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Impaired response to first SARS-CoV-2 dose vaccination in myeloproliferative neoplasm patients receiving ruxolitinib

To the Editor:

Covid-19, the disease caused by pandemic SARS-CoV-2 infection, had significant impact on patients with hematologic conditions[1]; a meta-analysis involving 3377 patients with hematologic malignancies who were affected by Covid-19 reported a mortality rate of 34%.[2] A similarly dismal outcome was documented among 175 patients with chronic myeloproliferative neoplasms (MPN), collected in a European observational study, where mortality rate was 30% for the entire cohort, reaching 48% in primary overt myelofibrosis (MF).[3] Covid-19 was also associated with higher incidence of thrombosis in patients with essential thrombocythemia (ET), compared to MF and polycythemia vera (PV) (20% vs 5% for both, respectively).[4] Finally, MPN patients surviving the acute phase may suffer from additional long-term sequelae from Covid-19, that furtherly increase mortality and morbidity.[5]

The JAK1 and JAK2 inhibitor (JAKi) ruxolitinib is approved for the treatment of patients with MF and hydroxyurea resistant/refractory PV.[6] By inhibiting JAK–STAT signaling, ruxolitinib has profound effects on different cell compartments of the immune system, including T cells, natural killer and dendritic cells, in addition to potently dampening inflammatory cytokine production.[7] These properties have been mechanistically linked to the increased rate of infections in MPN patients receiving ruxolitinib, and, conversely, were explored successfully in the setting of steroid-refractory acute graft vs host disease following allogeneic stem cell transplantation.[8] In the above cited European study in MPN, rapid discontinuation of the drug was implicated in 75% of deaths occurring in the ruxolitinib-treated cohort; these were ascribed to a previously described "discontinuation syndrome", a potentially fatal complication due to a cytokine storm that follows the abrupt suspension of ruxolitinib.[9,10] In fact, observational studies support the effectiveness of ruxolitinib to quench the hyper inflammatory reaction accompanying Covid-19 in the general population.[11,12] Due to the immunomodulatory properties of ruxolitinib, the question arises whether response to vaccination for SARS-CoV-2 in patients under stable ruxolitinib therapy might be impaired.

We prospectively assessed serologic response following the first injection of SARS-CoV-2 mRNA vaccine in 30 consecutive patients with PV, ET and MF who were referred to the Center of Research and Innovation of Myeloproliferative Neoplasms (CRIMM), Florence. Patients signed an informed consent to participate in the study, that was approved by the local Ethical Committee of Azienda Ospedaliera Careggi, Florence. Patients were eligible if they had a diagnosis of MPN according to the 2016 WHO criteria and all the following at the time of study entry: no history of positivity for SARS-CoV-2 by PCR on swab; negativity of serum anti-nucleoprotein antibodies; no clinical suspicion of Covid-19. A cohort of 14 healthy volunteers without prior SARS-CoV-2 infection was used as a reference group. The vaccines used were the Moderna and Pfizer vaccine in 25 (83%) and five (17%) MPN patients, and 10 (71%) and four (29%) healthy controls, respectively. Blood samples were collected before first vaccination (T0) and right before the second dose administration (T1, day 21 for Pifzer, day 28 for Moderna). Serologic tests for SARS-CoV-2 antibodies were performed to demonstrate presence of IgG antibodies against spike (S) protein, receptor binding domain (RBD) and neutralizing antibodies. A cut-off value of test positivity was established for each antibody type according to manufacturer's instructions; patients above the upper cut-off level were considered as "responders", and those below as "non responders". Categorical variables were expressed as frequency and percentage. Chi-square test was used to compare categorical variables.

Clinical characteristics of the patients are outlined in Table S1. There were 10, seven and 13 patients with PV, ET and MF (two and three patients were post-PV and post-ET MF, respectively). Of these, 18 were on a stable dose of ruxolitinib since at least 3 months (ruxo-patients), while 12 were not currently treated, nor had received before, ruxolitinib (no-ruxo patients), including five patients under watch-and-wait and five under hydroxyurea therapy since at least 3 years. The current median dose of ruxolitinib was 20 mg daily (range, 10–50 mg), and the median duration of ruxolitinib therapy was 7.3 years (range, 0.8–13.8 years). Figure 1 shows the levels of individual anti-SARS-CoV-2 antibodies at T0 and T1. Anti-S IgG, anti-RBD IgG and neutralizing Ab were not detected before vaccination in any of the three groups, accordingly to predefined cut-off levels. In general, the extent of specific antibody response after first dose vaccination (T1 time point), measured as binding antibody unit (BAU)/mL for anti-S and anti-RBD immunoglobulin, and relative index for neutralizing antibodies (Figure 1(A)–(C)), was significantly lower in

