Oxford-AstraZeneca ChAdOx1 COVID-19 Vaccine Does Not Alter Platelet Aggregation

Youness Limami, PhD[1,2] Loubna Khalki, PhD[3] Nabil Zaid, PhD[4] Meriem Khyatti, PhD[5] Joumana El Turk, PhD[6] Mounia Ammara, MSc[2] El Mostafa Mtairag, PhD[2] Mounia Oudghiri, PhD[2] Abdallah Naya, PhD[2] Mustapha Taberkant, MD[7] Younes Zaid, PhD[1,2,4]

[1] Research Center of Abulcasis, University of Health Sciences, Cheikh Zaïd Hospital, Rabat, Morocco
[2] Department of Biology, Immunology and Biodiversity Laboratory, Faculty of Sciences Ain Chock, Hassan II University, Casablanca, Morocco
[3] Faculty of Medicine, Department of Biological Sciences, Mohammed VI University of Health Sciences, Casablanca, Morocco
[4] Faculty of Sciences, Department of Biology, Mohammed V University in Rabat, Rabat, Morocco
[5] Laboratory of Viral Oncology, Institut Pasteur du Maroc, Casablanca, Morocco
[6] Faculty of Health Sciences, Department of Fundamental Sciences, International University of Casablanca, Casablanca, Morocco
[7] Department of Vascular Surgery, Mohammed V University in Rabat, Rabat, Morocco

Address for correspondence Younes Zaid, PhD, Faculty of Sciences, Department of Biology, Mohammed V University in Rabat, 10000, 4 Ibn Battouta Av, Rabat, Morocco (e-mail: y.zaid@um5r.ac.ma).

Semin Thromb Hemost 2022;48:109–111.

Since the emergence of SARS-CoV-2 in China in December 2019 and the subsequent coronavirus disease 2019 (COVID-19), and as a consequence of the rapid spread worldwide, the World Health Organization (WHO) declared the outbreak a serious public health emergency of international concern.[1]

COVID-19 is now considered a multisystem disease with deregulation of multiple physiological pathways, including hemostasis.[2] Indeed, several lines of evidence established a link between COVID-19 severity and thrombotic events, including thrombocytopenia, platelet hyperreactivity, and severe bleeding.[3–8]

To face the pandemic situation, development of vaccines seems to be crucial to prevent COVID-19 infection. In the past few months, numerous vaccines have been developed, among them the Oxford–AstraZeneca ChAdOx1 nCoV-19 (Oxford-AstraZeneca) vaccine (AZD1222). This vaccine is a chimpanzee adenoviral-vectored vaccine with full length SARS-CoV-2 spike insert, developed at the University of Oxford. Randomized trials have allowed the assessment of the Oxford-AstraZeneca vaccine safety and efficacy.[9,10] In the original protocol trial, volunteers aged 18 years and older received two standard doses of 5×10^{10} viral particles per dose administered 28 days apart. The protocol amendments allowed other trial participants to receive a booster more than 28 days after their first dose (up to 12 weeks).[9–11] Overall, vaccine efficacy after a single standard dose from day 22 to day 90 after vaccination was 76.0%. More than 14 days after the second dose, vaccine efficacy was 66.7%. Efficacy was higher in participants who received a longer prime-boost interval than in those with a short interval (<6 weeks). There were no hospital admissions for COVID-19 in the Oxford-AstraZeneca vaccine group after the initial 21-day exclusion period. There were two deaths in the Oxford-AstraZeneca vaccine group, but these were considered unrelated to vaccination.[9–11]

Recently, several cases of thrombosis, thrombocytopenia, and severe bleeding have been reported following administration of the Oxford-AstraZeneca vaccine.[12] Denmark was the first country to temporarily suspend the use of the Oxford-AstraZeneca vaccine as a precautionary move after reports of thrombosis in some people, including one person who developed pulmonary embolism and died 10 days after receiving at least one dose. Several other European countries soon followed suit.[12] In total, 37 thrombosis cases have been reported out of more than 17 million people vaccinated in the European Union and Britain. Five of the cases were deep vein thrombosis, and 22 were pulmonary embolisms.[13]

