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BRIEF REPORT

ChAdOx1 vaccination, blood coagulation, and inflammation: No effect on coagulation but increased interleukin-6

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Abstract

Background: Vaccination is the leading approach in combatting the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. ChAdOx1 nCoV-19 vaccination (ChAdOx1) has been linked to a higher frequency of rare thrombosis and thromboembolism. This study aimed to explore markers related to the blood coagulation system activation and inflammation, before and after ChAdOx1 vaccination.

Patients and Methods: An observational cohort study including 40 health care workers. Whole blood samples were collected before, and either 1 or 2 days after vaccination. Activated coagulation factors in complex with their natural inhibitors were determined by custom ELISAs, including thrombin:antithrombin (T:AT), kallikrein:C1-esterase-inhibitor (PKa:C1Inh), factor(F)IXa:AT, FXa:AT, FXIaAT, FXIa:alpha-1-antitrypsin (α1AT), FXIa:C1inh, and FVIIa:AT. Plasma concentrations of interleukin (IL)-6 and IL-18 were quantified via ELISA. Analyses were performed using Wilcoxon signed-rank test.

Results: Levels of FVIIa:AT decreased with a median (IQR) of 707 (549–1028) pg/ml versus 598 (471–996) pg/ml, $p = 0.01$; and levels of IL-6 increased, 4.0 (1.9–6.8) pg/ml versus 6.9 (3.6–12.2) pg/ml, $p = 0.02$, after vaccination. No changes were observed in T:AT, PKa:C1Inh, FIXa:AT, FXa:AT, FXIaAT, FXIa:α1AT, FXIa:C1inh, and IL-18.

Conclusion: ChAdOx1 leads to an inflammatory response with increased levels of IL-6. We did not observe activation of the blood coagulation system 1–2 days following vaccination.

KEYWORDS
blood coagulation, COVID-19, COVID-19 vaccines, SARS-CoV-2, thrombosis, vaccination

Essentials

- Vaccination is the leading approach in combatting the coronavirus disease 2019 pandemic.
- Blood coagulation and inflammation were studied before and after ChAdOx1 nCoV-19 vaccination.
- The ChAdOx1 nCoV-19 vaccine leads to an inflammatory response.
- The ChAdOx1 nCoV-19 vaccine does not induce activation of the blood coagulation system.

1 | BACKGROUND

Thrombosis is a frequent complication in patients during the acute and recovery phase of the coronavirus disease 2019 (COVID-19).[1-4] Reflecting this increased thrombotic risk, complexes of activated coagulation factors and their inhibitors were elevated in plasma of individuals with COVID-19. These factors similarly vary with disease severity.[5] Unpublished data from our group show that, ~3 months after COVID-19 infection, the same markers of blood coagulation system activation remain increased in 40%–50% of all patients, alongside elevated inflammatory cytokines [submitted for publication]. As novel variants of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continue to infect individuals worldwide, vaccination is the leading approach in mitigating this pandemic. By August 2021, four different COVID-19 vaccines have been approved by the European Medicines Agency. Among these, the ChAdOx1 nCoV-19 vaccine (ChAdOx1) of AstraZeneca, which has been shown to be safe and effective in randomized controlled trials.[6] Despite this evidence in favor of the use of ChAdOx1, the vaccination program was discontinued in several European countries because of observations of extensive thrombosis in atypical sites with associated thrombocytopenia occurring at a low frequency.[7] This manifestation, now known as vaccine-induced immune thrombotic thrombocytopenia (VITT), is associated with anti-platelet 4 antibodies activating platelets.[8,9] Besides the rare occurrence of VITT, researchers have described a higher frequency of general thrombosis and thromboembolism in individuals recently vaccinated with ChAdOx1 when compared with individuals vaccinated with other COVID-19 vaccines[10] and compared with the general population.[11] This raises the question whether ChAdOx1 could induce activation of the blood coagulation system, comparable to the activation of the blood coagulation system by SARS-CoV-2 itself. This study aimed to explore changes in circulating biomarkers of the activated blood coagulation system and proinflammatory cytokines before and after ChAdOx1 vaccination.

