## Relative hypercoagulopathy of the SARS-CoV-2 Beta and Delta variants when compared to the less severe Omicron variants is related to TEG parameters, the extent of fibrin amyloid microclots, and the severity of clinical illness.

**Lize M Grobbelaar BScHons1, Arneaux Kruger MBChB1, Chantelle Venter PhD1, EsteM Burger MEng2, Gert Jacobus Laubscher, MBChB, FCP(SA)3, Tongai G Maponga PhD4, Maritha J Kotze PhD5, Hau C. Kwaan MD, PhD6, Joseph B Miller MD7, Daniel Fulkerson MD8, Wei Huff MD, PhD8, Eric Chang BS9,Grant Wiarda10, Connor M Bunch MD7, Mark M Walsh MD9,10,11, Syed Raza MD12,Mahmud Zamlut MD12, Hunter B Moore MD, PhD15, Ernest E. Moore MD16, Matthew D Neal MD17, Douglas B Kell DPhil, DSc1,13,14\*, Etheresia Pretorius PhD1,14\***

1Department of Physiological Sciences, Faculty of Science, Stellenbosch University, Stellenbosch, Private Bag X1 Matieland, 7602, South Africa

2 BioCODE Technologies, Stellenbosch 7600

**3**Mediclinic Stellenbosch, Stellenbosch 7600, South Africa

4Division of Medical Virology, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, Cape Town, South Africa

5Division of Chemical Pathology, Department of Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University and National Health Laboratory Service, Tygerberg Hospital, Cape Town 8000, South Africa.

6Division of Hematology and Oncology, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA

7Departments of Emergency Medicine and Internal Medicine, Henry Ford Hospital, Detroit, MI 48202, USA

8Department of Neurosurgery, St. Joseph Regional Medical Center, Mishawaka, IN 46545, USA

9Indiana University School of Medicine South Bend Campus, Notre Dame, Indiana USA

10Department of Internal Medicine, Saint Joseph Regional Medical Center, Mishawaka, IN 46545, USA

11Department of Emergency Medicine, Saint Joseph Regional Medical Center, Mishawaka, IN 46545, USA

12Department of Critical Care Medicine, Saint Joseph Regional Medical Center, Mishawaka, IN 46545, IN

13Department of Biochemistry and Systems Biology, Institute of Systems, Molecular and Integrative Biology, Faculty of Health and Life Sciences, University of Liverpool, L69 7ZB, UK.;

14The Novo Nordisk Foundation Centre for Biosustainability, Technical University of Denmark, Kemitorvet 200, 2800 Kgs Lyngby, Denmark

15Department of Surgery, Division of Transplant Surgery, Denver Health and University of Colorado Health Sciences Center, Denver, CO 80204, USA

16Department of Surgery, Ernest E. Moore Shock Trauma Center at Denver Health and University of Colorado Health Sciences Center, Denver, CO 80204, USA

17 Pittsburgh Trauma Research Center, University of Pittsburgh Medical Center, Pittsburgh, PA 15213, USA

**\*Corresponding authors:**

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| * **\*Etheresia Pretorius**
* Department of Physiological Sciences, Stellenbosch University, Private Bag X1 Matieland, 7602, SOUTH AFRICA

resiap@sun.ac.za<http://www.resiapretorius.net/>ORCID: 0000-0002-9108-2384* **\*Douglas B. Kell**
* Department of Biochemistry and Systems Biology, Institute of Systems, Molecular and Integrative Biology, Faculty of Health and Life Sciences, University of Liverpool, L69 7ZB, UK.

dbk@liv.ac.ukORCID: 0000-0001-5838-7963 |

## Running title: Relative hypercoagulability among SARS-CoV-2 variants

## Abstract

Earlier variants of SARS-CoV-2 have been associated with plasma hypercoagulability (as judged by thromboelastography) and an extensive formation of fibrin amyloid microclots, which are considered to contribute to the pathology of the coronavirus 2019 disease (COVID-19). The newer Omicron variants appear to be far more transmissible, but less virulent, even when taking immunity acquired from previous infections or vaccination into account. We here show that while the clotting parameters associated with Omicron variants are significantly raised over those of healthy, matched controls, they are only raised to levels significantly lower than those seen with more severe variants such as Beta and Delta. We also observed that individuals infected with Omicron variants manifested less extensive microclot formation in platelet poor plasma compared to those harbouring the more virulent variants. The measurement of clotting effects between the different variants acts as a kind of ‘internal control’ that demonstrates the relationship between the extent of coagulopathies and the virulence of the variant of interest. This adds to the evidence that microclots play an important role in determining the severity of symptoms observed in COVID-19.