Although platelets from COVID-19 patients have shown hyperreactivity,[4–6] no scientific data have been able to

published online May 10, 2021

Issue Theme Maintaining Hemostasis and Preventing Thrombosis in COVID-19—Part III; Guest Editors: Emmanuel J. Favaloro, PhD, FFSc (RCPA) and Giuseppi Lippi, MD

Thieme Medical Publishers, Inc., 333 Seventh Avenue, 18th Floor, New York, NY 10001, USA

DOI https://doi.org/10.1055/s-0041-1728831.
ISSN 0094-6176.

demonstrate platelet dysfunction in people injected with the Oxford-AstraZeneca vaccine.

The WHO and the European Medicines Agency (EMA) stated that the association of the vaccine with the increased risk of blood clots is not justifiable and advised to continue vaccinations.[13]

Therefore, this study aimed to assess platelet function by measuring the aggregation of platelets from participants who had been injected with the Oxford-AstraZeneca vaccine compared with platelets from a cohort of healthy donors.

Participants vaccinated with a single dose ($n = 35$) and with two doses ($n = 42$) and patients with severe COVID-19 ($n = 10$) who were admitted to the Cheikh Zaïd Hospital of Abulcasis University (Rabat, Morocco) were included in this study. Of note, the second dose was injected 28 days after the first injection following the original protocol for administration of the Oxford-AstraZeneca vaccine. Sex- and age-matched healthy blood donors were used as controls. Recruitment was approved by the Ethics Committee of Cheikh Zaïd Hospital (CEFCZ/PR/2020/PR04) and complies with the Declaration of Helsinki. All participants gave their written informed consent. Patients who were receiving medications that interfere with platelet function within 2 weeks before the experiment started were excluded from the study. Vaccinated participants who previously had COVID-19 were also excluded from this study. ►**Table 1** provides basic demographic and clinical data for patients.

Washed platelets were prepared as previously described.[14] Briefly, platelet-rich plasma (PRP) was obtained by centrifugation of acid citrate dextrose (ratio of 1:5) anticoagulated blood at 200 g for 15 minutes. Platelets were then pelleted from PRP, to which 1 mg/mL of Prostaglandin E1 (PGE_1) was added, washed with HBSS-Hank's sodium citrate buffer (138-mM NaCl, 5-mM KCl, 0.34-mM Na_2HPO_4, 0.4-mM KH_2PO_4, 4.2-mM Na_2HCO_3, 5.6-mM glucose, 10-mM HEPES, 12.9-mM sodium citrate, pH 7.4), also containing PGE_1 (0.5 mg/mL), and finally resuspended in HBSS-Hank's buffer containing 2-mM $MgCl_2$ and 2-mM $CaCl_2$. Platelets were adjusted to 250×10^6/mL and allowed to rest at 37°C for 30 minutes before platelet aggregation tests. Platelet aggregation was monitored and recorded as previously described using an 8-channel optical aggregometer (SD Medical Innovation; Frouard, France).[15]

Our results show, as demonstrated in several published studies,[4–6] that platelets from COVID-19 patients are hyperreactive (►**Fig. 1A**). In addition, our findings document that vaccination by Oxford-AstraZeneca vaccine (one or two doses) was not associated with enhanced platelet aggregation.

Indeed, in response to a suboptimal α-thrombin concentration (0.05 U/mL), no significant difference in platelet aggregation was recorded (►**Fig. 1A**). At a higher concentration of α-thrombin (2 U/mL), platelets from vaccinated people aggregate at a maximum rate >90% (►**Fig. 1B**).

