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Case Report

Isolated nodal TBC reactivation in a patient with post-Thrombocythemia Myelofibrosis treated with Ruxolitinib: case report and review of the literature.

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Running head: Isolated nodal TBC reactivation in a ruxolitinib-treated MF

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Abstract:

Ruxolitinib side effects include the most frequent hematological toxicity along with a more recently evidenced immunosuppressive activity, interfering both on the innate and adaptive immunity, and several cases of reactivation of latent infections by opportunistic agents in patients in treatment with Ruxolitinib have been published in the last years. Several pathophysiological mechanisms may explain an association between Ruxolitinib and opportunistic infections. For what we know, the only case of an isolated lymph node TBC reactivation in a Ruxolitinib-treated MF patient was reported by Patil RV et al. in 2016. Other five cases describing TBC reactivations in MF patients assuming Ruxolitinib and successfully treated with four-drug anti-TBC therapy are available in literature to date.

The case we reported describes an isolated lymph nodal TBC reactivation in a patient with diagnosis of postET-MF after during Ruxolitinib treatment after a long course of IFN-α2b assumed for the previous diagnosis of ET. The case we report teaches that lymphadenopathy with or without constitutional symptoms developing during Ruxolitinib therapy should be considered as a possible manifestation of a TBC reactivation in patients with a previous positive TBC-exposure test. In these cases, Ziel-Nielsen testing on urine and sputum have to be performed to rule out infectiousness and eventually isolate the patient. Moreover, previous long-time exposition to IFN-α2b may be related with a higher risk for TBC reactivation in these subset of patients. We encourage re-evaluation of the cohorts of patients treated with Ruxolitinib in previous and current large prospective studies to study the possible correlation between previous exposition to IFN-α2b and TBC reactivation.

Introduction:

Myelofibrosis (MF) is a chronic Philadelphia-negative myeloproliferative neoplasm, characterized by bone marrow fibrosis, anemia, leukocytosis or leukopenia, thrombocytosis or thrombocytopenia, splenomegaly mostly due to extra-medullary hematopoiesis, hepatomegaly and constitutional symptoms (fever, fatigue, weight loss, night sweats, pruritus). MF is associated with a deregulation of JAK-STAT signaling, that confers a proliferative and neoplastic survival phenotype. The abnormal activation of the JAK-STAT pathway often comes from one of the three known driver genes (JAK2 V617F, MPL and CALR), frequently mutated in MF (1).

Despite the recent improvement in the knowledge of this disease and of its molecular mechanisms, the pathogenesis remains partially obscure. At the present date, the only curative strategy remains allogenic hematopoietic cell transplantation, which is not always an option, according to patient's and disease's features. Alternatively, the use of cytoreductive drugs (i.e. interferon or hydroxyurea) or JAK inhibitors, like Ruxolitinib, can improve constitutional symptoms and decrease the size of the spleen (2). Ruxolitinib is a selective JAK-1 and JAK-2 inhibitor, approved for the treatment of intermediate-risk and high-risk MF and high-risk Polycythemia Vera (PV) with resistance or intolerance for hydroxyurea. Its mechanism of action involves the reduction in signal transduction and cytokine levels, including interleukin-6 (IL-6) and tumor necrosis factor-alfa (TNF-α) (3). Ruxolitinib side effects include the most frequent hematological toxicity along with a more recently evidenced immunosuppressive activity, interfering both on the innate and adaptive immunity, and several cases of reactivation of latent infections by opportunistic agents in patients in treatment with Ruxolitinib have been published in the last years. Several pathophysiological mechanisms may explain an association between Ruxolitinib and opportunistic infections: 1- The reduction of NK cells number and activity, due to the decrease of cytokine signals that are important for NK maturation (4); 2- The downregulation of regulatory T-cells; 3- The quantitative reduction and impaired function of the dendritic cells (DC), with defective costimulatory properties and decreased cytokine production (5).

DC are essential antigen-presenting cells, that control adaptive immunity producing IL-12, and thus driving Th1-lymphocytes activation. Th1-cells produce cytokines, such as gamma-interferon (INF-γ) and TNF-α. TNF-α stimulates the Th1 cells against intracellular pathogens, IFN-γ gives protective immunity against Mycobacterium TB and INF-γ-activated macrophages release bactericidal superoxides that can limit Koch bacillus growth. Ruxolitinib may reduce this protective mechanism (6).