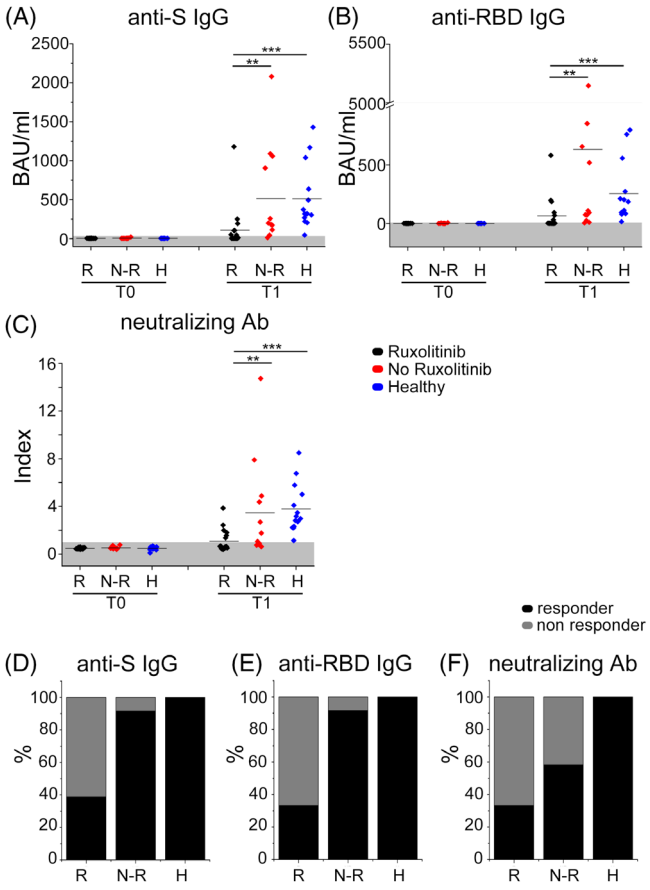

FIGURE 1 Serum levels of (A) anti-Spike IgG, (B) anti-RBD IgG, (C) neutralizing antibodies in 18 ruxolitinib-treated MPN patients (black diamonds), 12 no-ruxolitinib treated MPN patients (red diamonds), 14 healthy controls (blue diamonds), before the first (T0) or the second (T1) vaccine dose administration. Horizontal lines represent mean values. Gray area represent cut-off value. Data in A,B are expressed as binding antibody unit (BAU)/mL, while in C as Index. $^{**}p < 0.01$; $^{***}p < 0.01$ calculated with Mann–Whitney test. Percentage of ruxolitinib-treated (R), no-ruxolitinib-treated (N-R) MPN patients or healthy subjects (H) who developed antibodies D, (anti-S IgG, anti-RBD IgG, E, or neutralizing antibodies, F,) above (black, "responder") or below (gray, "non-responder") the predetermined cut-off levels

ruxo-patients compared to healthy controls and the no-ruxo group. The latter conversely did not differ significantly from controls, suggesting that the potentiality to mount adequate immune response is maintained in most MPN patients who were not receiving ruxolitinib. In detail, mean anti-S BAU levels/ml were 111.4, 513.4 and 510.8 for ruxo, no-ruxo and controls, respectively (Figure 1(A)); corresponding anti-RBD BAU levels/mL were 65.2, 631.7 and 254.2 (Figure 1(B)); and, for neutralizing antibodies, the mean relative index was 1.1, 3.5 and 3.8 (Figure 1(C)). All these values were statistically different when comparing ruxo patients vs no-ruxo and controls, but not between controls and no-ruxo patients (p values are reported in Figure 1(A)–(C)).

According to the predefined cut-off levels, all the 14 healthy volunteers were considered as responders to vaccination for the anti-S IgG, anti RBD IgG and neutralizing antibodies (100% each), compared to 38.8%, 33.3% and 33.3%, respectively, for the ruxo-patients ($p < 0.001$ vs controls for each antibody type), and 91.6% (p = not significant), 91.6% (p = not significant) and 58.3% (p = < 0.01) in the no-ruxo group (Figure 1(D)–(F)).

In summary, these findings, with the limitation of the small number of subjects included, make a strong and urgent argument for an impaired early response to SARS-CoV-2 vaccine in patients receiving ruxolitinib. Further and future studies are needed to address whether such unresponsive status persists after the second dose of vaccine, as suggested by a study performed in Israel, where the rate of seropositivity (anti-S1/S2 IgG) after complete vaccination in patients with MPN was 42% for those using JAKi.[13] It will be important to address whether also responses mediated by T-cell and other myeloid cells are impaired by ruxolitinib treatment, owing to their key role in SARS-CoV-2 infection.[14–16] Although clear-cut relationships between specific anti-SARS-CoV-2 immunoglobulin titers and protection against the virus has not been unequivocally established in the general population, MPN patients receiving ruxolitinib should be urged to continue to adopt the best preventive measures against Covid-19 even after receiving vaccination, in the light of the evidences presented herein. Furthermore, it is also suggested that, since MPN patients not receiving ruxolitinib overall developed antibody titers that were comparable to healthy volunteers, but a proportion of them not did actually produce neutralizing antibodies, initiation of ruxolitinib therapy in a naïve patient might be prudentially delayed after completion of vaccination, unless urgently needed. This notwithstanding, we reinforce that patients with MPN, as for any hematologic malignancies, should be vaccinated, since the possibility of protection at any extent outweighs minor risks,[2] as supported also by recommendations from the American Society of Hematology (https://www.hematology.org/covid-19).

