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Prevalence of Anti-PF4 Antibodies After First and Second ChAdOx1 nCoV-19 Vaccinations in Women With Adverse Events: A Brief Report and Literature Review

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Dear Editor,

During the coronavirus disease pandemic, the AstraZeneca ChAdOx1 nCoV-19 vaccine, a viral vector vaccine, was one of the first approved for global vaccination. Various vaccination-related adverse events (AEs) have been reported, including vaccine-induced thrombotic thrombocytopenia (VITT), characterized by unusual site thrombosis accompanied by thrombocytopenia [1]. Platelet-activating antiplatelet factor 4-dependent antibodies (anti-PF4 Abs) were detected in most cases of VITT [2]. We investigated the prevalence of anti-PF4 Abs in Korean women administered ChAdOx1 nCoV-19 and the correlations of anti-PF4 Ab positivity with AEs, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) IgG Abs, and SARS-CoV-2-neutralizing Abs. The Institutional Review Board of Chung-Ang University Hospital, Seoul, Korea, approved this study (IRB No. 2204-002-501).

Sera from vaccinated healthcare workers were tested for anti-PF4 Abs using an Asserachrom HPIA IgG ELISA kit (Stago, Asnieres, France) after first and second doses of ChAdOx1 nCoV-19. This study was conducted at Chung-Ang University Hospital and informed consent was obtained from all the subjects. Individuals who received the BNT162b2 vaccine (Pfizer, NY, USA and BioNTech, Mainz, Germany) and those diagnosed with COVID-19 were excluded from this study. Optical density (OD) values higher than 30% of the OD observed for control (i.e. reference lyophilized human plasma samples containing antibodies that are reactive to heparin-PF4 complexes) were considered positive, according to the manufacturer's instructions. The sera were also tested using a GenScript SARS-CoV-2 surrogate virus neutralization test (sVNT) kit (GenScript Biotech Corp., Piscataway, NJ, USA) and an Euroimmun anti-SARS-CoV-2 IgG ELISA kit (Euroimmun, Lübeck, Germany), as reported [3].

Demographic and AE data were collected using questionnaires. AE severity was subjectively graded from 1 to 5, according to the Faces Pain Scale [3, 4]. The severity of AEs was scored as the sum of severity scores of all events and as the sum of severity scores of all events multiplied by their duration [3]. Surveys and blood sampling were conducted from March 2, 2021, to June 15, 2021. Correlation between anti-PF4 Abs and SARS-CoV-2 IgG Abs was analyzed using Pearson's correlation test. Correlations of anti-PF4 Abs with SARS-CoV-2 neutralizing Abs and AEs were assessed using Spearman's rank correlation test.

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In total, 33 healthy female healthcare workers were included in this study (Table 1). Anti-PF4 Ab positivity after the first and second vaccinations was 3.0% (1/33), which was comparable to previous findings [5, 6].

One subject who tested anti-PF4 Ab-positive after the first dose tested negative after the second dose, although the OD value (0.21) was relatively high compared with that for other subjects after the second dose ($P<0.05$). Another subject, who

Table 1. Summary of the laboratory data of the 33 cases after the first vaccination

Case number	Age, yr	Anti-PF4 IgG Ab ELISA (OD)	EUROIMMUN IgG ELISA (OD)	sVNT Inhibition rate, %	Systemic SUM	Systemic SoM
1	24	0.10	2.08	27.68‡	24	67
2	42	0.09	2.70	48.61	9	27
3	30	0.10	4.75	93.00	22	60
4	30	0.08	2.24	46.27	23	69
5	45	0.11	7.11	91.39	17	35
6	26	0.08	4.03	61.65	8	12
7	49	0.09	1.92	53.98	12	16
8	24	0.08	2.52	59.15	19	41
9	36	0.12	1.84	35.95	18	27
10	27	0.26*	1.45	28.37‡	18	36
11	48	0.17	2.51	47.59	13	27
12	37	0.13	3.95	70.31	24	68
13	44	0.07	1.98	62.21	9	160
14	30	0.06	3.70	63.24	23	64
15	39	0.07	2.17	45.77	24	84
16	46	0.09	1.19	37.63	17	61
17	41	0.05	5.07	87.23	22	46
18	41	0.05	1.58	45.63	11	22
19	49	0.07	1.99	46.34	13	25
20	39	0.07	3.76	49.19	20	37
21	32	0.09	4.92	72.79	20	45
22	25	0.07	4.67	67.90	16	26
23	27	0.15	2.55	40.07	16	16
24	28	0.10	4.70	53.06	14	60
25	37	0.12	1.35	35.18	15	31
26	27	0.14	5.42	72.57	15	27
27	30	0.11	0.41†	8.42‡	9	24
28	46	0.11	7.03	68.52	8	16
29	33	0.15	3.66	47.50	19	57
30	37	0.07	5.19	68.77	3	3
31	27	0.07	3.38	65.74	9	28
32	40	0.07	2.21	58.80	14	14
33	29	0.20	2.45	43.52	16	86
Total	35.3±8.0	0.10±0.05	3.23±1.66	54.67±18.60	15.8±5.5	42.9±30.2

*Positive Anti-PF4 IgG Ab (OD of control: 0.20); †Negative Euroimmun IgG ELISA (cut-off: 0.80); ‡Negative sVNT inhibition rate (cut-off: <30%).
Abbreviations: OD, optical density; sVNT, surrogate virus neutralization test; SUM, sum of adverse event severity scores; SoM, sum of adverse event severity scores multiplied by event duration.

