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BRIEF REPORT

Prevalence of anti–platelet factor 4/polyanionic antibodies after COVID-19 vaccination with ChAdOx1 nCoV-19 and CoronaVac in Thais

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Abstract

Introduction: Vaccine-induced thrombotic thrombocytopenia (VITT) has been reported after vaccination with the adenoviral vector coronavirus disease 2019 (COVID-19) vaccine ChAdOx1 nCoV-19 in European countries. To date, two cases of VITT have been reported in Thais after COVID-19 vaccination. We determined the frequency of anti–platelet factor 4 (PF4)/polyanionic antibodies in the Thai population receiving the COVID-19 vaccines.

Methods: We conducted a cross-sectional study to evaluate the prevalence of anti-PF4/polyanionic antibodies in health care workers who received COVID-19 vaccination with ChAdOx1 nCoV-19 or CoronaVac within 7 to 35 days. A control population who had not been vaccinated was also included. Anti-PF4/polyanionic antibodies were detected using ELISA. Functional assay with platelet aggregation was performed for all positive anti-PF4/polyanionic antibody ELISA tests.

Results: A total of 646 participants were included in the study; 221 received ChAdOx1 nCoV-19, 232 received CoronaVac, and 193 participants were in the control group. The prevalence of anti-PF4 antibodies was 2.3% (95% confidence interval [CI], 0.7-5.2), 1.7% (95% CI, 0.5-4.4) in the ChAdOx1 nCoV-19 and CoronaVac groups, respectively. There was no positive test in the control group. None of the PF4/polyanionic positive sera induced platelet aggregation.

Conclusion: We found a low prevalence of anti-PF4 antibodies in Thais after vaccination with ChAdOx1 nCoV-19 and CoronaVac. None of the antibodies were functional and lacked an association with VITT.

KEYWORDS
COVID-19 vaccines, Platelet factor 4, Prevalence, Thailand, Vaccines

Essentials

- Anti–platelet factor 4 (PF4) is associated with vaccine-induced thrombotic thrombocytopenia (VITT).
- Low-titer PF4 antibodies following ChAdOx1 nCoV-19 and CoronaVac vaccination are observed.
- These PF4/polyanion antibodies do not activate platelets and lack an association with VITT.
- The significance of these low-titer PF4 antibodies remains unknown.

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) has resulted in devastating worldwide morbidity and mortality since late 2019. Vaccination against COVID-19 is a cornerstone measure in the control of the COVID-19 pandemic. Currently, ChAdOx1 nCoV-19 (AstraZeneca) and CoronaVac (Sinovac Biotech) are the only 2 available vaccines in Thailand. The ChAdOx1 nCoV-19 vaccine consists of a replication-deficient chimpanzee adenoviral vector, containing the severe acute respiratory syndrome (SARS-CoV-2) structural surface glycoprotein antigen (spike protein; nCoV-19) gene. The CoronaVac is an inactivated SARS-CoV-2 vaccine derived from virus grown in culture and then chemically inactivated, which delivers stably expressed, conformationally native antigenic epitopes.[1] Recent reports of vaccine-induced thrombotic thrombocytopenia (VITT) after ChAdox1 nCoV-19 vaccination have raised concern of using the COVID-19 vaccine.[2-4] Almost all of the cases presented with thrombosis in unusual sites, thrombocytopenia, and strongly positive anti–platelet factor 4 (PF4)/polyanionic antibodies. The syndrome resembles that of autoimmune heparin-induced thrombocytopenia (HIT). Several European countries have paused or restricted the use of the ChAdOx1 nCoV-19 vaccine due to the perceived risk of this severe adverse event. To date, two cases of VITT have been reported in Thailand after vaccination with ChAdOx1 nCoV-19 of about 9.1 million doses.[5] Case reports of this syndrome are also sparse in the Asian population. To support the vaccination campaign and to reassure the public about mass vaccination in Thailand, we conducted a cross-sectional study to evaluate the prevalence of postvaccination anti-PF4/polyanionic antibodies among Thais receiving ChAdOx1 nCoV-19 and CoronaVac to identify individuals who may be at risk of developing VITT, and to investigate the prevalence of subclinical anti-PF4/polyanionic antibodies. ELISA has been shown to reliably detect anti-PF4/polyanionic antibodies associated with VITT, and was thus used for antibody screening in this study.

