Title

Variant-derived SARS-CoV-2 spike protein does not directly cause platelet activation or hypercoagulability

Authors

Eriko Kusudo[1], Yutaka Murata[1,2], Shuji Kawamoto[1*], Moritoki Egi[1]

[1]Department of Anesthesia, Kyoto University Hospital

54 Shogoin-kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan

[2]Department of Anesthesia, Kitano Hospital, Osaka, Japan

2-4-20 Ohgimachi, Kita-ku, Osaka 530-8480, Japan

[*]Correspondence: skawamot@kuhp.kyoto-u.ac.jp

Abstract

Background: The occurrence of thrombosis has been reported to be associated with severity and mortality in COVID-19 patients. SARS-CoV-2 infects the host using its spike protein. However, there is no study to examine the direct effect of the spike protein derived from SARS-CoV-2 variants on platelet activity and coagulability.

Methods: This is an ethically approved *ex vivo* study under preplanned power analysis. Venous blood was collected from 6 healthy subjects who gave prior written consent. The samples were divided into 5 groups: without spike proteins (group N) and with spike proteins derived from SARS-CoV-2 variants (Alpha, Beta, Gamma, and Delta) (group A, B, C, and D, respectively). Platelet aggregability, P-selectin expression, platelet associated complement-1 (PAC-1) binding, platelet count, mean platelet volume (MPV), and thromboelastography (TEG) parameters were measured. TEG parameters were measured in 2 groups, N and D; all other parameters were measured in 5 groups. The percent change in each parameter of groups A to D was calculated using the values of group N as reference. These data were then analyzed by Friedman test for all but the TEG parameters and by Wilcoxon matched pairs test for the TEG parameters. $P < 0.05$ was considered to be statistically significant.

Results: In this study, we included 6 participants based on the power analysis. There were no significant differences in group A to D in platelet aggregability measured under adenosine diphosphate (ADP) 5 μg/ml stimulation, collagen 0.2 or 0.5 μg/ml, and Ser-Phe-Leu-Leu-

Arg-Asn-amide trifluoroacetate salt (SFLLRN) 0.5 or 1 μM stimulation in groups A-D compared to group N. There were also no significant differences in P-selectin expression and PAC-1 binding under either basal conditions or SFLLRN stimulation. There were as well no significant differences in platelet count, MPV and TEG parameters.

Conclusions: In this *ex vivo* study, 5 μg/ml of the spike proteins derived from SARS-CoV-2 variants (Alpha, Beta, Gamma, and Delta) did not directly cause either the platelet hyperactivity or blood hypercoagulability reported in COVID-19 patients.

Trial registration: This study was approved by the Ethics Committee of Kyoto University Hospital (R0978-1) on 6th March 2020.

Keywords: COVID-19, SARS-CoV-2, Variants, Spike protein, Platelet, Thrombosis

Background

COVID-19 has caused many infections and deaths throughout the world [1]. New infections and deaths still occur due to the appearance of viral variants, in particular, variants of concern (VOCs). The main difference among COVID-19 variants is in the viral spike protein. SARS-CoV-2 uses its spike protein to bind to proteins on human cells and then invade these cells [2].

COVID-19 is a systemic disease that affects multiple organs including the hematopoietic system causing blood hypercoagulability [3-12]. Thrombosis in COVID-19 patients is

common [11-16] and associated with disease severity and the risk of death [17]. Klok et al [13] reported that prothrombin time > 3 s or activated partial thromboplastin time >5 s were independent predictors of thrombotic complications in COVID-19 pneumonia patients admitted to the intensive care unit, thus hypercoagulation in COVID-19 and COVID-19 thrombosis seem to be related. In addition, Zhang et al. [18] conducted *ex vivo* study and showed that the spike protein from a wild-type strain directly promotes platelet activation. However, thromboembolism caused by variants has not been well examined. Recently, all wild strain-derived SARS-CoV-2 have been replaced by variants-derived viruses [19]. It is unfortunate that there is no study to assess the direct effect of the spike protein derived from SARS-CoV-2 variants on platelet activity and coagulability, which have been reported in a wild-type strain [18].

