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RAPID COMMUNICATION

Hemolysis induced by SARS-CoV-2 mRNA vaccination in patients with paroxysmal nocturnal hemoglobinuria

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Abstract

Autoimmune and complement-related hematological side effects have been observed with messenger ribonucleic acid (mRNA) vaccines. Here, we report the incidence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine-induced hemolysis in patients with paroxysmal nocturnal hemoglobinuria (PNH). We reviewed the medical records of seventeen patients with PNH visiting the University of Tsukuba Hospital who had received two doses of the SARS-CoV-2 mRNA vaccine between May 2021 and November 2021. Twelve patients were being treated with complement inhibitors. The median age of all patients was 62 years (range 29–89 years).; six were males and eleven were females. Fourteen patients received the BNT162b2 vaccine (Pfizer/BioNTech) and three received the mRNA-1273 vaccine (Moderna). The median percentages of PNH clones in erythrocytes and granulocytes were 37.61% (range 8.11–85.71%) and 59.73% (range 3.76–97.82%), respectively. Of the twelve patients receiving complement inhibitors, only one had a hemolytic reaction after vaccination, but it did not meet the definition of breakthrough hemolysis. By contrast, hemolytic attacks were observed in two of the five untreated patients with PNH, and one of them required a blood transfusion. Appropriate administration of complement inhibitors to patients with PNH may prevent hemolysis induced by SARS-CoV-2 mRNA vaccination.

Keywords PNH · mRNA vaccine · SARS-CoV-2 · Complement inhibitor · Hemolysis

Introduction

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a major public health problem. The efficacy of messenger ribonucleic acid (mRNA) vaccines has been reported, and hence, they have been widely administered worldwide [1, 2]. However, autoimmune and complement-related hematological side effects have been observed for mRNA vaccines [3]. Paroxysmal nocturnal hemoglobinuria (PNH) is a bone marrow failure disorder induced by acquired PIG-A gene mutations that lead to deficiencies in complement regulatory proteins CD55 and CD59, resulting in intravascular hemolysis and thrombosis [4]. Breakthrough hemolysis (BTH) and thrombosis have both been reported in patients with PNH following SARS-CoV-2 infections; thus, vaccination for these patients is generally warranted [5, 6]. Although anemia, hemolysis attacks, and thrombosis after mRNA vaccination have been reported in patients with PNH [7–9], the number of patients enrolled in those studies was small. Here, we describe SARS-CoV-2 vaccine-induced hemolysis in a larger cohort of patients with PNH with and without complement inhibitor administration.

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Patients and methods

With the approval of the Ethics Committee, we reviewed the medical records of all patients with PNH visiting the University of Tsukuba Hospital who had received two doses of a SARS-CoV-2 mRNA vaccine between May and November 2021. Patients whose physical examination, blood, and urine test data were available within four weeks of the first or second vaccine doses were included. For the cohort without complement inhibitor administration, patients with erythrocyte PNH clone size > 5% and lactate dehydrogenase (LDH) > 1.5 × upper limit of normal (ULN) were enrolled [10, 11]. Baseline patient characteristics included age, sex, type of complement inhibitor administered, time since anti-complement therapy, type of vaccine administered, and percentage of PNH clones within twelve months prior to the first vaccination. Before and after the first and second vaccinations, the following test results were retrieved to estimate the extent of hemolysis: hemoglobin (Hb), total bilirubin (T-bil) (normal range 0.3–1.2 mg/dL), LDH (normal range 124–222 U/L), and 50% hemolytic unit of complement (CH50) levels, reticulocyte counts, blood transfusion and hospitalization records, and gross hemoglobinuria. Complement inhibitor administration and blood sample collection were performed on the same day. BTH in patients on complement inhibitor therapy was defined as the presence of ≥ 1 new or worsening symptoms of intravascular hemolysis in the presence of LDH ≥ 2 times the ULN [10].

Results and Discussion

Seventeen patients with PNH were enrolled in this study. The clinical characteristics of the patients and the duration from anti-complement therapy to vaccination are summarized in Table 1. Twelve patients (unique patient number [UPN] 1 to 12) were on complement inhibitors, and five patients (UPN 13–17) were not. The median age of all patients was 62 years (range 29–89 years). Patients included six males and eleven females. Fourteen patients received the BNT162b2 vaccine (Pfizer/BioNTech) and three received the mRNA-1273 vaccine (Moderna). The median duration from anti-complement therapy to the first and second vaccination was 6.5 days and 21 days, respectively. The median percentages of PNH clones were 37.61% (range 8.11–85.71%) and 59.73% (range 3.76–97.82%) in erythrocytes and granulocytes, respectively. Of the twelve patients receiving complement inhibitors, two received eculizumab, eight received ravulizumab, and two received crovalimab (subcutaneous injection every 4 weeks). The two patients receiving crovalimab were enrolled in the phase 1/2 clinical study [12]. Five patients (UPN 13–17) did not receive complement inhibitor treatment either because their hemolysis was stable or because the patient was unwilling to receive the treatment. The adverse effects

