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SUPPORTING INFORMATION

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SARS-CoV-2 vaccination in patients with paroxysmal nocturnal hemoglobinuria: An Italian multicenter survey

To the Editor:

SARS-CoV-2 infection and vaccination have raised concern in paroxysmal nocturnal hemoglobinuria (PNH).[1] In fact, PNH patients carry an increased infectious risk secondary to therapy with complement inhibitors or to underlying bone marrow failure (BMF), and therefore, may benefit from preventive strategies such as vaccinations. On the other hand, vaccines may potentially trigger a hemolytic flare or a breakthrough hemolysis (BTH) by amplifying complement activation, as described for meningococcal serogroup B vaccine.[2] So far, isolated reports of hemolytic exacerbations following SARS-CoV-2 vaccines have been reported in PNH[3–5] and other complement-mediated disorders, such as cold agglutinin disease and atypical hemolytic uremic syndrome.[5] We conducted a systemic survey among 8 Italian reference centers to evaluate complications and hemolytic flares in PNH patients who received SARS-CoV-2 vaccinations.

Adult patients with PNH regularly followed at 8 Italian institutions who received SARS-CoV-2 vaccinations from January 2, 2021 until the time of writing (data cut-off: January 31, 2022) were included. Disease history and treatments were collected via clinical charts. Vaccine- and PNH-related symptoms, hematologic data and hemolytic parameters (i.e., lactate dehydrogenase, LDH) before and 7–10 days after each dose of vaccine were recorded. For patients on complement inhibitors, BTH was classified as per European Bone Marrow Transplant Severe Aplastic Anemia Working Party criteria in (a) clinical BTH (Hb drop ≥2 g/dL from usual values or clinical signs or symptoms of hemolysis, in combination with LDH >×1.5 upper limit of normality, ULN), or (b) subclinical BTH (Hb drop <2 g/dL without clinical symptoms or signs, except moderate hemoglobinuria). Hemolysis occurring in treatment-naïve patients was defined as hemolytic flare/exacerbation. Adverse events were graded according to CTCAE v5.0. All patients gave informed consent to the study, which was conducted according to the Declaration of Helsinki.

About 87 PNH patients (median age 47.5 years, range 18; 91, 57% females) received SARS-CoV-2 vaccination and were included in the analysis. According to the International PNH Interest Group classification, 63% of patients suffered from hemolytic PNH, whilst 30% from PNH in the context of BMF and only 7% from subclinical PNH. Of this, 70 subjects (80%) were on complement inhibition therapy, mainly anti-C5 compounds (i.e., eculizumab $N = 46$, ravulizumab $N = 15$, crovalimab $N = 3$), 3 of whom in combination with oral anti-factor D inhibitor, and three patients were receiving oral anti-factor B single agent. A total of 240 vaccine doses were administered (Comirnaty/Pfizer-BioNTech $N = 179$, mRNA-1273/Moderna $N = 57$, and ChAdOx1 nCov-19/AstraZeneca $N = 4$). A total of 66 patients received the three doses, three received only the first dose because of previous SARS-CoV-2 infection, and 18 subjects had not yet received the third one at data cut-off (<6 months from the second dose). During the observation period, 13 BTH/hemolytic exacerbations were registered in 12 patients (13.8%), only three of which classifiable as clinical BTH (Table 1). Most events ($N = 10$) occurred after the second/third dose, generally within 24–48 hours; nine episodes followed Moderna vaccines, whilst 4 occurred after Pfizer vaccines. The median delta variations from usual Hb and LDH values were −14% (range −40; +3.2%) and +23% (+14; +105%), respectively. Anti-complement drugs were not modified/discontinued in any of the 10 patients on treatment. The most severe episode occurred after the second dose of Moderna vaccine in a 46-year-old woman on subcutaneous ravulizumab (Patient #1) who experienced a Hb drop from 9.1 to 6.7 g/dL, with marked increase of LDH to 2.1xULN and hemoglobinuria. The patient required hospitalization for additional treatment with recombinant erythropoietin and anti-thrombotic/bacterial prophylaxis. The second more severe BTH was registered in a 28-year-old male on eculizumab (Patient #2) who needed transfusion with 2 units of red blood cells for a symptomatic Hb drop from 10.8 to 6.5 g/dL after his first dose of Moderna vaccine. Of note, the same patient had experienced a clinical BTH during coronavirus disease 2019 (COVID-19) bilateral pneumonia, requiring high-dose steroids, antibiotics, transfusions, additional eculizumab doses, and anti-thrombotic prophylaxis. The third severe clinical BTH occurred in a 27-year-old female on eculizumab (Patient #3) with Hb drop from 9.8 to 7.7 g/dL and dark urines 1 day after the third dose of vaccine; the episode resolved in few days with no need of interventions. The remaining episodes were mild. Of note, Patient #4, who was on oral anti-factor B treatment, required hospitalization for intravenous antibiotic therapy due to concomitant urinary tract infection. Additionally, Patient #11 had two mild hemolytic flares after both the second and the third dose of two different vaccines. Patients not experiencing BTH (86.2%) showed stable hematologic parameters after vaccination. Interestingly, 8 out of 10 patients with a previous SARS-CoV-2 infection completed the vaccination schedule without any complication/PNH exacerbation. Overall, vaccines were well-tolerated, with six non-hematologic adverse events after the first dose (4 fever, one grade 1 exercise-induced tachycardia, and one grade 2 dizziness), 5 after the second one (3 fever, accompanied by vomit in one patient, and 1 flu-like syndrome, all grade 1) and 7 after the third one (3 fever, 2 asthenia, 1 nausea, 1 headache). Anti-spike protein antibodies were available for 18 patients, of whom 16 on anti-complement treatment. In this, 15 showed protective titers (median 838 U/mL, range 26; 7500 U/mL), whilst 3 had a titer <80 U/mL (1 with an associated BMF, and 2 on complement inhibitors). Anti-complement treatment was not associated with impaired antibody response to vaccine ($p = .31$).