Therefore, we assessed the effects on platelet activation and coagulability of spike proteins from four SARS-CoV-2 variants, Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2), which were all designated as VOCs by the World Health Organization when the experiments were planned.

Methods

Subject selection

This study was approved by the Ethics Committee of Kyoto University Hospital under approval number R0978-1, and carried out according to the guidelines of the Declaration of Helsinki.

We included healthy volunteers who met the following three criteria: 1) healthy adults aged 20 to 65 years, 2) not taking blood coagulation-related medication, and 3) no blood coagulation-related diseases. Prior written informed consent was obtained from all subjects.

Measurement groups

The groups with spike proteins derived from Alpha, Beta, Gamma, and Delta variants were classified as groups A, B, C, and D, respectively. The group without spike protein is group N.

Materials and processing

Details of drugs and equipment used in this study, washed platelet preparation, and platelet-stimulating agent processing are described in Additional file 1. The details of structure and stability of the spike proteins used in the experiments are provided in Additional file 2.

In determining the concentration of the spike protein in the study blood, we first referred to previous studies [18], where Zhang et al. found that platelet aggregation was enhanced in a concentration-dependent manner using 0-2 μg/ml of spike protein from a wild-type strain.

They further showed that P-selectin expression was enhanced by maximum concentration (2 μg/ml) of spike protein used in the aggregation assay. We therefore hypothesized that the higher concentration of 5 μg/ml would further enhance platelet function, and conducted a preliminary study to examine platelet aggregability and P-selectin expression using 0, 1, 2, and 5 μg/ml of spike protein from a wild-type strain (Table S1, Figs. S1 and S2 [Additional file 3]). In this preliminary study, platelet aggregability tend to increase according to the concentration of spike protein, and it appeared to be maximized at 5 μg/ml. Finally, we determined to use 5 μg/ml of spike proteins in the main study.

Spike proteins from SARS-CoV-2 variants (Alpha, Beta, Gamma, and Delta) were diluted to 100 μg/ml in phosphate-buffered saline (PBS), divided into small portions, combined with 0.1% w/v bovine serum albumin, and refrozen at -80ºC. Just before the start of experiments, the spike protein was thawed and excess was discarded without refreezing.

Measured parameters

In the current study, we used the same experimental techniques that we had evaluated the effects of drugs on enhancing and inhibiting platelet function [20, 21].

Platelet aggregability

Spike proteins were added to washed platelets and left to stand at 37ºC for 5 min. Light transmittance rates were then measured for 10 min under adenosine diphosphate (ADP) 5 μM, collagen 0.2 or 0.5 μg/ml, and Ser-Phe-Leu-Leu-Arg-Asn-amide trifluoroacetate salt (SFLLRN) 0.5 or 1 μM stimulation. The control was purified water (100% light transmittance). Two consecutive measurements were made and the average of the maximum values was used as the result (details are in Additional file 1).

P-selectin expression

Spike proteins were added to washed platelets and left to stand at 37ºC for 5 min. These were added to PBS ± SFLLRN (final concentration 1 μM), left to stand at 22ºC for 15 min, and then fixed at 4ºC (details are in Additional file 1). Centrifugation at 4ºC and 1,600 g for 15 min and washing with PBS were repeated twice, followed by addition of 1 μl each of anti-CD61 antibody and anti-CD62P antibody. Flow cytometry was performed as previously reported [20] after standing in the dark at 22 ºC for 1 hour. Mean fluorescent intensity (MFI) of CD62P-positive platelets among 10,000 CD61-positive platelets was calculated under basal conditions (no platelet stimulant) or SFLLRN stimulation.

Platelet complement-1 (PAC-1) binding

Spike proteins were added to the whole blood after collection and the sample was left to stand at 37ºC for 5 min. A total of 2.5 µl of the blood plus spike proteins were added to 5 µl of anti-CD61 antibody, 5 µl of anti-PAC-1 antibody, and 37.5 µl of PBS with or without SFLLRN (final concentration 1 µM) and left to stand in the dark at 22ºC for 15 min, then fixed at 4ºC (details are Additional file 1). MFI of PAC-1-positive platelets among 10,000 CD61-positive platelets was calculated under basal conditions or SFLLRN 1 µM stimulation.