Table 1 Clinical characteristics of patients and duration from anti-complement therapy to vaccination

Patient	Age	Sex	RBC PNH clone %	Granulocyte PNH clone %	Anti-complement therapy	Vaccine	Duration from anti-complement therapy to 1st vaccination (days)	Duration from anti-complement therapy to 2nd vaccination (days)
UPN-1	67	M	36.31	30.22	Eculizumab	BNT162b2	12	19
UPN-2	77	F	33.11	3.76	Eculizumab	BNT162b2	7	6
UPN-3	58	M	36.86	82.29	Crovalimab	mRNA-1273	6	6
UPN-4	43	F	46.31	22.38	Crovalimab	BNT162b2	6	27
UPN-5	69	F	17.92	59.73	Ravulizumab	BNT162b2	2	23
UPN-6	62	M	8.11	66.39	Ravulizumab	BNT162b2	6	27
UPN-7	40	M	40.18	25.18	Ravulizumab	BNT162b2	6	27
UPN-8	80	F	70.09	5.60	Ravulizumab	BNT162b2	36	1
UPN-9	71	F	77.04	95.19	Ravulizumab	BNT162b2	12	33
UPN-10	47	F	45.63	80.39	Ravulizumab	BNT162b2	6	27
UPN-11	71	F	85.71	93.56	Ravulizumab	BNT162b2	50	15
UPN-12	71	F	8.28	35.76	Ravulizumab	BNT162b2	43	8
UPN-13	53	F	10.02	40.41	None	BNT162b2	N/A	N/A
UPN-14	31	M	34.38	97.82	None	BNT162b2	N/A	N/A
UPN-15	62	F	37.61	56.87	None	mRNA-1273	N/A	N/A
UPN-16	89	F	39.88	95.91	None	BNT162b2	N/A	N/A
UPN-17	29	M	73.87	94.65	None	mRNA-1273	N/A	N/A

RBC red blood cells, *PNH* paroxysmal nocturnal hemoglobinuria, *UPN* unique patient number, *N/A* not available

and intervals between vaccination and blood tests are shown in Table 2. Two and one patients in the complement inhibitor group and the corresponding untreated group presented with a fever over 38 degrees Celsius, respectively. All patients presented with injection site pain.

The clinical data before and after vaccination are summarized in Table 3. The twelve patients receiving complement inhibitors had no clear increase in LDH and T-Bil levels after vaccination. UPN-11 showed a decrease in the Hb level at the visit on day 7 after the first dose of the vaccine. Self-reported gross hemoglobinuria was observed on the second day after vaccination but ended within a day and the Hb level recovered the following week. This patient did not present LDH elevation or any hemolysis-related symptoms and, therefore, did not meet the BTH definition. In contrast, of the five patients who did not receive complement inhibitors, two (UPN-16, 17) showed hemolysis attacks after vaccination (Fig. 1). UPN-16 became aware of persistent gross hemoglobinuria and severe fatigue three days after the second dose of the vaccine. The LDH level increased from 870 U/L before vaccination to 3119 U/L on day 14 after the second dose of vaccine, and severe anemia (Hb 5.9 g/dL), jaundice, and gross hemoglobinuria were observed, leading to the need for hospitalization. Furthermore, D-dimer increased from 1.6 to 6.7 μg/mL in this patient after the second vaccination, even though she did not have thrombosis. She was treated with four units of red blood cell transfusion, rehydration, and rest, and her anemia and hemolytic findings improved. UPN 17 was chronically hyper hemolytic with an LDH level of 1150 U/L before vaccination; however, after receiving the first dose of the vaccine, the LDH level increased to 2039 U/L on day 14 after the first dose of vaccine. Although it had decreased to 1643 U/L a week later, it increased again to 1926 U/L after the second vaccination. The patient's reticulocyte counts increased after vaccination, and persistent gross hemoglobinuria was observed. However, his D-dimer level remained unchanged. In this cohort, most cases did not exhibit a change in platelet count; nevertheless, only UPN-14 showed a slight decrease after both vaccination doses (from 6.2×10^9/L to 3.0×10^9/L). None of the patients developed thrombosis. No clear correlation was inferred between hemolysis and other adverse events such as fever (Tables 2, 3).

All patients receiving anti-complement therapy had received the meningococcal vaccine prior to the initiation therapy, and fourteen of the seventeen patients received an annual influenza vaccine. No hemolysis attack was observed after these vaccinations. Compared with conventional vaccines, mRNA vaccines could be considered a risk factor for hemolysis in patients with PNH [9, 13, 14].