For what we know, the only case of an isolated lymph node TBC reactivation in a Ruxolitinib-treated MF patient was reported by Patil RV et al. in 2016. They described the case of an axillary nodal TBC occurred during Ruxolitinib therapy in an MF patient. Pulmonary involvement was only evaluated with an X-ray of the chest, and Ruxolitinib was continued during anti-TBC treatment. No Ziel-Nielsen test on sputum or urine was performed in the case reported by Patil and colleagues (7). Khalid et al. reported a case of pulmonary TBC in a 62 years old male with primary MF, after one year of treatment with Ruxolitinib. Anti-TBC therapy was

prescribed and pulmonary TBC resolved after ten months of therapy (8). Shamil et al. reported a case of a 78-years old female with primary MF who experienced miliary TBC during Ruxolitinib treatment. Ruxolitinib was stopped and the patient was successfully treated with a 12-months regimen of four drug anti TBC therapy with resolution of miliary nodules (9). Hopman RK et al. reported a case of a 62 years old man with primary MF on Ruxolitinib who developed a disseminated TBC two weeks after the beginning of therapy. The patient was treated with quadruple anti-TBC therapy for 9 months, obtaining the improvement of his clinical conditions (10).

Other five cases describing TBC reactivations in MF patients assuming Ruxolitinib and successfully treated with four-drug anti-TBC therapy are available in literature to date (11–14).

Anti-TBC therapies are known for their hematological adverse events, in particular Isoniazid-induced anemia, through the superoxides generation and the blockade of heme synthesis (15,16). Also Ruxolitinib may cause or contribute to anemia in MF. Their association is uncommon and myotoxicity in this setting is not well known.

We here describe the case of a patient diagnosed with post-Essential Thrombocythemia MF treated with Ruxolitinib, who developed the reactivation of TBC infection with isolate lymph node localization.

Case Presentation:

A 38-year-old female was diagnosed with JAK2 V617F positive Essential Thrombocythemia (ET) in 2011, according to 2008 WHO criteria. At the time of diagnosis, acetylsalicylic acid (ASA) 100 mg a day was started. One year after, in 2012, hydroxyurea (HU) therapy was started because of a platelet (PLT) value of more than 1.5 million per deciliter, obtaining the normalization of PLT count some months after the beginning of the drug.

HU dosage was gradually increased due to poor control of PLT value (PLT = 825,000/mmc), and in September 2015 Anagrelide was added to HU. In February 2016, due to lack of response to the double therapy and persistence of severe thrombocytosis (PLT = 849,000 per deciliter), HU and Anagrelide were stopped and Interferon-a (IFN-a) 3 million units subcutaneously every other day was prescribed, obtaining a discreet control of thrombocytosis, at the cost of a flu-like syndrome and occasional headaches. However, almost three years after the beginning of IFN-a therapy, in September 2018 the patient's clinical condition worsened (worsening asthenia and headaches), thrombocytosis reappears and spleen becomes palpable at 6 cm from the costal margin. For these reasons, in November 2018 IFN-a was withdrawn and the patient underwent a new bone marrow biopsy, which revealed post-ET Myelofibrosis with grade 1 reticulin fibrosis and the patient was diagnosed with a post-TE MF with Intermediate-1 risk according to the IPSS score. In January 2019 Ruxolitinib was prescribed at the dose of 15 mg twice a day. Serology for HBV, HCV, HIV was negative. A pre-

Ruxolitinib positive Mantoux test revealed a previous exposition to Mycobacterium Tuberculosis, with a negative chest-RX. No TBC prophylaxis was given.

In March 2019, the patient registered a sensible improvement in headache and the objective achievement of myeloproliferation signs, with platelet values ranging between 500000 and 600000 per microliter and spleen reduction at manual palpation during the first two months of therapy. Some days later the last control visit, the patient reported frequent night sweats, chills, asthenia, itchy lesions on the neck and face and a hard and painful lateral cervical right nodule appeared. The physical exam revealed an almost unmovable mass with a hard-parenchymal texture. A neck ultrasound was performed revealing rounded lymph nodes with maximum diameters of 24 and 12 mm. A total body CT scan was performed revealing the known cervical lymphadenopathies, with central colliquation, along with two sub-centimetric calcific lymph nodes at the right lung hilum. No other lymph node enlargement at other sites or splenomegaly/hepatomegaly were found. The patient had no fever at that time point. We then programmed lymph node excision with diagnostic purpose in order to exclude lymphoproliferative diseases, but the patient developed fever, malaise and severe asthenia, referred to our hospital's emergency unit and was sent to an urgent infective disease evaluation that indicated prompt withdrawal of Ruxolitinib and hinted a TBC reactivation. Urine and sputum search for Koch Bacillus with Ziel-Nielsen coloration was performed and resulted negative on three consecutive samples from both origins. Quantiferon TB gold was performed that confirmed the known positivity to the Mantoux test.