Platelet count, mean platelet volume (MPV) and thromboelastography (TEG) parameters

Spike proteins were added to whole blood and left to stand at 37ºC for 5 min before testing. Due to the large and costly amount of spike protein required for measurements with TEG 6s, we decided to compare TEG parameters in only groups N and D. TEG 6s represents platelet and fibrinogen function using four main parameters: R, K, MA and LY30. A Global Hemostasis cartridge contains four reagents: CK, CKH, CRT, and CFF. We measured the following five parameters in this study: R-CK, K-CK, MA-CK, MA-CFF, and MA-CRT minus MA-CFF. R-CK, K-CK, MA-CK are the R, K, and MA values measured using CK; MA-CFF is the MA values measured using CFF; and MA-CRT minus MA-CFF is the difference between the MA values measured using CRT and CFF. R is the time until clot formation starts; K is the rate of clot formation; and MA is the maximum clot strength. CK contains kaolin, a coagulation promoter; CRT contains kaolin and tissue factor which further

enhances the clotting reaction; and CFF contains kaolin, tissue factor, and glycoprotein IIb/IIIa receptor antagonist which inhibits platelet participation in coagulation and assesses fibrinogen-only clot strength. CRT minus CFF indicates the platelets-only clot strength.

Statistical analysis

For each test in each subject, the percentage changes of values in groups A, B, C and D were calculated using the values in group N as a reference: value in groups A, B, C or D/value in group N × 100 (%). Results are presented as medians and quartiles. Platelet aggregation rate, P-selectin expression, PAC-1 binding, and blood count were analyzed by Friedman test. TEG was analyzed by Wilcoxon matched pairs test. Both tests were performed with Prism 9 for macOS, ver. 9.3.1 (GraphPad Software, San Diego, CA, USA).

To obtain enough power of study, we performed a power analysis. Given a difference of 1.5 times of the standard deviation was considered to be clinically significant, a power of 0.80, and an α level of 0.05, we found that 6 subjects were seemed to be necessary. A two-sided $\alpha < 0.05$ was considered to be statistically significant. All data are shown in Tables S2-5 [Additional file 4].

Results

The current study included 6 participants according to the power analysis. The subjects in this study were voluntarily asked the date of their last COVID-19 vaccination prior to blood collection, and all of them were found to have been vaccinated 5 to 7 months after their second Pfizer mRNA vaccination. Platelet aggregability and P-selectin expression were measured using washed platelets without leukocytes in order to remove the effects of the vaccine. PAC-1 binding, platelet count, MPV and TEG were measured using whole blood with leukocytes. The percentage of granulocytes (%GR) after adding spike protein was not low, which suggests that the neutrophil activity of the blood collected was sufficient (Table S6 [Additional file 4]).

The medians and quartiles for the % changes in groups A to D relative to group N are shown in Figs. 1 to 5.

Platelet aggregability

Platelet aggregability was measured with ADP (5 μM), collagen (0.2 or 0.5 μg/ml), or SFLLRN (0.5 or 1 μM) stimulation (Fig. 1). The aggregation rate of each group when the aggregation rate of group N was set at 100% did not change significantly with any of the platelet stimulating agents ($p = 0.69$ for ADP 5 μM stimulation, $p = 0.37$ for collagen 0.2 μg/ml stimulation, $p = 0.31$ for collagen 0.5 μg/ml stimulation, $p = 0.28$ for SFLLRN 0.5 μM stimulation, $p = 0.63$ for SFLLRN 1 μM stimulation).

P-selectin expression, PAC-1 binding, platelet count and MPV

For P-selectin and PAC-1, the MFI of each group did not change significantly under either condition when the MFI of group N under basal conditions was set at 100% ($p = 0.78$ for P-selectin expression under basal conditions, $p = 0.50$ for P-selectin expression under SFLLRN 1 μM stimulation, $p = 0.92$ for PAC-1 binding under either condition) (Figs. 2, 3). There were also no significant differences in platelet count and MPV ($p = 0.56$ for platelet count, $p = 0.11$ for MPV) (Fig. 4).