## Keywords:

## COVID-19; Variants; Omicron; Coagulation; Fluorescence Microscopy; Microclots

## INTRODUCTION

## Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the pathogen responsible for coronavirus disease 2019 (COVID-19), has resulted in more than 6.3 million deaths (as of 23 June 2022) worldwide 1. Adaptive mutations in the SARS-CoV-2 viral genome can alter its pathogenic potential by effecting the ability of the virus to evade the immune system 2. As of December 11, 2021, the WHO has reported five SARS-CoV-2 variants of concern (VOCs): Alpha (B.1.1.7) in December 2020; Beta (B.1.351) in December 2020; Gamma (P.1) reported early January 2021; Delta (B.1.617.2)  reported in December 2020; and Omicron (B.1.1.529)  reported in November 2021 2-9 (see table 1).

COVID-19 has resulted in five distinct waves in South Africa 5-8,10,11. The first wave was caused by multiple lineages and peaked in July 2020 12,13. The Beta VOC (501Y.V2) drove the second wave of infections that started in Nelson Mandela Bay in October 2020. This was followed by a third Delta (B.1.617.2) VOC driven wave, that was determined to have ten mutations in the spike protein. The latest VOC, Omicron (B.1.1.529) (with more than 30 changes to the spike protein), was first identified in Botswana and South Africa in November 2021, and divided into sub-linages; BA.1 (the main clade), BA.2, and BA.3 5. The first wave associated with Omicron in South Africa was determined to have passed its peak by December 2021 14. This fourth wave of COVID-19 cases in South Africa was characterized by a higher and quicker peak with fewer hospital admissions 15. In April 2022 two new sub-lineages of the Omicron VOC, known as BA.4 and BA.5 were also discovered, resulting in a fifth wave 16,17. The WHO is closely tracking the sub-linages to determine the potential these sub-linages have for transmissibility and disease severity 9.

It is well-established that the earlier variants (before Omicron) caused severe disease and resulted in coagulopathies in critically ill patients 18-36. In contrast, the heavily mutated Omicron variants have been shown to have milder symptoms than the earlier variants. The five most prevalent symptoms during Omicron infection were reported to be runny nose, rhinitis headache, fatigue (either mild or severe), sneezing, and sore throat 37-40. Milder infections could be a result of other factors apart from Omicron variants, such as effects of previous infection and vaccination protection 41. However, multiple studies have indicated a reduced or no effect of different COVID-19 vaccines against Omicron variants 42-44.

Using point-of-care technologies such as thromboelastography (TEG®) poses an opportunity to improve early management of severe COVID-19-associated coagulopathy 29,45. In conjunction with this, our research group has suggested that fibrin amyloid microclot (currently only available as a research laboratory tool) might be of great importance to determine clotting pathology in individuals with acute COVID-19 infection 29,36. Microclots are defined as fibrinogen (and other plasma proteins) that clot into an anomalous ‘amyloid’ from of fibrin (‘fibrinaloid’) with higher than normal resistance to fibrinolysis, and a size range from 1-200µm when measured on the longest axis 27. Our group have previously shown amyloid fibrin deposits in numerous inflammatory conditions 46-53. However, the extent of the microclot presence in both acute COVID-19 and Long COVID was found to be significantly more than in other chronic systemic inflammatory conditions 32,36.

Due to the reduction in Omicron symptoms and fatalities, we investigated the differences between new Omicron VOCs and the previously circulating SARS-CoV-2 variants using TEG® clotting profiles and an assessment of the extent of microclots in the plasma by means of fluorescence microscopy 29,36. Because of the nature of the blood collections from patients who reported at our clinical collaborator’s practice, we did not focus on a specific sub-lineage of the Omicron variant.

