morphologic

evidence of MPAL. He was staged as IV_{E, S} DLBL (Cotswold's modification of Anna Arbor staging system).

He continued to follow-up with the stem cell transplant service and a 10/10 sibling donor was identified. Unfortunately he developed the DLBCL prior to proceeding to allogeneic transplant for the biphenotypic leukemia.

After discussion with lymphoma specialists, R-CHOP (rituximab, cyclophosphamide, Adriamycin, vincristine, prednisone) regimen was initiated. After one course he had significant shrinkage of palpable lymph nodes and significantly diminished FDG uptake on the PET scan (Figure 1A and 1B). A repeat EBV serology performed 1 week later showed undetectable EBV levels in serum. He received a total of two cycles of R-CHOP therapy for the DLBCL. The stem cell transplant service continued to follow him with the hope that we would proceed to allogeneic transplant as a potential therapy for both the biphenotypic leukemia and DLBCL. Unfortunately, before he could proceed to allogeneic stem cell transplant the patient developed a progressive fungal pneumonia with sepsis and expired in the intensive care unit.

Discussion

We report a case of EBV-positive DLBCL in a patient treated with clofarabine for MPAL. EBV-positive LPD have been reported in several clinical settings associated with primary or acquired immunosuppression.^{1,2,13,14} While cases of EBV-positive LPD have been documented in patients with low grade lymphoma's and hairy cell leukemia treated with fludarabine and cladribine,³⁻⁶ we did not find any reported cases of EBV-positive LPD in patients with MPAL or AML treated with purine analogs.

Our patient initially presented with MPAL which accounts for fewer than 4% of all acute leukemia.^{11,12} Several studies have suggested that patients with MPAL have a worse clinical outcome than AML/ALL.^{15,16} There is no consensus as to whether induction therapy should be with lymphoid drugs, myeloid drugs or combinations.¹⁷ A retrospective study reported that induction with combined AML and ALL drugs led to a high rate of early death in adult and pediatric MPAL, although most of these patients had t(9; 22) which on its own portends poor prognosis.¹⁶ In pediatric MPAL, induction with ALL-type therapy or combined therapy with agents that are active against lymphoid and myeloid leukemia's has shown to yield higher complete remission (CR) rates than AML-type induction therapy.^{18,19} It is unknown whether similar strategies are applicable to adults.

The mechanisms by which EBV stimulates lymphoid proliferation and malignant transformation are poorly understood. EBV binds to receptors on B cells and pharyngeal surface molecule CD21.²⁰ Initial EBV infection is often subclinical. It then persists in the memory cells as a latent infection through integration of viral DNA into B cell DNA. It is proposed that the EBV infection then induces upregulation of BCL2 and MYC genes associated with anti-apoptosis and cell proliferation, respectively.²¹ This transforms the B-cell into a constantly proliferating lymphoblastoid cell. NF- κ B and mTOR pathways may also play a role in this transformation. EBV has been associated with multiple B and T-cell

lymphoproliferative disorders including DLBL, Hodgkin's lymphoma, post-transplant lymphoproliferative diseases (PTLD), AIDS-associated lymphomas, Burkitt's lymphoma, primary central nervous system lymphoma, mucosa associated lymphoid tissue (MALT), angioimmunoblastic lymphoma and peripheral T-cell lymphoma.^{2,22,23}

Clofarabine (*Genzyme Corp.*) is a new generation purine nucleoside (deoxyadenosine) analog, and is closely related to other members of this class such as cladribine and fludarabine.²⁴ It has more cytotoxic properties and a favorable pharmacokinetic profile than its counterparts. Clofarabine was initially approved by FDA for treatment of pediatric acute lymphoblastic leukemia (ALL) in 2007.²⁵ The therapeutic advantage of clofarabine-based therapies in the treatment of refractory AML and high risk MDS in the elderly are well known.^{7,8}

Clofarabine primarily inhibits DNA polymerases resulting in impaired DNA repair and apoptosis. Treatment with purine analogs can induce prolonged suppression of CD4+ and CD8+ cells, sometimes exceeding 24 months.^{20,26} The resultant immunosuppression may allow EBV reactivation and occurrence of LPDs. Such phenomenon have been well documented in early rather than late post-transplant LPDs which are 100% EBV driven.^{20,23} A decrease in EBV-specific cytotoxic T lymphocytes (CTLs) and an increase in the EBV viral load have been found to be strongly associated with the development of PTLD.²⁷ Association of EBV with AML is unknown at this time. Clofarabine is being used with increasing frequency in the therapy of AML. To our knowledge, this is the first documented report of a lymphoproliferative disorder (LPD) occurring after purine analogue therapy for AML. With such limited experience it would be premature to recommend routine evaluation with EBV PCR for all patients exposed to purine analogue therapy. However, with close clinical follow-up and assessment with EBV PCR when clinically indicated we may identify similar cases in the near future.