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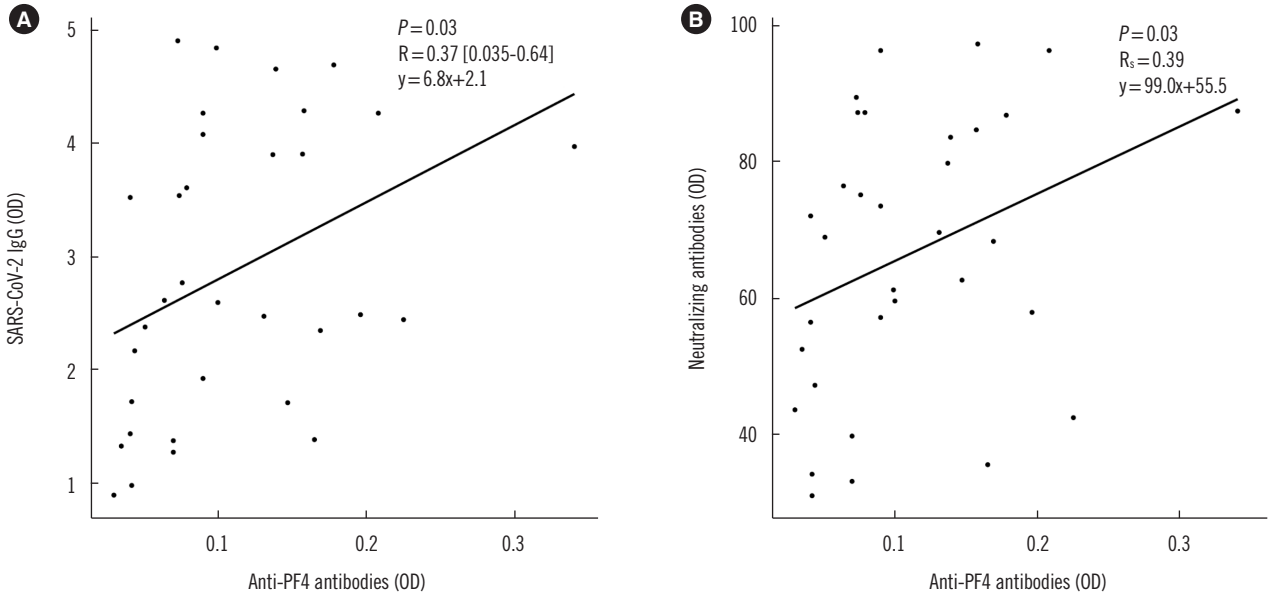

Fig. 1. Correlation between SARS-CoV-2 antibodies and anti-PF4 antibodies. (A) Correlation between Euroimmun anti-SARS-CoV-2 IgG and anti-PF4 antibodies after the second vaccination. (B) Correlation between sVNT neutralizing antibodies against anti-PF4 antibodies after the second vaccination. This line corresponds to the regression line describing the data pairs.
Abbreviations: R, Pearson correlation coefficient; Rs, Spearman's rank correlation coefficient; OD, optical density; sVNT, surrogate virus neutralization test.

tested anti-PF4 Ab-negative after the first dose (OD 0.13, cut-off 0.20), tested positive (OD 0.34, cut-off 0.25) after the second dose. She complained of severe headache, fever, chills, and fatigue after the first dose and previously had an antinuclear Ab titer of 1:80 and visited an outpatient clinic for suspected Sjögren's disease.

Anti-PF4 IgG Ab OD showed a weak positive correlation with Euroimmun IgG (Fig. 1A). Anti-PF4 IgG Ab titer and neutralizing Abs showed no significant correlation after the first dose, but weak correlation existed after the second dose (Fig. 1B). A study reported higher neutralizing Ab titer in the group with systemic side effects than in that without [7]; however, anti-PF4 IgG Ab titer and AEs showed no significant correlations in our study.

We found that ChAdOx1 nCov-19 can elicit anti-PF4 Ab production even in recipients without a clinical manifestation of thrombosis. One study reported that up to 8.0% of vaccinated healthy individuals presented anti-PF4 Abs [6]; however, another study reported a lower incidence of anti-PF4 Abs (1.4%) [8]. Variable laboratory assay sensitivities can be attributed to this difference [9].

The subject whose anti-PF4 Ab status converted to positive after the second dose was remarkable in that an autoimmune tendency was found. One study indicated that autoimmune patients have a higher prevalence of anti-PF4 Ab positivity than the normal population; however, the Abs did not affect platelet activation [10]. The risk of anti-PF4 Ab positivity may increase if repeated ChAdOx1 nCoV-19 vaccinations are administered to autoimmune patients.

This study had several limitations. First, the number of subjects was not large. Second, baseline data on PF4 Ab positivity before vaccination could not be obtained. Third, there were no data related to VITT, such as platelet count, coagulation, and imaging studies. Finally, functional studies were not conducted; therefore, we could not confirm whether the anti-PF4 Ab has thrombotic activity.

In conclusion, this study is the first to report the prevalence of anti-PF4 Abs in Korean women who received ChAdOx1 nCoV-19 vaccination, revealing Ab production after the first and second doses. In particular, in patients with autoimmune tendency, anti-PF4 Ab positivity may occur after repeated vaccinations; therefore, close observation is required.

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AUTHOR CONTRIBUTIONS

Conceptualization: Kim HR; Data curation: Choe KW; Funding acquisition: Kim HR; Methodology: Kim HR; Validation: Lim YK,

Kweon OJ, Lee MK, and Chung JW; Visualization: Choe KW; Writing-original draft: Choe KW; Writing–review and editing: Kim HR. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

None declared.

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