## MATERIALS AND METHODS

## Ethical considerations

Ethical approval for blood collection and analysis of participants with COVID-19 and healthy individuals was given by the Health Research Ethics Committee (HREC) of Stellenbosch University (reference #9521). This laboratory study was carried out in strict adherence to the International Declaration of Helsinki, South African Guidelines for Good Clinical Practice, and the South African Medical Research Council (SAMRC) Ethical Guidelines for research. Consent was obtained from all participants.

**Blood sample preparation**

Citrated blood samples were collected and centrifuged at 3000xg for 15 minutes where after the platelet poor plasma (PPP) were stored at -80°C, until analysed later, with the haematocrit being analysed on the day the sample was received.

## Participant demographics (see table 2)

## Healthy participants

Our healthy samples comprised 10 individuals who were SARS-CoV-2 negative, and with no signs of long COVID (in case they had previously been infected), or any known inflammatory or cardiovascular diseases.

## Participants with COVID-19: variants pre-October 2021

Whole blood (WB) samples were collected from 10 COVID-19-positive participants between October 2020 and September 2021 before treatment was administered. This group thus include individuals with the Beta and Delta COVID-19 variant and will be denoted in this paper as β/Δ. Six of the patients were hospitalized.

## Participants with COVID-19: Omicron infection (January 2022- onwards)

WB samples were collected from 10 outpatient COVID-19-positive individuals before treatment was started. Whole SARS-CoV-2 genome sequences of this subset of patients were generated from nasopharyngeal swabs using the Midnight protocol on the GridION device (Oxford Nanopore Technologies, Oxford, United Kingdom) 54. Of the 10 samples, six were confirmed to be Omicron (2 BA.1, 3 BA.2 and 1 BA.4). Four of the samples had inconclusive results, however we expect these samples to form part of the Omicron clade as it was the prevailing variant at the time of sample collection 8.

WHO Clinical Progression Scale assessment of COVD-19 patients

All participants in the study diagnosed with COVID-19 were scored by our clinical collaborators using the WHO Clinical Progression Scale. This scale serves as a minimum set of common outcome measures for clinical research on COVID-19 55. The scale ranges from 0 (uninfected) - 10 (dead). Scores 1-3 represent ambulatory mild disease ranging from asymptomatic to symptomatic needing assistance. Scores 4 – 5 are given to hospitalized patients with moderate disease: no oxygen support (score 4), and oxygen by mask or nasal prongs (score 5). Scores 6 – 9 categorize hospitalized patients with severe disease: oxygen by high flow or non-invasive ventilation (score 6), intubation and mechanical ventilation with partial pressure oxygen (pO2)/ fraction of inspired oxygen (FiO2) ≥ 150 or oxygen saturation (SpO2)/ FiO2 ≥ 200 (score 7), intubation and mechanical ventilation with pO2/FiO2 mm Hg < 150 or SpO2/FiO2 mm Hg < 200 (score 8), or extracorporeal membrane oxygenation (ECMO) (score 9) 55.

**Thromboelastography (TEG®) of Whole blood (WB)**

TEG® is a viscoelastic technique that allows for the quantitative measurement of the efficiency of blood coagulation. Table 2 summarizes the various parameters measured in the present study using this method.

WB TEG® was performed to assess the clot kinetics and viscoelastic properties of naïve WB samples from healthy individuals (n=10), individuals diagnosed with β/Δ (n =10), and individuals diagnosed with Omicron (n = 10). Sample preparation required the addition of 20μL 0.2M calcium chloride (CaCl2) (7003, Haemonetics®, Niles, IL, USA) to a disposable TEG® cup (HAEM 6211, Haemonetics®, Niles, IL, USA), followed by the addition of 340μL citrated WB. CaCl2 is responsible for the reversal of the anticoagulant action of sodium citrate and, consequently, activation of the coagulation cascade 46. Samples were loaded into the measuring channels of a TEG® 5000 Hemostasis Analyzer System (07-033, Haemonetics®, Boston, MA, USA) and allowed to run until the maximum amplitude (MA) was reached. All analyses were performed at 37°C.