2 | METHODS

An observational cohort study was performed including 40 health care workers of the Radboud University Medical Center (Radboudumc, Nijmegen, the Netherlands), scheduled for the first dose of ChAdOx1 vaccination, recruited by open invitation between April 13 and May 6, 2021. This study was approved by the regional ethics committee and the directory board of the Radboudumc. Individuals meeting any of the following criteria were excluded from participation: (1) individuals with a bleeding disorder; (2) individuals on vitamin K antagonists, low molecular weight heparin, or direct oral anticoagulants; or (3) individuals on immunosuppressant and/or anti-inflammatory therapy, including glucocorticoids, cytostatic agents, antibodies, immunophilins, interferons, tumor necrosis factor–binding proteins, mycophenolate, and interleukin antagonists. Written informed consent was obtained from all participants. Baseline demographics and clinical data regarding medical history and medication use were requested. Whole blood samples were collected prior to vaccination. A second whole blood sample collection was scheduled for either 1 or 2 days after vaccination, endeavoring to include equal numbers of participants in both groups and considering participants preferences for the day of second collection. Whole blood samples were collected and processed to platelet-poor plasma by standard procedures.[12] Participants were contacted by telephone 4 weeks after vaccination to inform about any health complaints experienced since vaccination, including thrombosis and thromboembolism. Activated coagulation factors in complex with their natural inhibitors, including thrombin:antithrombin (T:AT), kallikrein:C1-esterase-inhibitor, factor (F)IXa:AT, FXa:AT, FXIaAT, FXIa:alpha-1-antitrypsin, FXIa:C1inh, and FVIIa:AT were quantified by in-house developed ELISA.[13] Plasma concentrations of interleukin (IL)-6 and IL-18 were quantified using commercial ELISA kits (R&D, Minneapolis, MN). Data were analyzed by performed Wilcoxon signed-rank test to detect changes in levels of activated coagulation factor:inhibitor complexes and proinflammatory cytokines from baseline to after vaccination. Subanalyses were performed for the groups with the second blood sample collection 1 day after vaccination and 2 days after vaccination, separately. Analyses were performed using IBM SPSS Statistics 25 and p values below 0.05 were considered significant.

3 | RESULTS AND DISCUSSION

Forty health care workers, scheduled for the first dose of ChAdOx1 vaccination, were recruited by open invitation. One subject did not meet the in- and exclusion criteria and was replaced. Subjects were assessed for changes in circulating biomarkers of blood coagulation activation and proinflammatory cytokines, before and 1 or 2 days after exposure to ChAdOx1 vaccination. All participants were aged between 60 and 65 years, the mean body mass index was 26 kg/m^2, and 20% of the subjects were male. Comorbidities, most frequently hypertension (22.5%) and cardiovascular disease (22.5%), were

present in 65% of individuals. Nineteen subjects had their second blood sample collection 1 day after vaccination, and 21 subjects had their second blood sample collection 2 days after vaccination. No significant differences were established in baseline characteristics or medical history between the two groups (Table 1).

In vivo blood coagulation activity was measured and compared before and after ChAdOx1 vaccination. No changes in T:AT, kallikrein:C1-esterase-inhibitor, FIXa:AT, Fxa:AT, FXIaAT, FXIa:alpha-1-antitrypsin, and FXIa:C1inh were observed after exposure to ChAdOx1. FVIIa:AT was significantly decreased after vaccination, median (IQR) 707 (549–1028) pg/ml versus 598 (471–996) pg/ml, $p = 0.01$. Levels of IL-6 increased after ChAdOx1 vaccination (4.0 [1.9–6.8] pg/ml versus 6.9 [3.6–12.2] pg/ml, $p = 0.02$), whereas IL-18 levels were unchanged. Subanalyses were performed separately on the 1-day and 2-day postvaccination groups. Results of both groups were similar to the overall population with decreasing tendencies in FVIIa:AT levels and increasing tendencies in IL-6 levels (Table 2).