This multicenter survey showed a frequency of 13.8% BTH/hemolytic flares following SARS-CoV-2 vaccination among a cohort of 87 PNH patients. The inflammatory stimulus provided by the vaccine may act as a trigger for complement activation similar to what described during infections, including COVID-19.[1] Moreover, hemolytic flares with thrombotic events after *Neisseria meningitis* vaccination have been previously described in treatment-naive PNH patients.[6] Most of our flares were mild, in line with what reported in literature.[3–5] However, 3 of our patients, all on complement inhibitors, had clinically significant BTH requiring supportive therapy and hospitalization. In this view, the protective role of complement inhibitors against hemolytic triggers, such as vaccines, is matter of debate. It has been hypothesized that complement inhibition may exert a protective role against thrombo-inflammation during COVID-19 infection. Consistently, Gerber et al. reported on a case of small bowel microvascular thrombosis in a treatment-naive PNH subject,[4] and the patient described by Portuguese et al. started eculizumab because of the severity of the hemolytic flares following vaccination.[3] Most flares described in our survey and reported in literature occurred within few days after vaccine administration, suggesting close clinical monitoring and patients' education particularly in the first week following vaccine.[3–5] Moreover, most exacerbations occurred after the second or third dose, possibly indicating that booster doses may further amplify the inflammatory response. The frequency of BTH following vaccine is greater than that reported during COVID-19 (2.5%).[7] However, the risk/benefit of COVID-19 infection versus vaccination in PNH deserves some considerations. The Italian survey reported a mild course of COVID-19 infection, whilst the Leeds experience a more severe course with concomitant hemolytic flares.[1] This difference may be due to heterogeneous inclusion criteria: in the former study, all SARS-CoV-2 positive subjects were enrolled, whereas in the latter only in-hospital patients were considered. Overall, the benefits of vaccinating this patient population clearly outweigh the potential severity of COVID-19. In fact, vaccines represent a "programmed" hemolytic trigger, whilst COVID-19 infection is unpredictable and its course may range from asymptomatic to lethal.[1] Finally, we report protective antibody titers in almost all PNH patients tested, independently from treatment with complement inhibitors, at variance with B-cell depleting therapies administered in other hematologic diseases. The limited numbers of patients tested deserves larger confirmation studies.