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CONFLICT OF INTEREST

A.M.V., membership on Advisory Board, Novartis; P.G., membership on Advisory Board, Novartis. Other authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Paola Guglielmelli, Alessandro M Vannucchi, Francesco Annunziato designed research Paola Guglielmelli, Alessio Mazzoni, Laura Maggi, Seble Tekle Kiros, Lorenzo Zammarchi, Sofia Pilerci, Arianna Rocca, Michele Spinicci, Miriam Borella, Alessandro Bartoloni, Gian Maria Rossolini, Francesco Annunziato, Alessandro M Vannucchi collected patients and performed research. Paola Guglielmelli, Alessio Mazzoni, Francesco Annunziato, Alessandro M Vannucchi wrote the manuscript. All authors read and approved the final version.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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REFERENCES

1. Passamonti F, Cattaneo C, Arcaini L, et al. Clinical characteristics and risk factors associated with COVID-19 severity in patients with haematological malignancies in Italy: a retrospective, multicentre, cohort study. *Lancet Haematol*. 2020;7(10):e737-e745.
2. Vijenthira A, Gong IY, Fox TA, et al. Outcomes of patients with hematologic malignancies and COVID-19: a systematic review and meta-analysis of 3377 patients. *Blood*. 2020;136(25):2881-2892.
3. Barbui T, Vannucchi AM, Alvarez-Larran A, et al. High mortality rate in COVID-19 patients with myeloproliferative neoplasms after abrupt withdrawal of ruxolitinib. *Leukemia*. 2021;35(2):485-493.
4. Barbui T, De Stefano V, Alvarez-Larran A, et al. Among classic myeloproliferative neoplasms, essential thrombocythemia is associated with the greatest risk of venous thromboembolism during COVID-19. *Blood Cancer J*. 2021;11(2):21.
5. Barbui T, Iurlo A, Masciulli A, et al. Long-term follow-up of recovered MPN patients with COVID-19. *Blood Cancer J*. 2021;11(6):115.
6. Coltro G, Vannucchi AM. The safety of JAK kinase inhibitors for the treatment of myelofibrosis. *Expert Opin Drug Saf*. 2021;20(2):139-154.
7. McLornan DP, Khan AA, Harrison CN. Immunological consequences of JAK inhibition: friend or foe? *Curr Hematol Malig Rep*. 2015;10:370-379.
8. Zeiser R, Burchert A, Lengerke C, et al. Ruxolitinib in corticosteroid-refractory graft-versus-host disease after allogeneic stem cell transplantation: a multicenter survey. *Leukemia*. 2015;29:2062-2068.
9. Tefferi A, Pardanani A. Serious adverse events during ruxolitinib treatment discontinuation in patients with myelofibrosis. *Mayo Clin Proc*. 2011;86:1188-1191.
10. Coltro G, Mannelli F, Guglielmelli P, Pacilli A, Bosi A, Vannucchi AM. A life-threatening ruxolitinib discontinuation syndrome. *Am J Hematol*. 2017;92(8):833-838.
11. Vannucchi AM, Sordi B, Morettini A, et al. Compassionate use of JAK1/2 inhibitor ruxolitinib for severe COVID-19: a prospective observational study. *Leukemia*. 2020;35:1121-1133.
12. La Rosée F, Bremer HC, Gehrke I, et al. The Janus kinase 1/2 inhibitor ruxolitinib in COVID-19 with severe systemic hyperinflammation. *Leukemia*. 2020;34:1799-1804.
13. Tzarfati KH, Gutwein O, Apel A, et al. BNT162b2 COVID-19 vaccine is significantly less effective in patients with hematologic malignancies. *Am J Hematol*. 2021;96(10):1195-1203. https://doi.org/10.1002/ajh.26284.
14. Mazzoni A, Maggi L, Capone M, et al. Cell-mediated and humoral adaptive immune responses to SARS-CoV-2 are lower in asymptomatic than symptomatic COVID-19 patients. *Eur J Immunol*. 2020;50(12):2013-2024.
15. Mazzoni A, Salvati L, Maggi L, et al. Impaired immune cell cytotoxicity in severe COVID-19 is IL-6 dependent. *J Clin Invest*. 2020;28:138554. https://doi.org/10.1172/JCI138554
16. Vanderbeke L, Van Mol P, Van Herck Y, et al. Monocyte-driven atypical cytokine storm and aberrant neutrophil activation as key mediators of COVID-19 disease severity. *Nat Commun*. 2021;12(1):4117.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Serologic response to mRNA COVID-19 vaccination in lymphoma patients

To the Editor:

The development of effective COVID-19 vaccines has been essential in slowing the spread of SARS-CoV-2. However, unvaccinated populations as well as those who do not respond to vaccination still remain at risk. Very few cancer patients were included in the COVID-19 mRNA vaccine trials and any individuals receiving chemotherapy or immunotherapy within 6 months were excluded.[1] Consequently, we have an inadequate knowledge of how well these vaccines work in the cancer patient population. However, by extrapolation from other vaccines, we hypothesized that patients with hematologic malignancies, especially those on