2 | MATERIALS AND METHODS

2.1 | Study population and settings

A cross-sectional study was conducted at Ramathibodi Hospital, a tertiary care academic hospital in Bangkok, Thailand. Adult health care workers who had received COVID-19 vaccination with either ChAdOx1 nCoV-19 or CoronaVac within 7 to 35 days were included in the study. Healthy volunteer participants who had not been vaccinated were also included as a control group. All participants gave written informed consent. Baseline characteristics including age and sex were recorded. The study protocol was approved by the Human Research Ethics Committee of the Faculty of Medicine at Ramathibodi Hospital, Mahidol University.

2.2 | Blood collection and laboratory analysis

After informed consent, blood was collected in a citrate anticoagulant tube from participants. Anti-PF4/polyanionic antibodies were screened by IgG-specific ELISA (Hyphen Biomed Zymutest HIA IgG; Quadratech Diagnostics, Surrey, United Kingdom) according to the manufacturer's instructions. Results were interpreted as positive if the optical density (OD) was above 0.3. Positive samples in ELISA were tested by platelet aggregation on the CHRONO-LOG platelet aggregometer (Chrono-log Corporation, Havertown, PA, USA). Normal blood group O donor platelets were incubated with PF4/polyanion-positive sera in the presence of low-dose heparin (unfractionated heparin, 1.0 IU/mL), high-dose heparin (unfractionated heparin, 100 IU/mL), or saline buffer. A previously confirmed HIT serum was used as a positive control, and normal pooled plasma as negative control.

2.3 | Statistical analysis

Baseline characteristics were analyzed and presented with mean and standard deviation or median and interquartile range as appropriate. All statical analyses were performed on GraphPad Prism 9.1.1 (GraphPad Software, La Jolla, CA, USA) and Stata statistical software version 15.1 (StataCorp, College Station, TX, USA).

3 | RESULTS

A total of 453 health care workers and 193 controls were included; 221 participants received one dose of ChAdOx1 nCoV-19, and 232 received either one or two doses of CoronaVac with an interval of 21±7 days between doses. All participants who received ChAdOx1 nCoV-19 had the first dose of the vaccine. Of the 232 participants receiving CoronaVac, 149 (64.2%) and 83 (35.8%) had the first and the second dose of the vaccine. Median age (interquartile range) was 61 (38-68), 35 (30-42), and 49 (38-55) years in the ChAdOx1 nCoV-19, CoronaVac, and control groups, respectively. Women accounted

for 65.6%, 83.6%, and 29.5% in the ChAdOx1 nCoV-19, CoronaVac, and control groups, respectively. The median day (range) after first vaccination was 23 (18-27) and 18.5 (10-34), respectively (Table 1). No participant had a history of heparin exposure within 3 months.

Positive anti-PF4/polyanionic antibodies were detected in 5, 4, and 0 samples in the ChAdOx1 nCoV-19, CoronaVac, and control groups, respectively. Therefore, the prevalence of anti-PF4 antibodies was 2.3% (95% confidence interval [CI], 0.7-5.2), 1.7% (95% CI, 0.5-4.4), and 0% in the ChAdOx1 nCoV-19, CoronaVac, and control groups, respectively. Median OD (range) was 0.03 (0.00-0.70), 0.04 (0.01-0.93), and 0.04 (0.01-0.29) in the ChAdOx1 nCoV-19, CoronaVac, and control groups, respectively. Overall, the results were positive in 9 samples. The mean OD of the positive results was 0.72 (SD 0.17). None of the positive samples showed an OD >1.0. One positive test in the CoronaVac group occurred after receiving the first vaccine dose, and three positive tests after the second dose (Figure 1). None of the PF4/polyanion-positive sera induced platelet aggregation in the Chrono-Log platelet aggregation assay. None of the study participants developed thrombosis or clinically evident VITT.

4 | DISCUSSION

We report a low prevalence of anti-PF4/polyanionic antibodies in the Thai population receiving COVID-19 vaccination with ChAdOx1 nCoV-19 or CoronaVac. Participants who received ChAdOx1 nCoV-19 and CoronaVac had a similar prevalence of anti-PF4 antibodies, while there was no positive result in the control group. Our data suggest that vaccination against COVID-19 leads to low-titer anti-PF4 antibodies in some subjects and that this may occur after vaccination with either ChAdOx1 nCoV-19 or CoronaVac as part of the immunological response.