Gerber et al. reported BTH after SARS-CoV-2 mRNA vaccine administration in three of five patients with PNH on complement inhibitors [9]. According to their report, among the three patients whose anemia worsened, only one had elevated LDH levels, and in the others, BTH was plausibly not the only cause of anemia. By contrast, in our cohort, apparent hemolysis attacks were observed in only two patients not receiving anti-complement therapy. Furthermore, only

Table 2 Adverse effects and intervals between vaccination and blood tests

Patient	Fever	Headache	Pain	Gross hemoglobinuria	Interval between 1st vaccination and blood test (days)	Interval between 2nd vaccination and blood test (days)
UPN-1	No	No	Yes	No	2	16
UPN-2	No	No	Yes	No	7	8
UPN-3	No	No	Yes	No	22	22
UPN-4	No	Yes	Yes	No	N/A	2
UPN-5	No	No	Yes	No	N/A	28
UPN-6	No	No	Yes	No	8	8
UPN-7	No	No	Yes	No	N/A	1
UPN-8	No	No	Yes	No	3	27
UPN-9	No	No	Yes	No	2	N/A
UPN-10	Yes	No	Yes	No	N/A	8
UPN-11	No	No	Yes	Yes	6	6
UPN-12	Yes	No	Yes	No	13	N/A
UPN-13	Yes	N/A	Yes	No	N/A	16
UPN-14	No	No	Yes	No	N/A	15
UPN-15	No	No	Yes	No	N/A	4
UPN-16	No	Yes	Yes	Yes	20	15
UPN-17	No	No	Yes	Yes	6	7

UPN unique patient number, *N/A* not available

Table 3 Laboratory data before and after vaccination

Patient	Hb (g/dL)			T-bil (mg/dL)			LDH (U/L)			CH50 (U/mL)		
	Pre[a]	Post 1[b]	Post 2[c]	Pre[a]	Post 1[b]	Post 2[c]	Pre[a]	Post 1[b]	Post 2[c]	Pre[a]	Post 1[b]	Post 2[c]
UPN-1	9.0	9.1	8.8	2.3	1.6	1.6	302	271	326	12.4	< 10	14.9
UPN-2	8.3	9.1	9.1	0.6	0.5	0.5	360	363	367	< 10	< 10	< 10
UPN-3	12.6	12.6	13.0	1.1	1.0	0.9	248	231	261	< 10	N/A	< 10
UPN-4	8.9	N/A	9.4	0.3	N/A	0.4	255	N/A	270	< 10	N/A	N/A
UPN-5	10.0	N/A	9.3	1.1	N/A	0.9	235	N/A	256	13.8	N/A	16.0
UPN-6	9.9	9.6	9.8	3.4	N/A	2.9	257	258	274	< 10	15.0	10.4
UPN-7	8.5	N/A	8.3	3.7	N/A	4.3	389	N/A	350	20.4	N/A	12.0
UPN-8	9.4	10.1	9.3	1.0	1.3	1.3	226	240	228	< 10	11.6	< 10
UPN-9	11.5	11.0	N/A	2.2	2.4	N/A	182	170	N/A	15.9	12.6	N/A
UPN-10	11.8	N/A	10.7	1.3	N/A	1.1	216	N/A	196	< 10	N/A	< 10
UPN-11	8.4	7.6	8.0	1.0	0.8	0.7	254	238	295	< 10	11.7	< 10
UPN-12	14.9	15.2	N/A	0.8	1.3	N/A	396	430	N/A	< 10	15.2	N/A
UPN-13	9.0	N/A	9.6	2.2	N/A	3.2	383	N/A	448	N/A	N/A	N/A
UPN-14	11.5	N/A	11.0	1.3	N/A	1.1	665	N/A	568	56.3	N/A	56.5
UPN-15	11.5	N/A	11.9	0.7	N/A	0.5	249	N/A	255	62.0	N/A	60.9
UPN-16	9.6	8.8	5.9	1.1	0.9	3.0	870	674	3119	71.7	70.5	87.3
UPN-17	6.3	6.1	6.5	1.8	1.6	1.3	1150	2039	1961	53.2	53.9	36.8

Hb hemoglobin, *T-bil* total bilirubin, *LDH* lactate dehydrogenase, *CH50* 50% hemolytic unit of complement, *UPN* unique patient number, *N/A* not available