In consideration of the clinical worsening of general malaise and fever resistant to broad spectrum antibiotics, the patient was hospitalized in the infectious disease unit and an excisional lymph node biopsy was performed soon after. Histopathology revealed a chronic granulomatous necrotizing tuberculous lymphadenitis. In June 2019, the patient started four-drug anti-TBC therapy with Isoniazid 300 mg a day, Rifampicin 600 mg a day, Pyrazinamide 500 mg a day and Ethambutol 400 mg a day for six months. Isoniazid and Rifampicin were prescribed until November 2019 (six months), while Ethambutol and Pyrazinamide were prescribed for two months, until July 2019. In July 2019 the therapy was continuing, achieving general clinical improvement, the disappearance of sweats and fever, and complete resolution of lymphadenopathies swelling, without other remarkable signs at a new total body CT scan. In September 2019, physical exam revealed splenomegaly again and headache reappeared along with a gradual rise of the platelet count with a zenith value of 650000/mmc. For this reason, the patient restarted Ruxolitinib to control myeloproliferation symptoms and signs. In November 2019 Isoniazid and Rifampicin were suspended, as programmed.

Of note, during four months in which the four-drug anti-TBC therapy was assumed in the absence of the JAK inhibitor, the patient registered no haematological toxicity and the platelet count gradually increased, while it is usual that in active TBC platelets are higher than during TBC-treatment, probably because thrombocytosis (that is frequently found during active TBC) is due to acute inflammation and is finalized to anti-TBC immune

response, through cytokines and chemokines release. No anaemia or leukopenia were registered during the anti-TBC treatment, in the absence of Ruxolitinib assumption.

Otherwise, during the period of Isoniazid and Ruxolitinib co-administration, only a grade 1 anaemia was registered, with haemoglobin values no lower than 11 grams/liter, comparable to the toxicity grade reached by the patient during the first two months of Ruxolitinib assumption alone.

To date, the patient is still in treatment with the JAK-inhibitor, do not assume any TBC prophylaxis and has not registered any other infective adverse event.

Discussion and conclusions:

The case we reported describes an isolated lymph nodal TBC reactivation in a patient with diagnosis of postET-MF after almost 2 months of Ruxolitinib assumption, administered after a long course of IFN-α2b treatment for the previous diagnosis of ET.

The immunosuppression due to the use of Ruxolitinib plays an important role in the development of opportunistic infections or in the reactivation of latent infections. Several cases of TBC reactivation during Ruxolitinib therapy in patients with MF have been reported in literature (6-12). According to the references reported, the time of administration of Ruxolitinib before TBC reactivation ranges between 3 weeks and 22 months (17). M. tuberculosis can be found in latent state granulomas in a moderate to high percentage of individuals worldwide; however, the use of some immunosuppressive drugs and in particular of JAK inhibitors can reactivate a latent TBC infection (18).

It is widely known that TBC infection triggers the activation of alveolar macrophages responding with the production of a precise pattern of cytokines (IL-12, IFN-δ, IL-1, IL-6, IL-5, IL-12, IL-18) which limit the growth of the bacillus. The risk of TBC infection may increase with Ruxolitinib therapy, that downregulates these cytokines expression and makes this protective mechanism fail (6). IFN-α belongs to the group of the I class IFNs, that have an ambiguous role in the defense against TBC infection (19). Episodes of TBC reactivation during IFN-α therapy for HCV are widely described in literature (20,21).

Concerning the previous treatment with IFN-α2b assumed by the patient, we highlight the possibility that after long time assumption of this drug there has been a decrease in anti-TBC innate activity, but this remains only a hypothesis that cannot be confirmed.

The case we report teaches that lymphadenopathy with or without constitutional symptoms developing during Ruxolitinib therapy should be considered as a possible manifestation of a TBC reactivation in patients with a previous positive Mantoux test or Quantiferon TB test. A Mantoux skin test or a QuantiFERON TB serum test is strongly recommended before the start of a JAK inhibitor therapy. When suspecting a TBC infection or reactivation, even if presenting with isolated lymphadenopathy, Ziel-Nielsen testing on urine and

sputum have to be performed to rule out infectiousness and eventually isolate the patient. Moreover, previous long-time exposition to IFN-α2b may be related with a higher risk for TBC reactivation in these subset of patients. We encourage re-evaluation of the cohorts of patients treated with Ruxolitinib in previous and current large prospective studies to study the possible correlation between previous exposition to IFN-α2b and TBC reactivation.

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References:

1. Sant'Antonio E, Bonifacio M, Breccia M, Rumi E. A journey through infectious risk associated with ruxolitinib. Br J Haematol. 2019 Nov;187(3):286–95.

2. Cervantes F. How I treat myelofibrosis. Blood. 2014 Oct 23;124(17):2635–42.

3. Lussana F, Cattaneo M, Rambaldi A, Squizzato A. Ruxolitinib-associated infections: A systematic review and meta-analysis. Am J Hematol. 2018 Mar;93(3):339–47.

4. Schönberg K, Rudolph J, Vonnahme M, Parampalli Yajnanarayana S, Cornez I, Hejazi M, et al. JAK Inhibition Impairs NK Cell Function in Myeloproliferative Neoplasms. Cancer Res. 2015 Jun 1;75(11):2187–99.