The results of our study were comparable to a prior study from Norway[6] that reported a 1.2% prevalence of anti-PF4/polyanion antibodies after COVID-19 vaccination with ChAdOx1 nCoV-19. The anti-PF4/polyanion antibodies were also negative in all participants in the control group in that study. However, a study from Germany demonstrated a higher frequency of anti-PF4 antibodies among vaccinees receiving ChAdOx1 nCoV-19 (8.0%).[7] It also demonstrated seroconversion of anti-PF4 in two of six available samples, while preexisting antibodies were found in the other four available samples. These two studies used different PF4/polyanion ELISA assays. However, both studies also showed that ODs were mostly low (<1.0) and none of the PF4/polyanion ELISA-positive samples induced platelet activation.

There has been no report of VITT after vaccination with CoronaVac, which is an inactivated virus vaccine. However, the prevalences of anti-PF4/polyanionic antibodies after vaccination with ChAdOx1 nCoV-19 and CoronaVac were similar in this study. Since the titer of all positive samples is low, its significance is unknown. The link between vaccination and antibody formation is unknown. Sera from patients with clinically overt VITT are strongly positive by anti-PF4/polyanion ELISA (typically ODs >2) and cause strong platelet activation in the platelet activation assay.

To date, two cases of VITT have been reported in Thailand after vaccination with ChAdOx1 nCoV-19 after >9.1 million doses.[5] Case reports of this syndrome are also sparse in the Asian population. It is possible that the incidence of VITT might be lower in Asians. We previously reported a lower prevalence of positive anti-PF4/heparin antibodies and clinical heparin-induced thrombocytopenia after cardiac surgery in Thai patients than in Caucasian patients.[8] Recently, it was shown that HLA-DRB1*03:01 and DQB1*02:01 were significant risk factors for increased anti-PF4/heparin antibodies following heparin exposure among inpatients.[9] Frequency distribution of the DRB1*03:01-DQB1*02:01 haplotype across the world varies among different countries (0.1% in Thailand, 12.7% in the United Kingdom, and 14.4% in the United States).[9] Whether this can explain the low prevalence of anti-PF4/heparin antibodies in Thais needs to be further evaluated.

In conclusion, we found a low prevalence of anti-PF4/polyanion antibodies among Thais vaccinated with ChAdOx1 nCoV-19 or CoronaVac. None of the positive antibodies were functional. Clinical significance of low-titer PF4 antibodies remains unknown.

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TABLE 1 Baseline characteristics of study population

Characteristics	Vaccine type, n (%)				
	Normal control (n = 193)	ChAdOx1 nCoV−19 First dose (n = 221)	CoronaVac		
			First dose (n = 149)	Second dose (n = 83)	First + second dose (n = 232)
Median age, y (IQR)	49 (38.0-55.5)	61 (38.0-68.0)	35 (30.0-41.5)	35 (30.0-42.0)	35 (30.0-42.0)
Female, n (%)	57 (29.5)	145 (65.6)	124 (83.2)	70 (84.3)	194 (83.6)
Postvaccination PF4/heparin ELISA +, n (%)	N/A	5 (2.3)	1 (0.7)	3 (3.6)	4 (1.7)
Median day since first vaccination, days (IQR)	N/A	23 (18.0-27.0)	12 (9.0-17.5)	36[a] (33.0-39.0)	18.5 (10.0-34.0)

[a]Median day since second-dose vaccination (IQR) was 10 (7.0-17.0) days. IQR, interquartile range; N/A; not applicable; PF4, platelet factor 4.