TEG

The differences between groups N and D were not significant ($p = 0.44$ for R-CK, $p = 0.63$ for K-CK, $p > 0.99$ for MA-CK, $p = 0.84$ for MA-CFF, $p = 0.44$ for MA-CRT minus MA-CFF) (Fig. 5).

Discussion

This is the first study to assess the direct effects of spike proteins from four SARS-CoV-2 variants on human platelets and coagulability. Contrary to the past results with spike protein from a wild-type strain [18], addition of spike proteins from four SARS-CoV-2 variants had

no effects on platelet aggregability, platelet activity, platelet count, MPV, or TEG in this *ex vivo* study.

Most studies evaluating the platelet function and/or blood coagulability in COVID-19 patients have used blood from COVID-19 patients [3-12, 22-32]. Hence, it has not been clear whether the increased platelet function and coagulability observed in these patients are due to SARS-CoV-2 or the spike protein itself, or to indirect effects triggered by SARS-CoV-2 infection, such as systemic inflammation. There is one study to show the increased platelet activity and coagulability when spike proteins from COVID-19 wild-type strain are reacted with human platelets [18]. However, no studies have examined such an effect in variants. In this regard, our study might have some novelty and relevancy.

Most studies on platelet function and coagulability in COVID-19 have reached inconsistent conclusions, with some showing platelet hyperactivation and blood hypercoagulation, while others did not. There are several reports [22-24] that COVID-19 patients have significantly increased aggregability compared to healthy subjects. However, in contrast, Bertolin et al. [6] found no difference in ADP-stimulated aggregability in patients compared to healthy subjects, and Heinz et al. [7] found it rather low in patients. Herrmann et al. [11] measured aggregability in critically ill patients over a 2-week period and found it to be well below baseline levels under ADP stimulation.

There have been several reports of significantly increased P-selectin expression in COVID-19 patients compared to healthy controls [3,18,22,25,26], but the details differ slightly. On the other hand, some reports have found P-selectin expression to be similar in patients and healthy controls [6,27].

Regarding with PAC-1 binding, one report shows a significant increase in COVID-19 patients, especially in severe COVID-19, compared to healthy controls [18], whereas another shows a significant decrease [27]. For agonist-stimulated PAC-1 binding, one report indicates that patients have increased expression compared to healthy subjects [18], while another indicates that patients have decreased expression independent of severity [22].

For MPV, the conclusions among studies of COVID-19 patients are more consistent. Patients have higher MPV than healthy subjects [6,18,23], and critically ill patients have higher MPV than non-critically ill patients [18,28-30], but rarely vice versa. The increase of MPV may be a hallmark of severity of COVID-19 as well as the increase of platelet count [23,28-31]. However, there is a wide variety of methods for MPV measurement, which makes it difficult to determine whether the MPV of an individual patient is normal or slightly elevated [32].

Blood viscoelasticity tests, regardless of the measuring device, have concluded that patients have hypercoagulability (shortened clotting times, increased clot strength, and shortened fibrinolysis times) [5-11,27]. This seems to be greater effects in critically ill

patients than in moderately ill patients [27,33]. However, in these studies, the findings for hypercoagulation were seen in selected parameters [5-9,27,33] and specific patients [9,10].

One reason for the wide variation in findings among studies is that platelet hyperactivation and blood hypercoagulation may be due to multiple factors and mechanisms, rather than to SARS-CoV-2 itself [34,35]. Our results, indicating that neither platelet activity nor blood coagulability were altered by a change of the SARS-CoV-2 spike protein alone, support this view.