**Quantitative assessment of anomalous platelet and fibrin amyloid clotting by fluorescent microscopy of platelet poor plasma (PPP)**

In order to detect microclot presence in PPP, Thioflavin T was added to PPP of healthy individuals (n=10), β/Δ (n =10), and Omicron participants (n = 10). This method was previously described 47,57. Thioflavin T (ThT) is a fluorescent probe frequently used to detect amyloid fibrils at roughly 482nm with an excitation of 450nm 58,59. The ThT molecule comprises a pair of benzothiazole and benzaminic rings that freely rotates around a shared C-C bond and if the rotation is disturbed, the molecule will exhibit strong fluorescence (at 482nm) which appears green to the observer 60. The PPP was thawed from -80°C to room temperature, whereafter the samples were exposed to ThT (Sigma-Aldrich, St. Louis, MO, USA), at a final exposure concentration of 5 μM and for a period of 30 minutes. Following incubation, 3µL PPP of each exposed sample was placed on a glass slide and covered with a coverslip. A Zeiss Axio Observer 7 inverted fluorescence microscope equipped with a Colibri 7 LED light source, and a Plan-Apochromat 63x/1.4 Oil DIC M27 objective (Carl Zeiss Microscopy, Munich, Germany) was used to view the prepared samples. The excitation wavelength for ThT was set at 450nm to 488nm and the emission at 499nm to 529nm.

Microclot images were assessed by calculating the area of fluorescent microclots (identified by ThT). A total of five micrographs per sample were subjected to two separate thresholding scripts/algorithms. A Fiji® (Java 1.8.0\_172) and a Python® (Python 3.9.5) script were the quantitative methods used. The Fiji® methodology was adapted from as described in 61. A second Python® image processing script was developed and applied to the same five micrographs per sample to calculate the area of fluorescent microclots (identified by ThT). The Fiji® script was set to analyse particles of a size of 0.5µm – 200,000µm (to represent infinity). A minimum of 0.5µm was set to include smaller particles but also account for background fluorescence. The Python® script converts the images to grayscale and performs simple binary thresholding to eliminate low-level background fluorescence. A threshold value of 30 (pixel value) is used for this step. For a grayscale images, the pixel value is a single number that represents the brightness of the pixel. The most common pixel format is the byte image, where this number is stored as an 8-bit integer giving a range of possible values from 0 to 255. Typically, zero is taken to be black, and 255 is taken to be white. After grayscaling the images, Otsu’s thresholding method is used to binarize the image and separate the image into foreground and background pixels, with the foreground pixels being the fluorescent clot areas. The individual clot areas are then identified, labelled, and characterised using the skimage measure library (<https://scikit>-image.org/). The now labelled clot areas are used to calculate the total fluorescent area. The average fluorescent area measurement calculated by both the Fiji® and Python® scripts are converted to percentages through considering the total area per image. This allowed for direct comparison of the two methodologies (scripts are available on request).

**Statistical analysis**

GraphPad Prism 9 (version 9.3.1, San Diego, CA) was used to determine statistical differences of quantitative parameters. An unpaired student’s t-test was performed for parametric data (as determined by the Shapiro–Wilk normality test), while the Mann–Whitney U test was performed for nonparametric data. A Kruskal-Wallis test (nonparametric distribution) was performed on the age parameter to determine the statistical difference between the three representative groups. Parametric data were expressed as mean ± standard deviation, and nonparametric data were expressed as median and [Q1 – Q3 interquartile range].

## RESULTS

Demographic features and clinical characteristics of all participants are presented in Table 3 alongside WHO Clinical Progression scores for Omicron and β/Δ patients. Based on this prognostic scale, all 10 Omicron patients had a WHO score of 2. Likewise, using the same algorithm, the 10 β/Δ (which included in - and outpatients) had a mean WHO score of 4.9. Four of the β/Δ patients were outpatients, of which three had WHO scores of 2 and one patient who required assistance had a WHO score of 3. Two patients were on nasal cannula oxygen for WHO scores of 5, and one patient required high-flow nasal cannula for a WHO score of 6. Three β/Δ patients received mechanical ventilation, (pO2/FiO2 mm Hg < 150 or SpO2/FiO2 mm Hg < 200) for WHO scores of 8. This prognostic tool indicated that the disease severity of β/Δ patients were significantly higher than that of Omicron patients.

