Autoimmune Acquired Factor XIII/13 Deficiency after SARS-CoV-2 mRNA Vaccination

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SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) vaccines have been approved and administered globally. Although there has been increasing evidence concerning the clinical effects, the adverse effects have not yet been fully elucidated. Various autoimmune diseases have been reported to develop in some patients after SARS-CoV-2 vaccination. We, here, report a case of autoimmune-acquired factor XIII/13 (FXIII/13) deficiency (AiF13D) accompanied by multiple purpuras, thrombocytopenia, and proteinuria after the second dose of the SARS-CoV-2 mRNA (messenger RNA) vaccine**,** BNT162b2.

The patient was a 75-year-old woman. She had past medical history of surgery for gastrointestinal stromal tumor in the small intestine at the age of 65. She received two doses of BNT162b2 and side effects after each vaccination were limited to muscle pain at the injection site. After 2 weeks of the second dose, perceived multiple purpuras in the upper limbs were observed. When the purpura spread to lower limbs, she visited our hospital on day 54 after the second dose of vaccination. Palpable purpura was observed in her upper and lower limbs upon physical examination; however, most of them were brownish and regressed. Urinalysis revealed proteinuria with 1,668 mg/g Cre, accompanied by occult blood. Blood tests revealed the white blood cell count of 7,720/μL (neut 69.3%, eosino 4.7%, baso 1.0%, mono 6.5%, lymph 18.5%), hemoglobin 10.2 g/dL, Ht 31.9%, platelet count 39×10^3/μL, and immature platelet fraction 13.9%. Hemostatic tests revealed the prothrombin time international sensitivity index of 1.04, activated partial thromboplastin time of 34.7 seconds, fibrinogen degradation products to be 20 μg/mL, and FXIII/13 activity to be 7% on day 54. Chemical and immunological analyses of blood revealed absence of hepatitis virus infection and specific autoimmune antibodies (►**Table 1**). She did not have any abnormal bleeding even in

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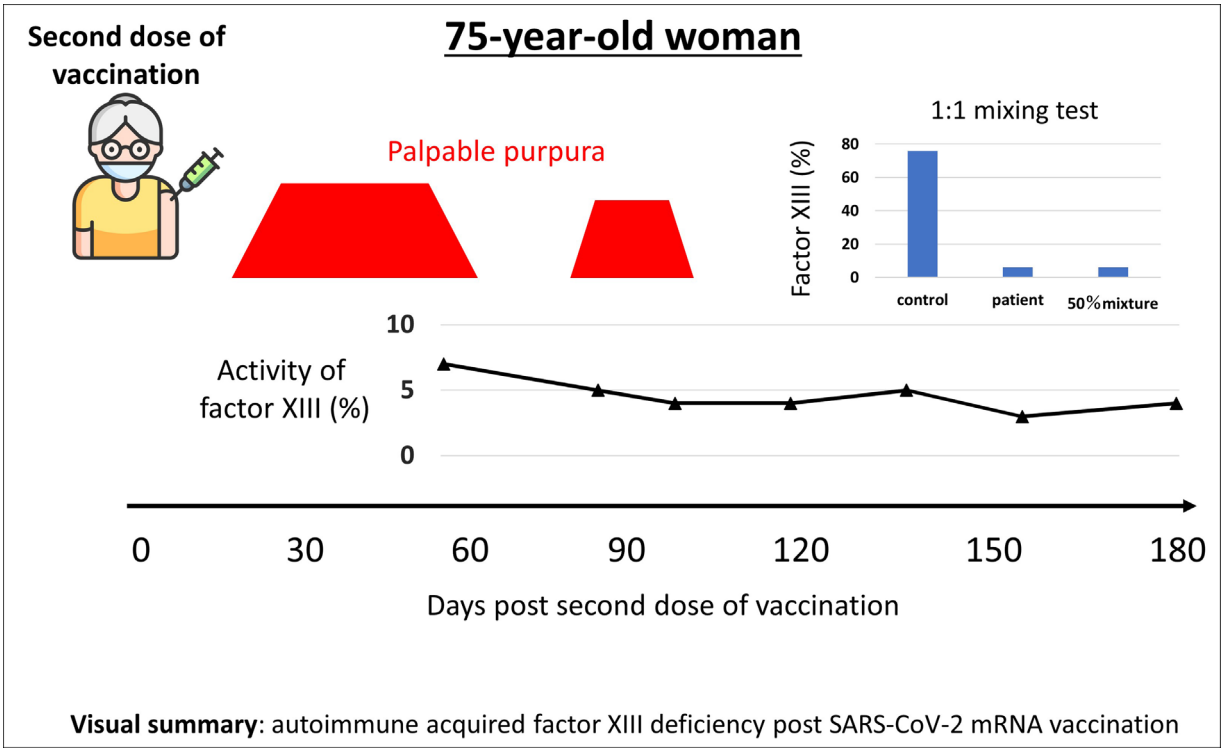