Since SARS-CoV-2 establishes infection by binding to the host angiotensin-converting enzyme 2 (ACE2) receptor [36] and thrombosis is common in COVID-19 [11-16], there must be a relationship between SARS-CoV-2 binding to the ACE2 receptor and COVID-19 thrombosis. In fact, many studies have shown that increased inflammatory cytokines through highly ACE2-positive organs and vascular endothelial cells can lead to a thrombogenic response and COVID-19 thrombosis [3,10,27,34]. There are two major pathways for this mechanism: direct infection of the vascular endothelium via ACE2 on vascular endothelial cells, resulting in endothelial damage [34]; and systemic ACE2 downregulation [37,38]. SARS-CoV-2 infection of the vascular endothelium via ACE2 on endothelial cells damages the endothelium and causes platelet aggregation [34,39], allowing thrombosis to occur [34,40]. Systemic ACE2 receptor downregulation occurs in the renin-angiotensin system pathway, an important mechanism that maintains systemic sodium levels and causes

vasoconstriction. This pathway is regulated by ACE2 to prevent excessive inflammation and thrombus formation [37]. When SARS-CoV-2 spike protein internalizes [34] and downregulates [37,38] ACE2, there is an elevation of inflammatory cytokines such as interleukin-1α, interleukin-6, and tumor necrosis factor-α, and endothelial biomarkers such as von Willebrand factor [41]. Hence, endothelial function is impaired, thrombus formation becomes uncontrolled, and tissue factors are released that amplify the platelet thrombotic response [40]. Thus, COVID-19 thrombosis can be explained by the above two mechanisms by direct or indirect SARS-CoV-2 infection of ACE2 on organs or the endothelium.

Our study has several limitations. First, the number of subjects was small, which may be one of the reasons for the lack of significant difference in our study. However, our study is conducted under power analysis using pair comparisons. Second, it is unclear whether the concentration of spike protein used was adequate. It is uncertain whether 5 μg/ml of spike protein is too much or too little, or what the concentration of spike protein is in patients with severe COVID-19. Therefore, our finding should be confirmed or refute with difference concentration of spike protein. Nonetheless, we should note that the concentration of spike protein was determined with referring previous study [18] and our own preliminary study (Additional file 2). Third, the concentrations of platelet stimulants might have been too high in measurements of aggregation rate and platelet activation markers. Collagen- or SFLLRN-stimulated aggregability was measured at two concentrations, low and high, but there may

have been other concentrations at which differences in results between the spike protein and control groups could have been observed. Fourth, extra platelet activation or consumption may have occurred during the process of preparation of washed platelets, which may have affected the results. Fifth, the spike proteins used were not glycosylated, which could have increased protein instability during dilution and thawing of the proteins and prevented accurate responses. Sixth, in our study, platelet aggregability and P-selectin expression were measured using washed platelets without leukocytes. Therefore, it is unclear how cell-mediated immunity such as neutrophils, nitric oxide releasing, or production of related molecules, which may contribute to the platelet function and coagulopathy, affect platelet aggregability and P-selectin expression. Seventh, our study was performed *ex vivo* and could not evaluate the effects of blood flow, blood vessels, shear stress on platelets, and the interactions between platelets and other blood cells. Eighth, we did not confirm platelet binding of spike protein as demonstrated in a previous study [18]. However, there is still little evidence to show the fact of "ACE2 on platelets" [22,23,34]. In this regard, our main focus was whether various spike proteins from variants directly impact the platelet function.

Within these limitations, our results contribute to reducing the likelihood that the spike proteins derived from SARS-CoV-2 variants contribute to development of COVID-19 thrombosis.

Conclusions

In this *ex vivo* study, 5 μg/ml of the spike proteins derived from SARS-CoV-2 variants (Alpha, Beta, Gamma, and Delta) did not directly cause either the platelet hyperactivity or blood hypercoagulability.

Abbreviations

PAC-1: platelet associated complement-1; MPV: mean platelet volume; TEG: thromboelastography; ADP: adenosine diphosphate; SFLLRN: Ser-Phe-Leu-Leu-Arg-Asn-amide trifluoroacetate salt; VOCs: variants of concern; PBS: phosphate-buffered saline; MFI: mean fluorescent intensity; ACE2: angiotensin-converting enzyme 2.

Declarations

Ethics approval and consent to participate

The research protocol, written instructions to the subjects, and consent form were approved by the Ethics Committee of Kyoto University Hospital (R0978-1) and carried out according to the guidelines of the Declaration of Helsinki. Prior written informed consent was obtained from all subjects.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional file.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by a Grant-in-Aid for Scientific Research (KAKENHI) (21K09044).