Increased systemic levels of IL-6 have been previously observed following various types of vaccination, such as foot and mouth disease vaccination,[14] Bacillus Calmette-Guérin,[15] diphtheria toxoid vaccination,[16] and influenza vaccination.[17] An explanation has been proposed by Farsakoglu et al.,[17] who demonstrated elevations in IL-6 secretion by CD11b+ dendritic cells following influenza vaccination, that this response was initiated by interferon-γ production from natural killer cells. A natural killer cell response has been previously established for single-dose ChAdOx1[18] and potentially justifies the elevated levels of IL-6 after vaccination in the current study.

IL-6 is known to induce the expression of tissue factor (TF), which plays a key role in the regulation of hemostasis.[19] TF forms complexes with activated FVII where TF:FVIIa complexes activate FIX and FX, which subsequently results in thrombin generation. Binding of FVII to TF could have reduced the availability of FVIIa to bind to AT, its natural inhibitor, thus resulting in the observed decrease in levels of FVIIa:AT. However, a type I error cannot be excluded. We found no further evidence of extrinsic pathway activation as reflected by comparable levels of FIXa:AT, Fxa:AT, and T:AT, before and after ChAdOx1 vaccination.

All participants were followed for health complaints until 4 weeks after vaccination. Thirty-two (80%) of the subjects reported health complaints, including injection site tenderness, myalgia, headache, malaise, fever, chills, nausea, or diarrhea. All health complaints resolved within 4 days following vaccination with ChAdOx1. No complications related to thrombosis or thromboembolism were reported.

This study had some limitations. All participants were aged 60–65 years, and the sample size was small, which limits the generalizability of the study results. Changes in markers related to blood coagulation system activation and inflammation were measured 24–48 h after vaccination. Our results, therefore, represent the immediate response of the blood coagulation and inflammatory system. In previous literature, the immediate inflammatory response following vaccination is measurable at 24 h after the trigger.[17] Coagulation system activation is related to inflammation and, more specific, to IL-6 release.[19] The onset of thrombosis and thromboembolism following ChAdOx1 vaccination usually occurs after the first 24 h. By measuring changes in markers related to blood coagulation system activation and inflammation at 24 and 48 h after vaccination, both the immediate inflammatory response and an eventually increasing trend in coagulation system activation, should be noticed. The

TABLE 1 Baseline characteristics and medical history

	All, $n = 40$	Second visit at +1 day, $n = 19$	Second visit at +2 days, $n = 21$	p value
Baseline characteristics				
Male, *n* (%)	8 (20)	5 (26.3)	3 (14.3)	0.34
Age, y, mean ± SD	61.2±1.3	61.2±1.4	61.2±1.2	0.96
Body mass index, mean ± SD	26.0±4.6	26.7±3.7	25.5±5.3	0.396
Smoking behavior, *n* (%)				
Never	13 (32.5)	7 (36.8)	6 (28.6)	0.60
Former	23 (57.5)	11 (57.9)	12 (57.1)	
Current	4 (10)	1 (5.3)	3 (14.3)	
Race, *n* (%)				
Caucasian	40 (100)	19 (100)	21 (100)	NA
Medical history				
Hypertension, *n* (%)	9 (22.5)	6 (31.6)	3 (14.3)	0.19
Hyperlipidemia, *n* (%)	3 (7.5)	2 (10.5)	1 (4.8)	0.49
Cardiovascular disease, *n* (%)	9 (22.5)	5 (26.3)	4 (19.0)	0.58
Diabetes mellitus, *n* (%)	1 (2.5)	0 (0.0)	1 (4.8)	0.34
Past COVID-19 infection, *n* (%)	3 (7.5)	2 (10.5)	1 (4.8)	0.49
Antiplatelet therapy, *n* (%)	6 (15)	1 (10.5)	4 (19.0)	0.45

Abbreviations: COVID-19, coronavirus disease 2019; NA, not applicable; SD, standard deviation.