Overall, our findings demonstrate that platelets from vaccinated participants are not hyperreactive. To the best of our knowledge, this is the first report that implies a normal platelet function of vaccinated participants. These findings

Table 1 Demographic and clinical data of study participants

Index	Healthy donors	AZ one dose	AZ two doses	*p*-Value		
				Healthy vs. one dose	Healthy vs. two doses	One dose vs. two doses
No. of patients	10	35	42			
Female/male	4/6	16/19	23/19	–	–	–
Age, y	51.57 ± 14.98	56.15 ± 8.12	54.15 ± 13.10	0.62	0.69	0.86
Weight, kg	83.26 ± 23.14	76.44 ± 10.10	75.68 ± 9.38	0.37	0.35	>0.99
Platelet number, $\times 10^9$/L	217 ± 81.28	230 ± 88.93	208 ± 101.14	0.83	0.18	0.22
Lymphocyte number, $\times 10^9$/L	1.11 ± 0.82	1.18 ± 0.66	1.24 ± 0.89	0.34	0.21	0.91
ALT, U/L	18.32 ± 6.03	15.32 ± 6.80	18.16 ± 4.57	0.49	>0.99	0.49
AST, U/L	16.49 ± 4.24	15.91 ± 3.42	15.44 ± 4.96	0.96	0.92	>0.99
LDH, U/L	362.86 ± 115.71	404.66 ± 149.92	329.50 ± 178.25	0.38	0.24	0.07
C-reactive protein, mg/L	9.13 ± 4.08	14.59 ± 6.52	16.19 ± 7.89	**0.05**	**0.04**	0.90
D-dimers, mg/L	0.42 ± 0.32	0.96 ± 0.21	0.89 ± 0.58	**0.01**	**0.01**	0.76
Fibrinogen, mg/dL	314.36 ± 91.52	330.18 ± 80.07	326.10 ± 96.15	0.44	0.46	0.88

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; AZ, AstraZeneca; LDH, lactate dehydrogenase.
Note: Data are presented as mean ± standard deviation. Statistical analysis: unpaired Student's *t*-test was used to calculate *p*-values. Bold numbers indicate statistical significance at $p \leq 0.05$.

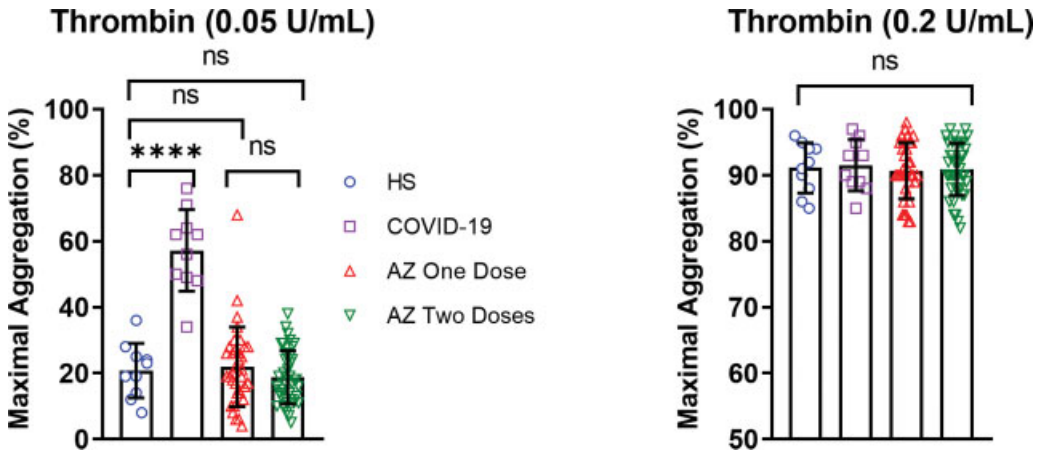

Fig. 1 Oxford-AstraZeneca vaccine does not affect platelet aggregation. Platelet aggregation was evaluated in healthy donors ($n = 10$), patients with severe coronavirus disease 2019 (COVID-19) ($n = 10$), participants who received a single dose of AstraZeneca vaccine ($n = 35$), and participants who received two doses of AstraZeneca vaccine ($n = 42$). Quantification (%) of maximal platelet aggregation after stimulation with (**A**) 0.05 or (**B**) 2 U/mL of α-thrombin. Data are represented as mean ± SD. Statistical analysis: data were normally distributed (Shapiro – Wilk test). One-way ANOVA (analysis of variance) with subsequent Sidak multiple comparisons test. $^{****}p < 0.0001$.

do not support any link between thrombosis and vaccination with the Oxford-AstraZeneca vaccine.