Visual summary: autoimmune acquired factor XIII deficiency post SARS-CoV-2 mRNA vaccination

the blood collection site and no after-bleeding, except for the purpura. Thrombosis was not detected by ultrasonography. During follow-up, exacerbations of fresh purpura (►**Fig. 1A**), proteinuria, and thrombocytopenia ($24 \times 10^3/\mu L$) were observed along with persistent low FXIII/13 activity (5% on day 82 after the second dose of SARS-CoV-2 vaccination). Histological analysis of skin biopsy showed slight infiltration of lymphocytes around the dermal vasculature and no deposition of immunoglobulin G (IgG), IgA, IgM, and complement, as per a direct immunofluorescence antibody technique. Gradual amelioration of purpura, proteinuria, and thrombocytopenia occurred after intravenous injection of immunoglobulin (20 g per day for 2 days); however, the activity of FXIII/13 did not improve until 180 days after the second dose (►**Fig. 1B**). During clinical course, until day 201, we had not administered FXIII/13 concentrate. Anti-platelet factor 4 (anti-PF4) antibody was not detected in the serum on day 82 by enzyme linked immunosorbent assay. The titer of serum anti-SARS-CoV-2 IgG antibody was 561.1 AU/mL, as measured by ARCHITECT SARS-CoV-2 IgG II Quant (Abbott Japan LLC, Tokyo, Japan) with ARCHITECT analyzer i 2000SR (Abbott Japan LLC, Tokyo, Japan) on day 82 after the second dose of vaccination. Her FXIII/13 activity was persistently low at 5% even on day 201.

In an experiment conducted by the Japanese Collaborative Research Group (JCRG) for autoimmune coagulation factor deficiencies (AiCFD), results of a 1:1 dilution mixing test with healthy control plasma did not show an increase of FXIII/13 activity (control 76%, patient 6%, 1:1 mixture 6%), as measured by ammonia release assay using Berichrom FXIII Kit (Sysmex Corporation, Kobe, Japan). In addition, other coagulation parameters are also measured at a commercial laboratory service (SRL Ltd., Hachioji, Japan) (►**Supplementary Table S1**, available in the online version); this hinted at the occurrence of FXIII/13 inhibition in the patient plasma.[1]

While the FXIII/13 A-subunit (FXIII/13-A) antigen level was moderately reduced (0.29 U/mL; reference range: 0.67–1.63 U/mL), its activity was extremely low (0.03 U/mL; reference range: 0.76–1.55 U/mL), as measured by an in-house amine incorporation assay.[2] A five-step dilution mixing test with healthy control plasma showed an FXIII/13 inhibitor pattern (►**Fig. 1C**), measured by the amine incorporation assay, and anti-FXIII/13-A autoantibodies were detected by both immunoblotting[2] and immunochromatography[3] (►**Fig. 1D,E**). Thus, a definite diagnosis of AiF13D was made, based on the International Society on Thrombosis and Haemostasis/Scientific and Standardization Committee criterion 2015.[4]