TABLE 2 Circulating concentrations of coagulation markers and inflammatory cytokines

	All, $n = 40$			Second visit at +1 day, $n = 19$			Second visit at +2 days, $n = 21$		
	Before, $n = 40$, median (IQR)	After, $n = 40$, median (IQR)	p	Before, $n = 19$, median (IQR)	After, $n = 19$, median (IQR)	p	Before, $n = 21$, median (IQR)	After, $n = 21$, median (IQR)	p
Coagulation enzyme: inhibitor complexes									
T:AT, μg/L	1.2 (1.2–1.9)	1.2 (1.2–1.5)	0.15	1.2 (1.2–2.2)	1.2 (1.2–1.5)	0.26	1.2 (1.2–1.7)	1.2 (1.2–1.4)	0.33
Pka:C1Inh, ng/ml	2.6 (2.2–4.1)	2.5 (2.2–4.5)	0.53	3.2 (2.2–4.9)	3.1 (2.2–6.7)	0.69	2.5 (2.3–3.7)	2.5 (2.2–3.5)	0.59
FIXa:AT, pg/ml	195 (195–212)	195 (195–197)	0.15	195 (195–260)	195 (195–247)	0.31	195 (195–199)	195 (195–195)	0.35
FXa:AT, pg/ml	200 (185–221)	200 (182–215)	0.55	209 (188–222)	203 (182–218)	0.81	191 (184–220)	195 (181–215)	0.46
FXIa:AT, pg/ml	22.4 (17.2–35.5)	22.3 (15.0–32.8)	1.00	25.1 (14.8–51.0)	28.5 (15.6–52.2)	0.98	21.1 (18.0–26.6)	20.7 (14.7–28.3)	0.96
FXIa:α1AT, pg/ml	50 (50–151)	50 (50–128)	0.50	50 (50–227)	50 (50–181)	0.26	50 (50–55)	50 (50–63)	0.87
FXIa:C1Inh, pg/ml	76 (76–308)	76 (76–322)	0.43	76 (76–748)	76 (76–722)	0.09	76 (76–112)	76 (76–146)	0.17
FVIIa:AT, pg/ml	707 (549–1028)	598 (471–996)	**0.01**	717 (563–1275)	620 (478–1083)	**0.02**	705 (525–874)	577 (454–936)	0.099
Inflammatory cytokines									
IL-6, pg/ml	4.0 (1.9–6.8)	6.9 (3.6–12.2)	**0.02**	5.0 (2.2–7.0)	9.1 (4.6–13.9)	0.05	3.9 (1.3–5.9)	4.2 (3.0–9.9)	0.29
IL-18, pg/ml	56 (5–107)	71 (0–155)	0.45	73 (15–158)	98 (0–170)	0.796	38 (2–96)	61 (0–145)	0.50

Abbreviations: α1AT, alpha-1-antitrypsin; C1Inh, C1-esterase-inhibitor; F, factor; IL, interleukin; IQR, interquartile range; Pka, kallikrein; T:AT, thrombin:antithrombin.

occurrence of VITT, which is generally diagnosed 4 or more days after vaccination, was not studied. Also, not all possibilities for immediate blood coagulation activation were assessed, such as platelet aggregation, which was not altered in previous literature.[20]

In conclusion, the current study found no evidence of immediate activation of the blood coagulation system 1–2 days following ChAdOx1 vaccination. ChAdOx1 leads to an inflammatory response with increased levels of IL-6, as seen previously with other types of vaccinations. The increase in IL-6, however, does not coincide with extrinsic pathway activation.

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RELATIONSHIP DISCLOSURE

None.

AUTHOR CONTRIBUTIONS

Loes H. Willems and Michiel C. Warlé contributed to the concept of the study. Loes H. Willems, Danielle Meijer, Saskia Middeldorp, Laszlo A. Groh, and Michiel C. Warlé contributed to the design of the study. Loes H. Willems, Lotte M. C. Jacobs, Maaike van Leeuwen, and Josephine Kranendonk contributed to the data collection. Loes H. Willems, Magdolna Nagy, Danielle Meijer, Saskia Middeldorp, and Laszlo A. Groh contributed to the data analysis. All authors contributed to the interpretation of the data. Loes H. Willems wrote the first draft. All authors criticiqued and revised the intellectual content. All authors approved the final version to be published.

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