Authors' contributions

EK performed all experiments, analyzed all data, wrote the initial draft, prepared all figures and an additional file, and edited the manuscript. YM edited the manuscript. SK designed the study and edited the manuscript. ME supervised the research and revised the manuscript.

Acknowledgements

Not applicable.

Additional file 1. Methods Appendix.

Additional file 2. The details and stability of spike protein structure.

Additional file 3. The methods and results of the preliminary study using spike proteins from the wild-type strain. **Table S1.** The measured values, medians, and quartiles obtained from the preliminary study. **Figure S1.** Maximum platelet aggregation rate with 1-5 μg/ml of wild-type strain-derived spike proteins (n = 2-4). **Figure S2.** P-selectin expression with 1-5 μg/ml of wild-type strain-derived spike proteins (n = 2-4).

Additional file 4. The data obtained from the study. **Table S2.** The measured values, medians, and quartiles of the results other than TEG parameters. **Table S3.** The measured values, medians, and quartiles of TEG parameters. **Table S4.** Ratio (%) of measured values in groups A to D to group N (except TEG). **Table S5.** Ratio (%) of TEG parameters in group D to group N. **Table S6.** Leukocyte fraction of blood collected.

References

[1] World Health Organization. WHO Coronavirus (COVID-19) Dashboard. https://covid19.who.int. Accessed 23 April 2023.

[2] Howes L. Structure of novel coronavirus spike protein solved in just weeks. Chemical & Engineering News. 2020;98(8). https://cen.acs.org/articles/98/i8/Structure-novel-coronavirus-spike-protein.html. Accessed 23 April 2023.

[3] Hottz ED, Azevedo-Quintanilha IG, Palhinha L, et al. Platelet activation and platelet-monocyte aggregate formation trigger tissue factor expression in patients with severe COVID-19. Blood. 2020;136(11):1330-41.

[4] Terpos E, Ntanasis-Stathopoulos I, Elalamy I, et al. Hematological findings and complications of COVID-19. Am J Hematol. 2020;95(7):834-47.

[5] Ranucci M, Ballotta A, Di Dedda U, et al. The procoagulant pattern of patients with COVID-19 acute respiratory distress syndrome. J Thromb Haemost. 2020;18(7):1747-51.

[6] Bertolin AJ, Dalçóquio TF, Salsoso R, et al. Platelet Reactivity and Coagulation Markers in Patients with COVID-19. Adv Ther. 2021;38(7):3911-23.

[7] Heinz C, Miesbach W, Herrmann E, et al. Greater Fibrinolysis Resistance but No Greater Platelet Aggregation in Critically Ill COVID-19 Patients. Anesthesiology. 2021;134(3):457-67.

[8] Bocci MG, Maviglia R, Consalvo LM, et al. Thromboelastography clot strength profiles and effect of systemic anticoagulation in COVID-19 acute respiratory distress syndrome: a prospective, observational study. Eur Rev Med Pharmacol Sci. 2020;24(23):12466-79.

[9] Yuriditsky E, Horowitz JM, Merchan C, et al. Thromboelastography Profiles of Critically Ill Patients With Coronavirus Disease 2019. Crit Care Med. 2020;48(9):1319-26.

[10] Panigada M, Bottino N, Tagliabue P, et al. Hypercoagulability of COVID-19 patients in intensive care unit: A report of thromboelastography findings and other parameters of hemostasis. J Thromb Haemost. 2020;18(7):1738-42.

[11] Herrmann J, Notz Q, Schlesinger T, et al. Point of care diagnostic of hypercoagulability and platelet function in COVID-19 induced acute respiratory distress syndrome: a retrospective observational study. Thromb J. 2021;19(1):39.

[12] Helms J, Tacquard C, Severac F, et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. Intensive Care Med. 2020;46(6):1089-98.

[13] Klok FA, Kruip MJHA, van der Meer NJM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res. 2020;191:145-7.

[14] Middeldorp S, Coppens M, van Haaps TF et al. Incidence of venous thromboembolism in hospitalized patients with COVID-19. J Thromb Haemost. 2020;18(8):1995-2002.