New-onset or exacerbation of autoimmune disease after SARS-COV-2 vaccination has been reported previously, namely vaccine-induced immune thrombotic thrombocytopenia (VITT)/thrombosis with thrombocytopenia syndrome (TTS), myocarditis, nephritis, and immune thrombocytopenia.[5] The mechanisms by which the vaccine triggers autoimmunity seem to involve molecular mimicry, production of particular autoantibodies, and role of certain vaccine adjuvants.[5] Our case revealed thrombocytopenia, purpura, and massive proteinuria at initial manifestation. Purpura appeared like palpable purpura, rather than petechiae, which is typical of thrombocytopenia. Serum anti-PF4 antibody was negative, and thrombosis was not clinically observed by ultrasonography; therefore, our case seemed to be different from VITT/TTS. Collagen

Table 1 Laboratory findings

	Urinalysis	Reference range			Reference range
Protein	(3 +)	(–)	ALT	19 U/L	5–40
Blood	(2 +)	(–)	LDH	270 U/L	124–222
Glucose	(–)	(–)	ALP	99 U/L	38–113
Protein	1,668 mg/g Cre	<150	γ-GTP	26 U/L	<30
Peripheral blood			TP	6.6 g/dL	6.7–8.3
Hb	10.2 g/dL	11.3–15.2	Alb	3.4 g/dL	3.8–5.2
Ht	31.9%	33.4–44.9	BUN	18 mg/dL	8.0–22.0
WBC	7,720 /μL	3,500–9,100	Cr	0.92 mg/dL	0.17–1.00
Neutro	69.3%	40.0–74.0	T-cho	135 mg/dL	150–219
Eosino	4.7%	0–6.0	HDL-C	30 mg/dL	40–96
Baso	1.0%	0–2.0	TG	95 mg/dL	50–149
Mono	6.5%	0–8.0	*Serological*		
Lymph	18.5%	18.0–59.0	CRP	0.40 mg/dL	<0.05
Plt	39 $\times$,10^3/μL	13.0–36.9	ANA	40 times	<40
IPF	13.9%	NA	MPO-ANCA	<3.5 U/mL	<3.5
Hemostasis			PR3-ANCA	<3.5 U/mL	<3.5
PT-INR	1.04	0.85–1.15	C3	113 mg/dL	86–160
APTT	34.7 s	24.3–36.0	C4	22 mg/dL	17–45
Fib	360 mg/dL	150–400	CH50	53.4 U/mL	25.0–48.0
FDP	20 μg/mL	<5	ASO	22 IU/mL	<239
FXIII	7%	70–140	HBsAg	<0.005 IU/mL	<0.005
Blood chemistry			HCVAb	<1.0 COI	<1.0
AST	26 U/L	10–40	IMP	M peak (–)	(–)

Abbreviations: ANA, antinuclear antibody; APTT, activated partial thromboplastin time; ASO, antistreptolysin O; FDP, fibrin/fibrinogen degradation product; IMP, immunoelectrophoresis; MPO-ANCA, myeloperoxidase-antineutrophil cytoplasmic antibodies; NA, not available; PR3-ANCA, proteinase-3-antineutrophil cytoplasmic antibodies.

disease, acute glomerulonephritis, and acute infection were thought as differential diagnosis, but laboratory findings were negative for anti-streptolysin O and other autoimmune antibodies. Based on the cutaneous findings, a vasculitis-like pathological condition was preferably considered, although skin biopsy did not show significant or typical findings for IgA vasculitis or other types of vasculitis. Thrombocytopenia, purpura, and proteinuria were improved by intravenous immunoglobulin and with lapse of time, implicating the involvement of infections that might have modulated the patient's pathophysiology in addition to vaccination. However, low FXIII/13 activity persisted, and molecular and biological approaches revealed inhibition of FXIII/13. Hemorrhagic history of abdominal surgery, 10 years ago, was not evident, and family history of congenital FXIII/13 deficiency and drug history leading to hemorrhage were not observed; therefore, we diagnosed the condition as acquired disease, not congenital FXIII/13 deficiency.[4] Thrombocytopenia and purpura were observed, but not those typical for AiF13D; therefore, multiple autoimmune reactions targeting multiple organs could have developed.