5. Heine A, Held SAE, Daecke SN, Wallner S, Yajnanarayana SP, Kurts C, et al. The JAK-inhibitor ruxolitinib impairs dendritic cell function in vitro and in vivo. Blood. 2013 Aug 15;122(7):1192–202.

6. Colomba C, Rubino R, Siracusa L, Lalicata F, Trizzino M, Titone L, et al. Disseminated tuberculosis in a patient treated with a JAK2 selective inhibitor: a case report. 2012;2.

7. Patil V, S C, Wasekar N, Jijina F, Mohite A. Ruxolitinib-associated tuberculosis - A rare complication of a novel drug! Int J Med Sci Public Health. 2017;6(3):1.

8. Khalid F, Damlaj M, AlZahrani M, Abuelgasim KA, Gmati GE. Reactivation of tuberculosis following ruxolitinib therapy for primary myelofibrosis: Case series and literature review. Hematol Oncol Stem Cell Ther. 2020 Mar;S1658387620300327.

9. Shamil E, Cunningham D, Wong BLK, Jani P. Ruxolitinib Associated Tuberculosis Presenting as a Neck Lump. Case Rep Infect Dis. 2015;2015:1–3.

10. Hopman RK, Lawrence SJ, Oh ST. Disseminated tuberculosis associated with ruxolitinib. Leukemia. 2014 Aug;28(8):1750–1.

11. Branco B, Metsu D, Dutertre M, Marchou B, Delobel P, Recher C, et al. Use of rifampin for treatment of disseminated tuberculosis in a patient with primary myelofibrosis on ruxolitinib. Ann Hematol. 2016 Jun 1;95(7):1207–9.

12. Chen Y-H, Lee C-H, Pei S-N. Pulmonary tuberculosis reactivation following ruxolitinib treatment in a patient with primary myelofibrosis. Leuk Lymphoma. 2015 May 4;56(5):1528–9.

13. Lescuyer S, Ledoux M-P, Gravier S, Natarajan-Amé S, Duval C, Maloisel F, et al. Tuberculosis and atypical mycobacterial infections in ruxolitinib-treated patients with primary or secondary myelofibrosis or polycythemia vera. Int J Infect Dis. 2019 Mar 1;80:134–6.

14. Abidi MZ, Haque J, Varma P, Olteanu H, Guru Murthy GS, Dhakal B, et al. Reactivation of Pulmonary Tuberculosis following Treatment of Myelofibrosis with Ruxolitinib. Case Rep Hematol [Internet]. 2016 [cited 2020 Jul 14];2016. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5097796/

15. Kassa E, Enawgaw B, Gelaw A, Gelaw B. Effect of anti-tuberculosis drugs on hematological profiles of tuberculosis patients attending at University of Gondar Hospital, Northwest Ethiopia. BMC Hematol [Internet]. 2016 Jan 8 [cited 2020 Jul 6];16. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4706672/

16. Amagon KI, Awodele O, Akindele AJ. Methionine and vitamin B-complex ameliorate antitubercular drugs-induced toxicity in exposed patients. Pharmacol Res Perspect. 2017 Oct;5(5):e00360.

17. Tsukamoto Y, Kiyasu J, Tsuda M, Ikeda M, Shiratsuchi M, Ogawa Y, et al. Fatal Disseminated Tuberculosis during Treatment with Ruxolitinib Plus Prednisolone in a Patient with Primary Myelofibrosis: A Case Report and Review of the Literature. Intern Med. 2018 May 1;57(9):1297–300.

18. Pepeler MS, Özkurt ZN, Güzel ÖT, Akyürek N. Tuberculosis reactivation related with ruxolitinib in a patient with primary myelofibrosis. J Infect Dev Ctries. 2018 Oct 31;12(10):926–8.

19. Donovan ML, Schultz TE, Duke TJ, Blumenthal A. Type I Interferons in the Pathogenesis of Tuberculosis: Molecular Drivers and Immunological Consequences. Front Immunol [Internet]. 2017 Nov 27 [cited 2020 Jul 6];8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5711827/

20. Sabbatani S, Manfredi R, Marinacci G, Pavoni M, Cristoni L, Chiodo F. Reactivation of severe, acute pulmonary tuberculosis during treatment with pegylated interferon-alpha and ribavirin for chronic HCV hepatitis. Scand J Infect Dis. 2006 Jan;38(3):205–8.

21. de Oliveira Uehara SN, Emori CT, Perez RM, Mendes-Correa MCJ, de Souza Paiva Ferreira A, de Castro Amaral Feldner AC, et al. High incidence of tuberculosis in patients treated for hepatitis C chronic infection. Braz J Infect Dis. 2016 Mar;20(2):205–9.

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