Corticosteroids at high doses can induce significant immunosuppression that may potentiate the occurrence of EBV-lymphoproliferative diseases (LPDs). However, our patient received solumedrol at a dose of 40 mg/day for five days. At this dose we felt it unlikely that steroids alone caused sufficient immunosuppression to be implicated as the primary cause of the DLBCL. Clofarabine is known to induces a more prolonged immunosuppression than cytarabine. Viral infections were noted with greater frequency with single agent clofarabine at a dose of 30mg/m² as compared to clofarabine 30mg/m² in combination with cytarabine 20mg/m².⁷ In a phase III trial that compared clofarabine in combination with cytarabine to cytarabine alone, severe infections and deaths related to adverse events were significantly more in the clofarabine alone arm.²⁸ These studies suggest that clofarabine produces a more potent and prolonged suppression of lymphocyte mediated immunity than cytarabine. Hence, we felt it to be the primary culprit.

EBV-positive LPDs represent a wide spectrum of entities ranging from reactive follicular or para-cortical hyperplasia to full blown DLBCL or Hodgkin's lymphoma. The prognosis depends on the morphologic entity, being excellent for reactive conditions and dismal for DLBCL and Hodgkin's lymphoma.^{2,22} Currently, most EBV+ DLBL are treated with standard DLBCL regimens using a combination of anthracycline, steroids, alkylating agents

and vincristine (CHOP).²⁹ Addition of rituximab has improved 3-year and 5-year overall survival in comparison to CHOP alone.⁹ Feugier et al have documented improved outcomes specifically in patients with CD20+, EBER+ DLBL treated with rituximab in combination with standard CHOP therapy.¹⁰ Newer therapeutic approaches including treatment of PTLs with autologous EBV-specific cytotoxic T-lymphocytes (CTLs) have been shown to reduce viral loads and shrink tumor size.³⁰ Current clinical trials are exploring the efficacy of administering such EBV CTLs expressing CD30 chimeric receptors in CD30+ Non-Hodgkin's Lymphoma ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01192464) Identifier: NCT01192464) and others (NCT00058617, NCT00779337).

Although antivirals (acyclovir, ganciclovir and valacyclovir) have been proposed for prevention and treatment of EBV driven LPDs, latent B-cells often do not express thymidylate kinase, which is the target for these antivirals. Thus, their role in prevention may be limited. This may have been the case in our patient, who was on valacyclovir prophylaxis and yet developed EBV infection. A recent phase I/II trial has shown promising response with simultaneous use of arginine butyrate (a thymidylate kinase inhibitor) and ganciclovir (an antiviral) in the treatment of patients with EBV associated lymphoid disorders.³¹

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Clinical practice points

1. EBV-positive lymphoproliferative disorders have been reported in several clinical settings associated with immunosuppression such as primary immune deficiency, HIV infection, bone marrow or solid organ transplantation, methotrexate or tumor necrosis factor-therapy, and aging. We did not identify any reported cases describing EBV-positive lymphoproliferative disorders post purine analog therapy for acute myeloid leukemia (AML) or mixed-phenotype leukemia (MPAL).
2. Acute myeloid leukemia patients with persistent or progressive lymphadenopathy should undergo fine needle aspiration to rule out extramedullary leukemia (granulocytic sarcoma) or concurrent lymphoma.
3. Identifying the ideal regimen for MPAL patients requires large clinical trials. However, our patient with MPAL achieved complete remission with clofarabine, idarubicin, cytarabine and solumedrol therapy.
4. Although antivirals (acyclovir, ganciclovir and valacyclovir) have been proposed for prevention and treatment of EBV driven lymphoproliferative (LPDs), the infected latent B-cells often do not express thymidylate kinase, which is the target for these antivirals. This is clearly seen in our patient as prophylactic valacyclovir was clearly ineffective in preventing occurrence of EBV driven lymphoma.

Fig 1.

A) Pretreatment PET-CT; B) PET-CT after 1 cycle of RCHOP; C) lung biopsy, hematoxylin-eosin, $\times 500$; D) CD20, $\times 500$; E) CD30, $\times 500$; F) In situ hybridization for Epstein-Barr virus -encoded small RNA (EBER), $\times 500$.