AiF13D has been reported to cause grade III bleeding, as high as 86%.[6] However, our case showed mild bleeding with purpura, despite the complete inhibition of fibrin cross-linking reaction (►**Fig. 1F**). Although the cause was not evident, it could probably be because FXIII/13 activity was maintained around 5%, even with FXIII/13 inhibitor. Immunosuppressive therapy and FXIII/13 concentrate replacement therapy were planned when her hemorrhagic tendency exacerbated.

Around 10 years ago, a JCRG on AiCFD started a nationwide survey for definitive diagnosis of patients with bleeding disorders in Japan. Although many patients with SARS-CoV-2 infection- and/or vaccination-related thrombosis were reported, only few had developed autoimmune bleeding disorder. However, other autoimmune bleeding disorder, acquired hemophilia A, was reported post vaccination.[7–10] AiF13D post-SARS-CoV-2 vaccination is extremely rare except for another case[11]; more clinical experiences are warranted in the future.

We concluded that AiF13D may develop after SARS-CoV-2 mRNA vaccination, and clinicians should keep the possibility in mind.

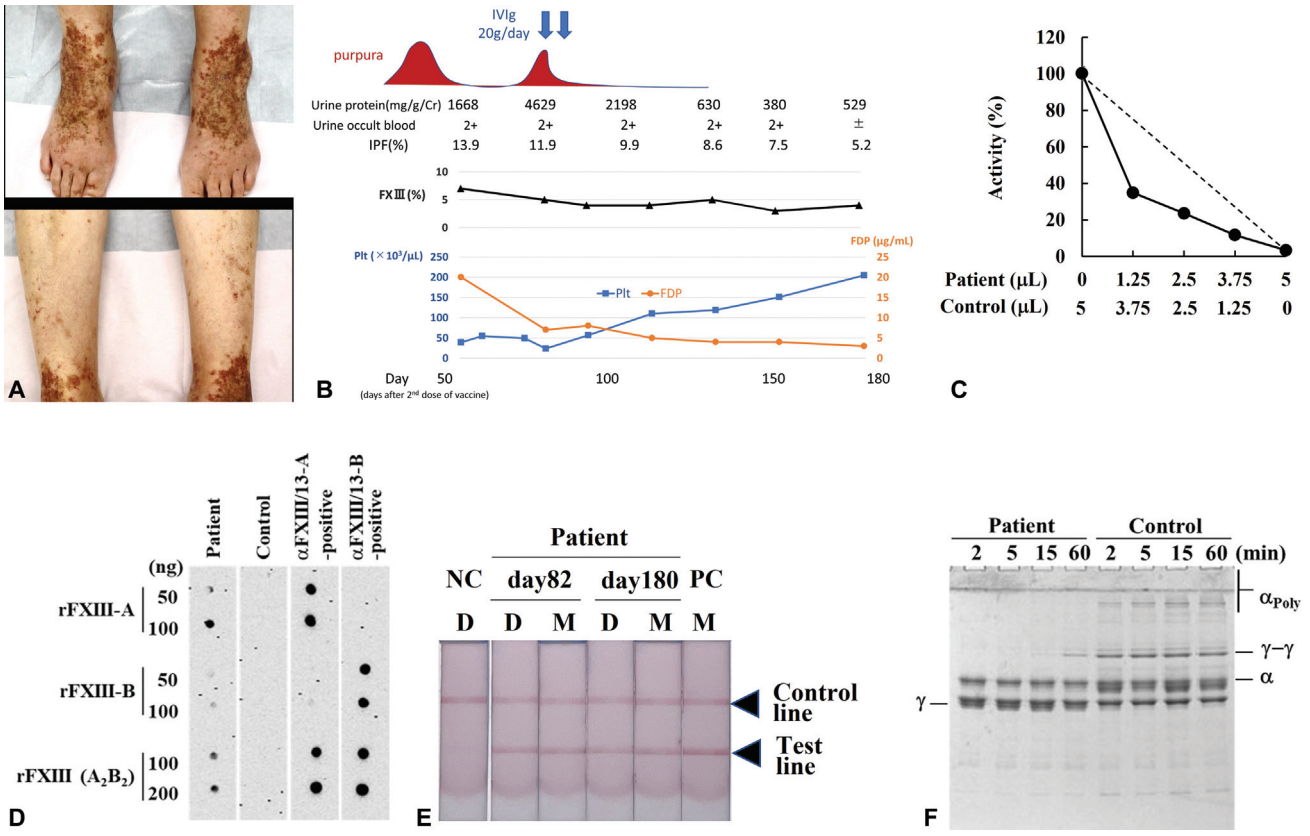

Fig. 1 (**A**) Purpura of the patient. Purpura of lower legs on day 82 after the 2nd dose of anti-SARS-CoV-2 vaccination. Palpable purpura was observed at the bilateral dorsum of foot (*upper*) and pretibial skin (*lower*). (**B**) Clinical course. FXIII/13 deficiency was recognized on day 54 with 7% activity (reference range: 70–140%). Purpura of the patient regressed spontaneously but persisted. Proteinuria, thrombocytopenia, purpura, and FXIII/13 deficiency were exacerbated on day 82. After intravenous immunoglobulin injection, proteinuria, thrombocytopenia, and purpura improved, but FXIII activities did not recover. (**C**) The Japanese Collaborative Research Group's (JCRG's) detailed analyses of the patient's FXIII/13 and anti-FXIII/13 antibodies. The five-step dilution cross-mixing test by an amine incorporation assay was performed using the patient's plasma in the ratios 0:1, 1:3, 1:1, 3:1, and 1:0 with normal plasma. The mixed