



Letters to the Editor

EBV reactivation mimicking a lymphoproliferative disorder associated with ruxolitinib therapy for myelofibrosis

TO THE EDITOR: The Jak-2 inhibitor ruxolitinib reduces constitutional symptoms and splenomegaly in patients with myelofibrosis and has been shown to increase overall survival [1, 2]. The JAK-STAT pathway has an important role in host defense and autoimmunity. There are ongoing trials using ruxolitinib as an immune modulator for ameliorating graft versus host disease in allogeneic hematopoietic stem cell transplant (allo-HCT). Recently, attention has been drawn to the increase in opportunistic infections and viral reactivations in patients receiving ruxolitinib [3]. Here, we describe a patient receiving ruxolitinib for myelofibrosis who was diagnosed with symptomatic Epstein-Barr virus (EBV) reactivation.

Case report

Our patient had a history of essential thrombocythemia, which was treated with anagrelide and hydroxyurea, and it progressed to Jak-2 positive myelofibrosis over a 20-year period. He had high-risk myelofibrosis according to the MIPSS 70 scoring system due to high white blood cell (WBC) count (around $25\text{--}30 \times 10^9/\mu\text{L}$), red cell transfusion dependence, circulating peripheral blood blasts, and presence of *ASXL1* mutation. He underwent splenectomy for increased transfusion dependence and constitutional symptoms shortly after starting ruxolitinib 20 mg bid for myelofibrosis. He continued hydroxyurea 1 g bid along with ruxolitinib. During the course of his treatment with ruxolitinib, he developed multiple infections, including disseminated Herpes zoster infection with secondary pneumonia after 5 months of treatment with ruxolitinib; community-acquired pneumonia and oral and peri-anal Herpes simplex infection after 8 months; respiratory syncytial virus pneumonia after 10 months; and recurrent paranasal sinus infections (2 episodes over the preceding year). He was not

neutropenic or lymphopenic during his treatment course (Fig. 1 shows the trend in lymphocyte counts).

There was no objective evidence of disease as response to ruxolitinib, and he continued to require transfusions; however, the transfusion frequency had decreased after splenectomy. Due to the high-risk features of his disease, he was scheduled for allo-HCT. On admission for the same treatment (around 14 months after treatment with ruxolitinib was first started), he was found to have altered liver function test (LFT) and palpable cervical lymphadenopathy (ALP 644 IU/L, total bilirubin 4 mg/dL, SGOT 57 IU/L, and SGPT 89 IU/L). The blood counts at the time revealed a hemoglobin of 8 g/dL, platelet count $136 \times 10^9/\mu\text{L}$, total WBC count $75.7 \times 10^9/\mu\text{L}$, neutrophils $4.8 \times 10^9/\mu\text{L}$, lymphocytes/monocytes $65 \times 10^9/\mu\text{L}$, blast count $2 \times 10^9/\mu\text{L}$, basophils $1.4 \times 10^9/\mu\text{L}$, meta-myelocytes $0.7 \times 10^9/\mu\text{L}$, myelocytes $0.7 \times 10^9/\mu\text{L}$, and pro-myelocytes $0.7 \times 10^9/\mu\text{L}$. Peripheral blood lymphocyte count had increased rapidly to $65 \times 10^9/\mu\text{L}$ (previous lymphocyte count ranged from 1.5 to $15 \times 10^9/\mu\text{L}$), and he was found to have EBV reactivation with 824,000

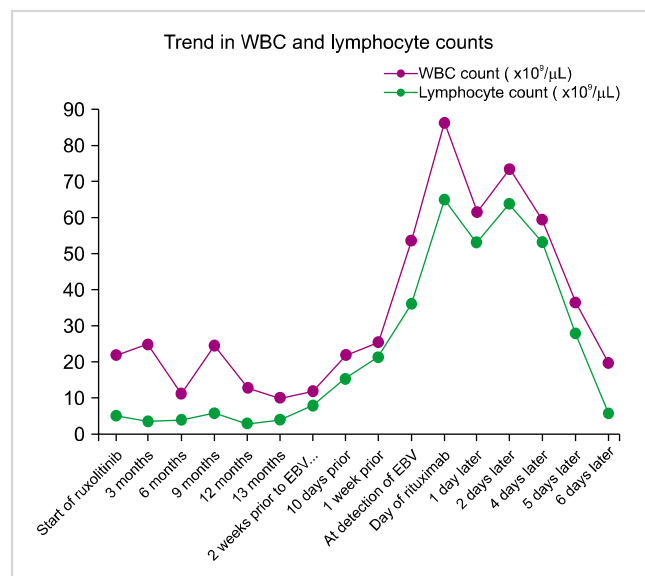


Fig. 1. Trend in total WBC and lymphocyte counts prior to, during and after episode of EBV re-activation.