[15] Lodigiani C, Iapichino G, Carenzo L, et al. Venous and arterial thromboembolic complications in COVID-19 patients admitted to an academic hospital in Milan, Italy. Thromb Res. 2020;191:9-14.

[16] Avruscio G, Camporese G, Campello E, et al. COVID-19 and Venous Thromboembolism in Intensive Care or Medical Ward. Clin Transl Sci. 2020;13(6):1108-14.

[17] Miesbach W, Makris M. COVID-19: Coagulopathy, Risk of Thrombosis, and the Rationale for Anticoagulation. Clin Appl Thromb Hemost. 2020;26:1076029620938149.

[18] 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(1):120.

[19] World Health Organization. Weekly epidemiological update on COVID-19 – 10 August 2022, Edition 104. https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---10-august-2022. Accessed 23 April 2023.

[20] Kawamoto S, Hirakata H, Sugita N, Fukuda K. Bidirectional effects of dexmedetomidine on human platelet functions in vitro. Eur J Pharmacol. 2015;766:122-8.

[21] Murata Y, Kawamoto S, Fukuda K. Rocuronium Has a Suppressive Effect on Platelet Function via the P2Y12 Receptor Pathway In Vitro That Is Not Reversed by Sugammadex. Int J Mol Sci. 2020;21(17):6399.

[22] Manne BK, Denorme F, Middleton EA, et al. Platelet gene expression and function in patients with COVID-19. Blood. 2020;136(11):1317-29.

[23] Barrett TJ, Bilaloglu S, Cornwell M, et al. Platelets contribute to disease severity in COVID-19. J Thromb Haemost. 2021;19(12):3139-53.

[24] 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):1404-18.

[25] Bongiovanni D, Klug M, Lazareva O, et al. SARS-CoV-2 infection is associated with a pro-thrombotic platelet phenotype. Cell Death Dis. 2021;12(1):50.

[26] Apostolidis SA, Sarkar A, Giannini HM, et al. Signaling Through FcγRIIA and the C5a-C5aR Pathway Mediate Platelet Hyperactivation in COVID-19. Front Immunol. 2022;13:834988.

[27] Nicolai L, Leunig A, Brambs S, et al. Immunothrombotic Dysregulation in COVID-19 Pneumonia Is Associated With Respiratory Failure and Coagulopathy. Circulation. 2020;142(12):1176-89.

[28] Aydınyılmaz F, Aksakal E, Pamukcu HE, et al. Significance of MPV, RDW, and PDW with the Severity and Mortality of COVID-19 and Effects of Acetylsalicylic Acid Use. Clin Appl Thromb Hemost. 2021;27:10760296211048808.

[29] Atik D, Kaya HB. Evaluation of the Relationship of MPV, RDW and PVI Parameters with Disease Severity in Covid-19 Patients. Acta Clin Croat. 2021;60(1):103-14.

[30] Comer SP, Cullivan S, Szklanna PB, et al. COVID-19 induces a hyperactive phenotype in circulating platelets. PLoS Biol. 2021;19(2):e3001109.

[31] Güçlü E, Kocayiğit H, Okan HD, et al. Effect of COVID-19 on platelet count and its indices. Rev Assoc Med Bras (1992). 2020;66(8):1122-7.

[32] Noris P, Melazzini F, Balduini CL. New roles for mean platelet volume measurement in the clinical practice? Platelets. 2016;27(7):607-12.

[33] Boscolo A, Spiezia L, Correale C, et al. Different Hypercoagulable Profiles in Patients with COVID-19 Admitted to the Internal Medicine Ward and the Intensive Care Unit. Thromb Haemost. 2020;120(10):1474-77.

[34] Koupenova M. Potential role of platelets in COVID-19: Implications for thrombosis. Res Pract Thromb Haemost. 2020;4(5):737-40.

[35] Koupenova M, Freedman JE. Platelets and COVID-19: Inflammation, Hyperactivation and Additional Questions. Circ Res. 2020;127(11):1419-21.

[36] Tai W, He L, Zhang X, et al. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cell Mol Immunol. 2020;17(6):613-620.