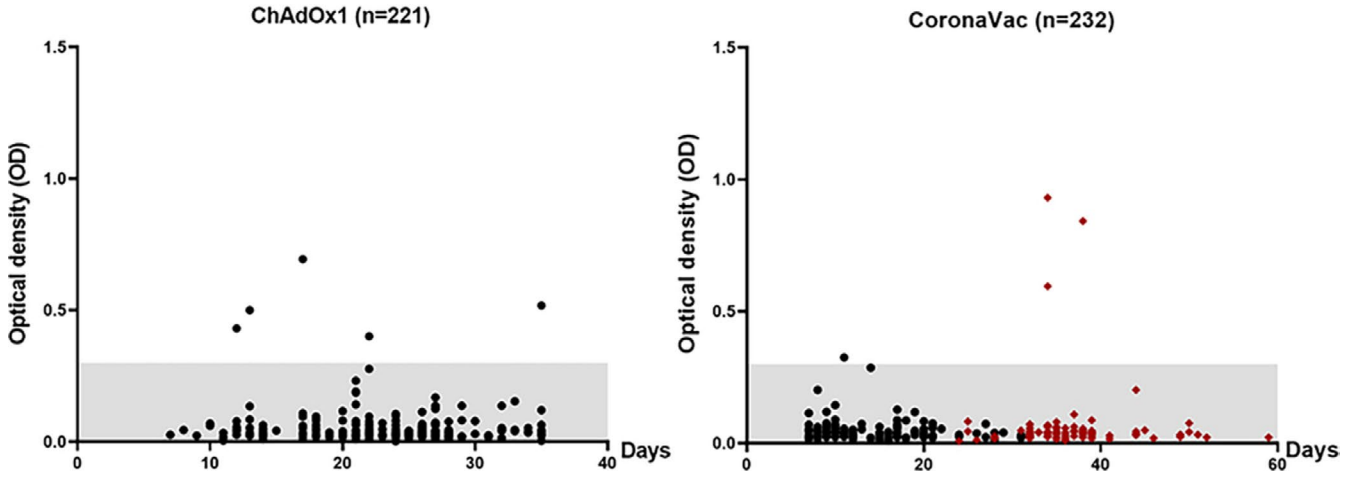

FIGURE 1 Anti-PF4/polyanionic antibodies were positive in five participants in the ChAdOx1 nCoV-19, and four participants in the CoronaVac group by ELISA. The highest OD was 0.9. OD of >0.3 indicates positive result. Black dots indicate OD after first-dose vaccination, and red diamonds indicate OD after second-dose vaccination. Gray shading indicates negative range. OD; optical density; PF4, platelet factor 4

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RELATIONSHIP DISCOSURE

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

P. Noikongdee, PP, and T. Phojanasenee recruited participants, performed the laboratory tests and ran the project. PP and KB performed the statistical analyses. PC, T. Puavilai, P. Niparuck, and AP collected the specimen. AP critically revised the manuscript. PA and KB designed the study and wrote the manuscript.

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REFERENCES

1. Creech CB, Walker SC, Samuels RJ. SARS-CoV-2 vaccines. *JAMA*. 2021;325:1318-1320. doi:10.1001/jama.2021.3199
2. Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination. *N Engl J Med*. 2021;384(22):2092-2101. doi:10.1056/NEJMoa2104840
3. Schultz NH, Sørvoll IH, Michelsen AE, et al. Thrombosis and thrombocytopenia after ChAdOx1 nCoV-19 vaccination. *N Engl J Med*. 2021;384(22):2124-2130. doi:10.1056/NEJMoa2104882.
4. Scully M, Singh D, Lown R, et al. Pathologic antibodies to platelet factor 4 after ChAdOx1 nCoV-19 vaccination. *N Engl J Med*. 2021;384(23):2202-2211. doi:10.1056/NEJMoa2105385.
5. Adverse effect after COVID-19 vaccination in Thailand. https://ddc.moph.go.th/doe/news.php?news=20032&deptcode=
6. Sorvoll IH, Horvei KD, Ernstsen SL, et al. An observational study to identify the prevalence of thrombocytopenia and anti-PF4/polyanion antibodies in Norwegian health care workers after COVID-19 vaccination. *J Thromb Haemost*. 2021;19(7):1813-1818. doi:10.1111/jth.15352.
7. Thiele T, Ulm L, Holtfreter S, et al. Frequency of positive anti-PF4/polyanion antibody tests after COVID-19 vaccination with ChAdOx1 nCoV-19 and BNT162b2. *Blood*. 2021;138(4):299-303. doi:10.1182/blood.2021012217.
8. Boonyawat K, Angchaisuksiri P, Aryurachai K, Chaiyaroj S, Ahmadi Z, Chong BH. Low prevalence of heparin-induced thrombocytopenia after cardiac surgery in Thai patients. *Thromb Res*. 2014;134:957-962. doi:10.1016/j.thromres.2014.08.020.
9. Allele frequency net database (AFND) 2020 update.

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