copies/mL which was confirmed on repeat EBV assay (he had a documented negative EBV PCR 3 months prior to this event). He did not have any concomitant viral infections at that time. Cervical lymph node biopsy showed extramedullary hematopoiesis and was negative for EBV antigens on immunohistochemistry. Computed tomography of the chest and abdomen showed an increase in the size of pre-existing para-aortic and mesenteric lymphadenopathy and ill-defined hepatic lesions. Flow cytometry to further delineate the etiology of peripheral blood lymphocytosis revealed the following: T lymphocytes, 41%; B lymphocytes, <1%; monocytes, 35%; natural killer (NK) cells, <1%; and a low CD4/CD8 ratio of 1.26; this was suggestive of a reactive lymphocyte population. Considering the elevated EBV viral load, altered LFT, and nodal and hepatic lesions, the patient was diagnosed with reactive lymphocytosis secondary to EBV reactivation. Ruxolitinib had already been tapered and discontinued 1 day prior to his admission for allo-SCT, and he was treated with rituximab 375 mg/m². The trends in total white blood cell count and lymphocyte count prior to, during, and in the first 7 days after detection of EBV reactivation and receiving rituximab are shown in Fig. 1. His lymphocyte count and liver enzymes returned to baseline levels over the next 2 weeks. The repeat PCR was negative for EBV 1 week later, and the patient was re-admitted for an allo-HCT 2 weeks after his liver enzyme levels had returned to baseline.

Discussion

There is no mention of EBV in the JAKAVI (ruxolitinib) product monograph [4], though the “serious warnings and precautions” section in the monograph states that serious bacterial, mycobacterial, fungal, and viral infections (in some cases fatal or rare such as progressive multifocal leukoencephalopathy) have been reported in patients receiving ruxolitinib.

In the pivotal COMFORT-I and COMFORT-II trials on ruxolitinib in myelofibrosis [1, 2], herpes zoster infections were reported at higher rates in patients treated with ruxolitinib than in those treated with the placebo, and the incidence increased with increase in the duration of exposure (0–12 mo exposure -2.1%; ≥48 mo exposure -10.3%). Other infections, including pneumonia, sepsis, upper respiratory tract infection, and urinary tract infection occurred at similar or lower rates in ruxolitinib-treated patients than in those treated with the placebo. Pneumonia was the most common new-onset grade 3 or 4 adverse event observed after 48 months of treatment. Leukocyte subpopulations, lymphocyte functions, or antibody deficiency were not documented in these studies.

There are two cases of EBV infection associated with ruxolitinib reported in the literature [5, 6]. The first was a rapidly fatal, EBV-driven lymphoproliferative disorder of the central nervous system in a patient with post-polythemia myelofibrosis 9 weeks after starting ruxolitinib. The patient had a documented normal brain magnetic reso-

nance imaging 3 weeks prior to initiation of ruxolitinib suggesting that the disorder developed after starting ruxolitinib. The second report was that of a patient with myelofibrosis receiving ruxolitinib, who presented with severe diarrhea related to a gastric ulcer, which was positive for EBV by PCR in biopsy but negative in the blood. In this patient, the authors were able to document a serial decrease in the number of NK cells and CD4+ T cells in the patient’s blood while receiving ruxolitinib. The EBV infection responded to ganciclovir, and ruxolitinib was discontinued, but the level of T and NK cells continued to decline for the next 3 months after stopping ruxolitinib.

Even though we did not have pathological evidence of EBV on lymph node biopsy in our patient, the acute lymphoproliferative response was seen in association with the rise in serum bilirubin and alkaline phosphatase, which is similar to a post-transplant lymphoproliferative disorder seen in association with EBV in immunocompromised patients.

JAK mutations are associated with primary immunodeficiency, and complete STAT-1 deficiency can lead to lethal viral and bacterial infection [7]. Several pre-clinical or phase 1/2 studies [8–11], have shown decreases in pro-inflammatory cytokines, reduced dendritic cell function, impaired CD4+ and CD8+ T cell priming *in vitro* and *in vivo*, and even marked and long-lasting decrease in regulatory T cells and NK cells in patients receiving ruxolitinib, some of which were linked to clinically relevant infections like herpes virus and cytomegalovirus.

In our patient, the asplenia could have potentiated the immune-suppressive effects of ruxolitinib, though the spectrum of atypical infections seen in our patient points to defective cell-mediated immune responses rather than defective humoral responses as is seen in asplenia. Similarly, it is unlikely that hydroxyurea could be responsible for his infections as he had been on hydroxyurea for 20 years prior to starting ruxolitinib without any increase in infections.

The most frequent infections described with ruxolitinib are tuberculosis and Herpes zoster, Hepatitis B virus, cryptococcal, and *Pneumocystis jiroveci* infections [12, 13]. In a report from the French pharmacovigilance database [14], 22 patients had infections while receiving ruxolitinib. The median dose in these patients was 30 mg/d (10–60 mg), and the median time to onset of infection was 424.5 d (98–1,550 d). These included bacterial, mycobacterial, viral (including 1 patient with EBV), fungal (including *Pneumocystis*, *Cryptococcus*, and *Aspergillus*), and parasitic (*Toxoplasma gondii*) infections. Some authors have suggested [15] that anti-infective prophylaxis may be indicated to offset the potent immunosuppressive effects of ruxolitinib, especially for the prevention of herpes simplex and varicella zoster virus reactivation, in addition to careful monitoring for other atypical infections.