## Thromboelastography (TEG®) of Whole blood (WB)

TEG® was performed on WB samples to assess coagulation sufficiency of our three groups. Four WB clot parameters were assessed by TEG® in this study: reaction time (R), kinetics (K), alpha angle (A) and maximum amplitude (MA) (see Table 3). Significant differences in all four TEG® parameters were found when comparing data from healthy individuals and individuals diagnosed with β/Δ COVID-19, with all parameters of β/Δ indicating to a hypercoagulable state. Overall significances were also established between healthy individuals and individuals with Omicron. The MA of the β/Δ group was significantly higher than the Omicron group. Despite the fact that significance was not established in three of the parameters when directly comparing Omicron and β/Δ, majority of the p values in the β/Δ to healthy individuals comparison were lower than the p values in the Omicron to healthy individuals comparison. The distribution of WB TEG® parameters between all groups are shown in Table 4.

## Fluorescence microscopy to detect aberrant fibrin amyloids in platelet poor plasma (PPP) stained with Thioflavin T (ThT)

Plasma from both β/Δ COVID-19 and Omicron COVID-19samples demonstrated a significantly higher percentage area of amyloid when compared to control samples. In addition, here we could also differentiate between the microclotting seen in acute Omicron COVID-19 samples and acute β/Δ COVID-19 samples. Following the % microclot area analysis using Fiji®, we subjected the same set of micrographs to a similar image processing script we developed using Python®. The results of the analysis were in agreement between the two scripts. Table 5 shows the statistical analysis of the area of fluorescent particle results obtained from the two different scripts.

Figure 1 shows a cartoon on how the PPP samples were prepared for the detection of microclots and representative examples of microclots in PPP. We observed less extensive microclot formation in Omicron PPP compared to the more virulent β/Δ variants.

## DISCUSSION

Mutations to the SARS-CoV-2 viral genome, resulting in the rise of new variants, have been associated with increased risk of hospitalization, ICU admission, morbidity, and mortality 62,63. The Beta and Delta variants posed a greater risk in terms of the above mentioned factors when compared to the less virulent Alpha and Gamma variants 63. Interestingly, estimates of the severity of the newest Omicron VOCs propose a lower risk of serious infection (per person infected) requiring hospitalization when compared to the previous dominant variant, Delta 64,65. The impact of SARS-CoV-2 genome mutations on COVID-19 associated coagulopathy is not well documented. In this study, we compared WB TEG® blood clotting parameters, and prevalence of microclots of healthy individuals to COVID-19 participants that have been infected by different SARS-CoV-2 variants.

 A spectrum of hypercoagulability is predicted to present among different COVID-19 variants. The early Alpha variants, and successive Beta and Delta variants, caused more hypercoagulability as documented by the incidence of venous thromboembolism, and reflected by hypercoagulopathic parameters of TEG®s including fibrinolytic shutdown 28,66 than what is presumed to be seen in subsequent Omicron variants. In our patient population, the majority of the β/Δ variants were sicker and included recruitment from the inpatient population, whereas all the Omicron variants were less ill and were recruited only from the outpatient population. Using the WHO Clinical Progression Scale, we determined that our Omicron population experienced significantly less severe disease states compared to our β/Δ population. This study did not calculate APACHE II & IV, or SOFA scores for the COVID-19 positive population, yet it is clear that these scores were much lower for the non-hospitalized Omicron patients. Thus, there has been significant literature published regarding the validity of these prediction tools as well as precision-based proteomics algorithms which rely on machine-learning 55,67-70. In this study, we demonstrated TEG® results and their ability to distinguish differing degrees of hypercoagulability among the variants. A large proportion of critically ill COVID-19 patients will present with hypercoagulable TEG® profiles 45,71,72. Omicron patients are met with reduced odds of developing severe disease 73, leading to the assumption of less hypercoagulable TEG® profiles. The results presented here do indeed indicate lesser hypercoagulability in our Omicron population when compared to healthy individuals versus our β/Δ population when compared to the same set of healthy individuals. A direct comparison between the Omicron population and β/Δ population indicated a significantly higher maximum amplitude in the β/Δ population.