In conclusion, BTH/hemolytic flares following SARS-CoV-2 vaccines were observed in about 14% of PNH patients, were generally mild, and manageable if clinically relevant. Thus, a possible hemolytic

TABLE 1 Clinical and hematologic characteristics of patients with paroxysmal nocturnal hemoglobinuria (PNH) experiencing breakthrough hemolysis (BTH)/hemolysis exacerbation following SARS-CoV-2 vaccination

Pt	Age/sex	PNH type	Anti-complement therapy	Baseline Hb (g/dL)/ LDH (× ULN)	Vaccine type, dose	Hb nadir (g/dL)/LDH zenith (× ULN)	BTH classification[a]	Time of BTH occurrence (days after vaccine administration)	Treatment provided	Outcome
#1	46/F	BMF-associated	Ravulizumab SC weekly	9.1/1.02	Moderna, 2^	6.7/2.1	Clinical	1	Hospitalization, IV antibiotics, LMWH prophylaxis, rhEPO 40 000 IU/week	Discharged after 5 days, Hb 8.9 g/dL
#2	28/M	Hemolytic	Eculizumab	10.8/1.08	Moderna, 1^	6.5/1.87	Clinical	2	2 pRBC units	Resolved in 48 hours
#3	27/F	Hemolytic	Eculizumab	9.8/0.9	Moderna, 3^	7.7/1.16	Clinical	1	—	Resolved in 72 hours
#4	62/M	Hemolytic	Iptacopan BID	14.2/1.34	Moderna, 1^	12.7/1.43	Subclinical	10	Hospitalization for concomitant febrile UTI, no PNH treatment changes	UTI responsive to IV antibiotics, discharged after 6 days
#5	55/M	Hemolytic	Ravulizumab SC weekly	14.1/0.85	Moderna, 2^	13.9/1.1	Subclinical	1	—	Dark urines resolved in 24 hours
#6	18/M	Hemolytic	Eculizumab	12.9/1.76	Pfizer, 1^	11.1/2	Subclinical	2	—	Resolved in 24 hours
#7	49/M	Hemolytic	Eculizumab	11/1.1	Moderna, 2^	9.6/1.32	Subclinical	1	—	Resolved in 24 hours
#8	55/F	Hemolytic	Eculizumab	8.8/1.28	Moderna, 2^	8.3/1.57	Subclinical	1	—	Resolved in 24 hours
#9	39/F	Hemolytic	Eculizumab	11.5/0.87	Moderna, 2^	10.4/1.02	Subclinical	1	—	Resolved in 24 hours
#10	30/F	BMF-associated	Eculizumab	10/1.14	Pfizer, 3^	9.6/1.3	Subclinical	1	—	Resolved in 24 hours
#11	54/F	BMF-associated	—	12.3/5.4	Pfizer, 2^	12.7/9.7	Subclinical	2	—	Resolved in 24 hours
				12/4.7	Moderna, 3^	11.5/5.5	Subclinical	1	—	Resolved in 24 hours
#12	60/M	Hemolytic	—	10.3/6.4	Pfizer, 3^	8.9/7.2	Subclinical	1	—	Resolved in 24 hours

Abbreviations: BID, twice daily; BMF, bone marrow failure; Hb, hemoglobin; IV, intravenous; LDH, lactate dehydrogenase; LMWH, low molecular weight heparin; pRBC, packed red blood cells; rhEPO, recombinant human erythropoietin; SC, subcutaneous; ULN, upper limit of normal; UTI, urinary tract infection.

[a]Definition of clinical/subclinical BTH was performed according to Risitano, et al., Front Immunol, 2019.

flare should not discourage vaccination, provided a close clinical and laboratory monitoring.

CONFLICT OF INTERESTS

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

Juri Alessandro Giannotta, Bruno Fattizzo and Wilma Barcellini conceived the study and wrote the paper. All authors followed the patients, collected data, and revised the paper for intellectual content.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Outcomes of *TP53*-mutated AML with evolving frontline therapies: Impact of allogeneic stem cell transplantation on survival

To the Editor:

TP53 mutations occur in 10%–20% of patients with acute myeloid leukemia (AML) and are predominantly associated with therapy-related AML and complex cytogenetics (CG).[1] *TP53*-mutated (m) AML is considered a high-risk disease, which is resistant to conventional chemotherapy and confers poor prognosis.[1] Recently, several novel therapies have been approved, providing an opportunity to have a risk-adapted approach to AML treatment. Among the newer therapies, CPX-351, a liposomal formulation of cytarabine and daunorubicin, was approved for the therapy of adults with newly diagnosed AML with myelodysplasia-related changes (AML-MRC) and therapy-related AML based on an improvement in OS when compared with 3 + 7 chemotherapy.[2] Similarly, in the