[37] Almutlaq M, Alamro AA, Alroqi F, Barhoumi T. Classical and Counter-Regulatory Renin-Angiotensin System: Potential Key Roles in COVID-19 Pathophysiology. CJC Open. 2021;3(8):1060-74.

[38] Fraga-Silva RA, Sorg BS, Wankhede M, et al. ACE2 activation promotes antithrombotic activity. Mol Med. 2010;16(5-6):210-5.

[39] Koupenova M, Clancy L, Corkrey HA, Freedman JE. Circulating Platelets as Mediators of Immunity, Inflammation, and Thrombosis. Circ Res. 2018;122(2):337-51.

[40] Koupenova M, Kehrel BE, Corkrey HA, Freedman JE. Thrombosis and platelets: an update. Eur Heart J. 2017;38(11):785-91.

[41] Satta S, Lai A, Cavallero S, et al. Rapid Detection and Inhibition of SARS-CoV-2-Spike Mutation-Mediated Microthrombosis. Adv Sci. (Weinh). 2021;8(23):e2103266.

[42] Salem N, Atallah B, El Nekidy WS, Sadik ZG, Park WM, Mallat J. Thromboelastography findings in critically ill COVID-19 patients. J Thromb Thrombolysis. 2021;51(4):961-5.

[43] Tomerak S, Khan S, Almasri M, et al. Systemic inflammation in COVID-19 patients may induce various types of venous and arterial thrombosis: A systematic review. Scand J Immunol. 2021;94(5):e13097.

Figure legends

Fig. 1 Maximum platelet aggregation rates shown as % changes relative to group N.

Medians and quartiles are shown on the graphs. There were no significant differences among the variants with any of the platelet stimulants. (a) Aggregation rates under ADP 5 µM stimulation (n = 6). (b) Aggregation rates under collagen 0.2 or 0.5 µg/ml stimulation (n = 5). (c) Aggregation rates under SFLLRN 0.5 or 1 µM stimulation (n = 5). ADP: adenosine diphosphate; SFLLRN: Ser-Phe-Leu-Leu-Arg-Asn-amide trifluoroacetate salt.

Fig. 2 MFI of P-selectin shown as % changes relative to group N under basal conditions. SFLLRN 1 μM was used as the platelet stimulant. Medians and quartiles are shown on the graphs. There were no significant differences among the variants under no stimulation or SFLLRN 1 μM stimulation (n = 6). MFI: mean fluorescent intensity; SFLLRN: Ser-Phe-Leu-Leu-Arg-Asn-amide trifluoroacetate salt.

Fig. 3 MFI of PAC-1 shown as % changes relative to group N under basal conditions. SFLLRN 1 μM was used as the platelet stimulant. Medians and quartiles are shown on the graphs. There were no significant differences among the variants under no stimulation or SFLLRN 1 μM stimulation (n = 6). PAC-1: platelet associated complement-1; MFI: mean fluorescent intensity; SFLLRN: Ser-Phe-Leu-Leu-Arg-Asn-amide trifluoroacetate salt.

Fig. 4 Blood counts shown as % changes relative to group N. Medians and quartiles are shown on the graphs. There were no significant differences in platelet count and MPV among the variants (n = 6). MPV: mean platelet volume.

Fig. 5 TEG parameters shown as % changes in group D relative to group N. Medians and quartiles are shown on the graphs. The differences between groups N and D were not significant (n = 6). (a) R is the time until clot formation starts and CK is a reagent containing

kaolin. R-CK is the R values measured using CK and refers to the rate of coagulation initiation. (b) K is the rate of clot formation. K-CK is the K values measured using CK and refers to the rate of the coagulation process. (c) MA is the maximum clot strength. MA-CK is the MA values measured using CK and refers to the blood clot strength. (d) CFF is a reagent containing kaolin, tissue factor, and glycoprotein IIb/IIIa receptor antagonist. MA-CFF is the MA values measured using CFF and indicates fibrinogen contribution to MA-CK. (e) CRT is a reagent containing kaolin and tissue factor. MA-CRT minus MA-CFF is the difference between the MA values measured using CRT and CFF, and indicates platelet contribution to MA-CK. TEG: thromboelastography.