Fibrinolytic abnormalities can occur in COVID-19 with fibrin deposits previously seen in the lungs 74 and hearts 75 of positive patients. The present study found a significant amount of fibrin amyloid microclots in the PPP of β/Δ samples and to a lesser extent Omicron samples. Microclots have previously been proven to be highly resistant to fibrinolysis and it was also shown that inflammatory molecules and plasmin inhibitors can become entrapped in them 32. Similar to this, Wygrecka *et. al.* found that abnormal fibrin structure and dysregulated fibrinolysis collectively contribute to a high incidence of thrombotic events in COVID-19 76. Abnormal fibrinogen levels are a prominent factor associated with COVID-19 induced coagulopathy 77-79. This study did not measure fibrinogen levels; however, previous studies show that elevated fibrinogen correlates with excessive inflammation, disease severity and ICU admission in COVID-19 patients 80. Our results show statically significant tapering in amount of microclots from β/Δ to Omicron to healthy individuals. The TEG® parameters did indicate a spectrum of hypercoagulability among the different COVID-19 variants, however to a lesser extent than the microclot results since direct comparison of the Omicron population to β/Δ population indicated significance.

We suggest that TEG/ROTEM and plasma microclot analysis, as personalized point-of-care medicine tools, may fill the gaps in these evidence-based recommendations for safely and effectively titrating thromboprophylaxis or anticoagulation. Although we did not directly show a lower risk of clinically significant macro-thrombosis for Omicron variants, this study is the first of its kind to directly study differing degrees of hypercoagulability among the less virulent Omicron and more virulent β/Δ variants. The severity of COVID-19 associated coagulopathy is largely due to severity of illness and correlates well with disposition; thrombotic and hemorrhagic events correlate positively to intensive-level care 81. Since Omicron less frequently causes severe illness and hospitalization, it was thought also to confer lesser hypercoagulability along the spectrum of COVID-associated coagulopathy. This study supports that hypothesis. Beyond the hospitalized COVID-19 patient, these results also have implications for the surgical patient undergoing elective surgery while afflicted with acute or convalescent COVID-19 82. The surgeon may use TEG/ROTEM and plasma microclot analysis to contextualize the patient’s coagulopathy and thrombohemorrhagic risk in the perioperative period. TEG/ROTEM has established operative use in cardiac surgery, liver transplantation, and trauma resuscitation 83,84. We propose that acute or convalescent COVID-19 should be a relative indication for adjunctive TEG/ROTEM use for the perioperative patient undergoing an emergent or elective procedure.

In the present study we did not focus on platelet activity. The role of platelets in COVID-19 associated coagulopathy is complex. Platelet hyperactivation fuels the thrombo-inflammatory milieu associated with disease severity in moderate to severe COVD-19 85-89. Similarly, SARS-CoV-2 can directly bind to platelet angiotensin-converting enzyme 2 (ACE2) via its spike glycoprotein to enhance thrombotic activity 90. Thrombocytopenia is another important platelet associated complication of COVID-19 91-93. The pathophysiology of thrombocytopenia in COVID-19 is not fully understood, but has been proposed to involve several mechanisms 94. As the mechanism may differ, the strategies to remedy this thrombocytopenia might be different, rendering the need for serious caution when approaching treatment 95. Traditional platelet functional assays are reliable, but show very limited potential in clarifying platelet phenotypic heterogeneity and interactions 96. Considering this in combination with the lack of pre-existing knowledge distinguishing the impact of differing SARS-CoV-2 variants on coagulation, and the multifaceted role platelets may play in COVID-19, highlights the need for alertness when approaching research in this uncharted territory. Advancements in techniques such as flow cytometry, electron microscopy, mass spectrometry, and ‘omics’ have started to open up new avenues in platelet research 96, paving the way for follow up studies to expand and add to the personalized-based medicine tools presented here.

**CONCLUSION**

Omicron variants present with less severe symptoms and a lower likelihood of hospitalization when compared to earlier variants such as Beta and Delta. Less is known about the impact of SARS-CoV-2 genome mutations on COVID-19 associated coagulopathy. The results presented here show differing degrees of hypercoagulability among SARS-CoV-2 variants determined by a combined approach of TEG® and fluorescent PPP microclot analysis. This study does not of itself infer a direct lower risk of clinically significant macro-thrombosis for Omicron variants, but it is the first of its kind to focus on studying and indicating differing levels of hypercoagulability among the less virulent Omicron and more virulent Beta and Delta variants. The use of TEG/ROTEM and plasma microclot analysis are suggested as personalized-based medicine tools, that may have potential in successfully facilitating safe and effective titrating thromboprophylaxis or anticoagulation. Future research should focus on establishing how SARS-CoV-2 infection from newer variants influence platelet behavior in the diseased state, compared to older variants. Additional development of easy-to-use screening tools should continue with an overlapping clinical and translational approach. Access to a variety of relevant screening tools will assist clinicians in choosing optimal treatment during COVID-19 associated coagulopathy.