Authors' Contributions

Y.L., A.A.K., L.K., N.Z., M.T., and Y.Z. conceived and designed the study; J.E.T, M.A., E.M.M, M.O., A.N., and Y.Z. helped design the study, performed experiments, and helped with data extraction; Y.L., M.K., M.T., and Y.Z. contributed critical reagents, biospecimens, and instruments; Y.L., A.A.K., and Y.Z. wrote the manuscript; and all authors read and approved the final manuscript.

Funding

This study was funded by Cheikh Zaïd Foundation and was approved by the Local Ethics Committee of Cheikh Zaïd Hospital, Rabat, Morocco [project: CEFCZ/PR/2020-PR04].

Conflict of Interest

None declared.

References

1 Mahase E. Covid-19: WHO declares pandemic because of "alarming levels" of spread, severity, and inaction. BMJ 2020; 368:m1036
2 Lauretani F, Ravazzoni G, Roberti MF, et al. Assessment and treatment of older individuals with COVID 19 multi-system disease: clinical and ethical implications. Acta Biomed 2020;91 (02):150–168
3 Lippi G, Favaloro EJ. D-dimer is associated with severity of coronavirus disease 2019: a pooled analysis. Thromb Haemost 2020;120(05):876–878
4 Zaid Y, Guessous F, Puhm F, et al. Platelet reactivity to thrombin differs between patients with COVID-19 and those with ARDS unrelated to COVID-19. Blood Adv 2021;5(03):635–639
5 Zaid Y, Puhm F, Allaeys I, et al. Platelets can associate with SARS-Cov-2 RNA and are hyperactivated in COVID-19. Circ Res 2020; 127(11):1404–1418
6 Zhang S, Liu Y, Wang X, et al. SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19. J Hematol Oncol 2020;13(01):120
7 Lippi G, Sanchis-Gomar F, Favaloro EJ, Lavie CJ, Henry BM. Coronavirus disease 2019-associated coagulopathy. Mayo Clin Proc 2021;96(01):203–217
8 Christensen B, Favaloro EJ, Lippi G, Van Cott EM. Hematology laboratory abnormalities in patients with coronavirus disease 2019 (COVID-19). Semin Thromb Hemost 2020;46(07):845–849
9 Knoll MD, Wonodi C. Oxford-AstraZeneca COVID-19 vaccine efficacy. Lancet 2021;397(10269):72–74
10 Voysey M, Clemens SAC, Madhi SA, et al; Oxford COVID Vaccine Trial Group. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet 2021;397(10269):99–111
11 Hung IFN, Poland GA. Single-dose Oxford-AstraZeneca COVID-19 vaccine followed by a 12-week booster. Lancet 2021;397 (10277):854–855
12 Wise J. Covid-19: European countries suspend use of Oxford-AstraZeneca vaccine after reports of blood clots. BMJ 2021;372:n699
13 Mahase E. Covid-19: WHO says rollout of AstraZeneca vaccine should continue, as Europe divides over safety. BMJ 2021;372:n728
14 Bou Khzam L, Hachem A, Zaid Y, Boulahya R, Mourad W, Merhi Y. Soluble CD40 ligand impairs the anti-platelet function of peripheral blood angiogenic outgrowth cells via increased production of reactive oxygen species. Thromb Haemost 2013;109(05):940–947
15 Zaid Y, Marhoume F, Senhaji N, et al. Paraphenylene diamine exacerbates platelet aggregation and thrombus formation in response to a low dose of collagen. J Toxicol Sci 2016;41(01):123–128