samples were incubated at 37°C for 2 hours before the assay. The patient's sample showed a downward concave "inhibitor" pattern. The straight broken line depicts a theoretical "deficient" pattern. (**D**) Immunoblotting test for anti-FXIII/13 autoantibodies. The assay was performed using recombinant FXIII/13-A subunit (rFXIII/13-A), recombinant FXIII/13-B subunit (rFXIII/13-B), and their complexes (A2B2) at the indicated amounts shown as antigen (ng). The results showed presence of anti-FXIII/13-A antibodies. The positive control (PC) and negative control (NC) stand for AiF13D patient's plasma and healthy individual's plasma, respectively. (**E**) Immunochromatographic test for anti-FXIII/13-A subunit autoantibodies with (spiked; M) or without (direct; D) mixing patient's and normal control's plasma showed positive results both on day 82 and day 180. (**F**) Fibrin cross-linking reaction. Gamma-chain dimerization was extremely retarded and γ-chain monomer remained even after 60 minutes. Alpha-chain polymerization was almost absent. FDP, fibrinogen degradation product; IPF, immature platelet fraction; IVIG, intravenous immunoglobulin; Plt, platelet.

Author Contributions

S.N. collected clinical data and samples, wrote the draft, and proofread the manuscript. M.S. performed experimental work related to FXIII/13 and proofread the manuscript. K.A. wrote the draft and proofread the manuscript. A.I. designed the study, wrote, edited, and proofread the manuscript. A.Y. and E.M. assayed serum anti-PF4 antibody. M.S. advised hemostasis tests and interpreted data. H.A. and M.S. biopsied skin and collected patient data. T.H. and H.Y. collected and interpreted clinical data. M.T. and M.A. interpreted data and proofread the manuscript.

Ethical Approval

This study was approved by the institutional review boards of Yamagata University of Medicine and Anan Medical Center. All procedures were conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from the patient.

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Conflict of Interest

None declared.

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