Conclusion

Patients receiving ruxolitinib should be counseled regarding the increased risk of infections, and careful monitoring

for opportunistic infections is advisable. Future studies on ruxolitinib may benefit from prospective monitoring of CD4, CD8, NK, and B cell profiles of patients for improved understanding of the pathophysiology of the immunodeficiency associated with ruxolitinib.

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REFERENCES

- Verstovsek S, Mesa RA, Gotlib J, et al. Long-term treatment with ruxolitinib for patients with myelofibrosis: 5-year update from the randomized, double-blind, placebo-controlled, phase 3 COMFORT-I trial. *J Hematol Oncol* 2017;10:55.
- Cervantes F, Vannucchi AM, Kiladjian JJ, et al. Three-year efficacy, safety, and survival findings from COMFORT-II, a phase 3 study comparing ruxolitinib with best available therapy for myelofibrosis. *Blood* 2013;122:4047-53.
- Caocci G, Murgia F, Podda L, Solinas A, Atzeni S, La Nasa G. Reactivation of hepatitis B virus infection following ruxolitinib treatment in a patient with myelofibrosis. *Leukemia* 2014;28:225-7.
- Novartis Europharm Limited. Summary of product characteristics: Jakavi, INN-ruxolitinib. Amsterdam, The Netherlands: European Medicines Agency, 2017. (Accessed October 31, 2018, at https://www.ema.europa.eu/en/documents/product-information/jakavi-epar-product-information_en.pdf).
- Pálmason R, Lindén O, Richter J. Case-report: EBV driven lymphoproliferative disorder associated with ruxolitinib. *BMC Hematol* 2015;15:10.
- Kusano Y, Terui Y, Ueda K, Hatake K. Epstein-Barr virus gastric ulcer associated with ruxolitinib. *Ann Hematol* 2016;95:1741-2.
- O'Shea JJ, Holland SM, Staudt LM. JAKs and STATs in immunity, immunodeficiency, and cancer. *N Engl J Med* 2013;368:161-70.
- Massa M, Rosti V, Campanelli R, Fois G, Barosi G. Rapid and long-lasting decrease of T-regulatory cells in patients with myelofibrosis treated with ruxolitinib. *Leukemia* 2014;28:449-51.
- Heine A, Held SA, Daecke SN, et al. The JAK-inhibitor ruxolitinib impairs dendritic cell function in vitro and in vivo. *Blood* 2013;122:1192-202.
- Parampalli Yajnanarayana S, Stübiger T, Cornez I, et al. JAK1/2 inhibition impairs T cell function in vitro and in patients with myeloproliferative neoplasms. *Br J Haematol* 2015;169:824-33.
- Schönberg K, Rudolph J, Vonnahme M, et al. JAK inhibition impairs NK cell function in myeloproliferative neoplasms. *Cancer Res* 2015;75:2187-99.
- Lussana F, Cattaneo M, Rambaldi A, Squizzato A. Ruxolitinib-associated infections: a systematic review and meta-analysis. *Am J Hematol* 2018;93:339-47.
- Dioverti MV, Abu Saleh OM, Tande AJ. Infectious complications in patients on treatment with ruxolitinib: case report and review of the literature. *Infect Dis (Lond)* 2018;50:381-7.
- Sylvine P, Thomas S, Pirayeh E; French Network of Regional Pharmacovigilance Centers. Infections associated with ruxolitinib: study in the French Pharmacovigilance database. *Ann Hematol* 2018;97:913-4.
- Heine A, Brossart P, Wolf D. Ruxolitinib is a potent immunosuppressive compound: is it time for anti-infective prophylaxis? *Blood* 2013;122:3843-4.

Retrospective screening for Philadelphia-negative myeloproliferative neoplasms in patients with cerebral infarctions as revealed using the revised 2016 World Health Organization diagnostic criteria

TO THE EDITOR: Arterial and venous thromboses are major clinical events in patients with Philadelphia-negative myeloproliferative neoplasms (MPNs) including essential thrombocythemia (ET) and polycythemia vera (PV) [1, 2]. Some MPN patients suffer from vascular complications even prior to diagnosis [3]. In some cases, MPN is evident in individuals newly diagnosed with cerebral infarction (CI) which is a type of thrombosis [4].

The World Health Organization (WHO) revised the MPN diagnostic criteria in 2016 [5]. Most notably, in the revised criteria, the hemoglobin/hematocrit threshold values for the diagnosis of PV were lowered. This has markedly changed the diagnostic landscape, and consequently, the treatment options and outcome of this disorder. However, the revised criteria were not widely used to evaluate patients with CI until recently. Thus, we retrospectively evaluated the likelihood of MPN in CI patients using the revised criteria. The medical records of CI patients admitted to the Chungnam National University Hospital from January 2016 to December 2017 were retrospectively reviewed. Patients with erythrocytosis or thrombocytosis were divided into those with a reactive case and possible, probable, or proven MPN. "Possible MPN" indicates that a reactive increase