[a]Baseline value

[b]Value after first dose of vaccine

[c]Value after second dose of vaccine

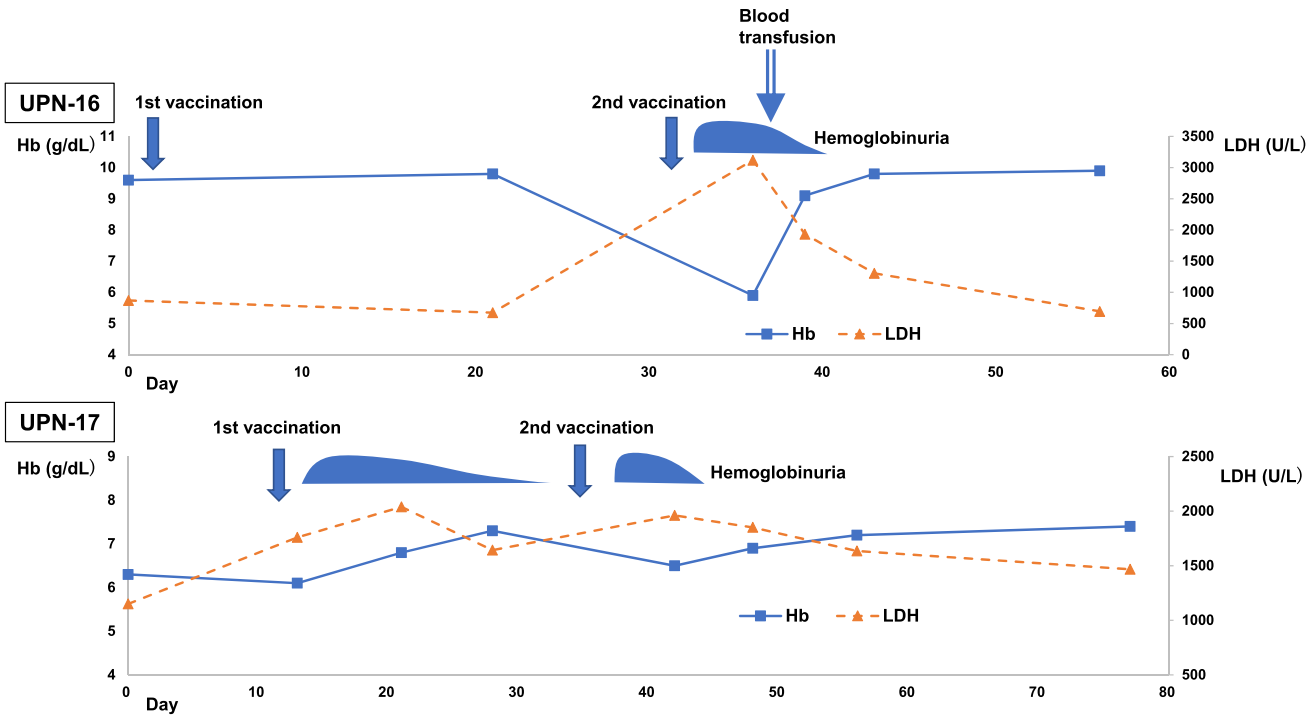

Fig. 1 Of the five patients who did not receive complement inhibitors, two (UPN-16, 17) had hemolysis attacks after vaccination for SARS-CoV-2

one out of the twelve patients receiving anti-complement therapy developed anemia. This patient had received ravulizumab 50 days before the initial vaccination, which may have decreased the concentration of the complement inhibitor and slightly increased intravascular hemolysis.

The spike protein of SARS-CoV-2 has been reported to compete with complement factor H for heparan sulfate binding and amplify the alternative complement pathway on the cell surface [15]. Recent data, however, suggest that BTH after the SARS-CoV-2 vaccination is not due to the direct effect of the spike protein [9]. In this report, it was speculated that increased complement activity due to the inflammatory response after the vaccination was the cause of clinically observed hemolysis [9]. In our cohort, UPN-16, who had required red blood cell transfusion, originally had a high CH50 value and a constantly high complement activity; the patient's CH50 values were further increased after the mRNA vaccination (Table 3). Conversely, UPN-11, who had self-reported gross hemoglobinuria after vaccination but did not have elevated LDH levels, did not show any increase in CH50 values after vaccination.

Our data suggest that the appropriate administration of complement inhibitors to patients with PNH may suppress hemolysis induced by SARS-CoV-2 vaccines. However, this was a retrospective analysis and was limited due to the possibility of missed asymptomatic hemolysis because of the lack of uniformity in the timing of post-vaccination blood tests. Case studies with larger cohorts are needed to clarify the detailed relationship between vaccination and complement inhibitors in patients with PNH.

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Declarations

Conflict of interest YK, TS, YY, and HN, declare no conflicts of interest. NO received research funding from Kyowa Kirin, Alexion Pharma outside the submitted work. MS-Y received research funding from Eisai, Otsuka Pharmaceutical, and Bristol Myers Squibb outside the submitted work. SC received research funding from Astellas, Ono, Kyowa Kirin, Sanofi, Takeda, and Chugai, outside the submitted work.

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