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**ETHICS STATEMENT**

Ethical clearance for microclot analysis was obtained from the Health Research Ethics Committee (HREC) of Stellenbosch University (South Africa): N19/03/043, project ID 9521. Confirmation of Omicron variants: N20/04/008\_COVID-19.

**CONFLICT OF INTEREST STATEMENT**

MJK is a non-executive director and shareholder of Gknowmix (Pty) Ltd. EP is the managing director of BioCODE Technologies. E.E.M., H.B.M., M.D.N. have received research grants from Haemonetics Corporation outside the submitted work. M.D.N. has received an honorarium from Haemonetics Corporation for speaking engagements, as well as research support from Janssen Pharmaceuticals (Beerse, Belgium) and Noveome Biotherapeutics (Pittsburgh, PA, USA) outside the submitted work. He has served as a consultant to Janssen and CSL Behring (King of Prussia, PA, USA) and serves on the Scientific Advisory Board of Haima Therapeutics (Cleveland, OH, USA). M.M.W. has received honoraria from Alexion Pharmaceuticals (Boston, MA, USA).

**REFERENCES**

1. WHO. WHO Coronavirus (COVID-19) Dashboard. Accessed 23 June, 2022. <https://covid19.who.int/>

2. Aleem A, Akbar Samad AB, Slenker AK. Emerging Variants of SARS-CoV-2 And Novel Therapeutics Against Coronavirus (COVID-19). *StatPearls*. StatPearls Publishing

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3. Meo SA, Meo AS, Al-Jassir FF, et al. Omicron SARS-CoV-2 new variant: global prevalence and biological and clinical characteristics. *Eur Rev Med Pharmacol Sci*. Dec 2021;25(24):8012-8018. doi:10.26355/eurrev\_202112\_27652

4. Ong SWX, Chiew CJ, Ang LW, et al. Clinical and virological features of SARS-CoV-2 variants of concern: a retrospective cohort study comparing B.1.1.7 (Alpha), B.1.315 (Beta), and B.1.617.2 (Delta). *Clin Infect Dis*. Aug 23 2021;doi:10.1093/cid/ciab721

5. Viana R, Moyo S, Amoako DG, et al. Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. *Nature*. 2022;603(7902):679-686. doi:10.1038/s41586-022-04411-y

6. Tegally H, Wilkinson E, Giovanetti M, et al. Detection of a SARS-CoV-2 variant of concern in South Africa. *Nature*. 2021;592(7854):438-443. doi:10.1038/s41586-021-03402-9

7. Tegally H, Wilkinson E, Lessells RJ, et al. Sixteen novel lineages of SARS-CoV-2 in South Africa. *Nat Med*. Mar 2021;27(3):440-446. doi:10.1038/s41591-021-01255-3

8. Tegally H, Moir M, Everatt J, et al. Continued Emergence and Evolution of Omicron in South Africa: New BA.4 and BA.5 lineages. Cold Spring Harbor Laboratory; 2022.

9. WHO. Tracking SARS-CoV-2 variants. Accessed 25 June, 2022. <https://www.who.int/activities/tracking-SARS-CoV-2-variants>

10. Salyer SJ, Maeda J, Sembuche S, et al. The first and second waves of the COVID-19 pandemic in Africa: a cross-sectional study. *The Lancet*. 2021;397(10281):1265-1275. doi:10.1016/s0140-6736(21)00632-2

11. Jassat W, Mudara C, Ozougwu L, et al. Difference in mortality among individuals admitted to hospital with COVID-19 during the first and second waves in South Africa: a cohort study. *The Lancet Global Health*. 2021;9(9):e1216-e1225. doi:10.1016/s2214-109x(21)00289-8

12. Giandhari J, Pillay S, Wilkinson E, et al. Early transmission of SARS-CoV-2 in South Africa: An epidemiological and phylogenetic report. Cold Spring Harbor Laboratory; 2020.

13. Engelbrecht S, Delaney K, Kleinhans B, et al. Multiple Early Introductions of SARS-CoV-2 to Cape Town, South Africa. *Viruses*. 2021;13(3):526. doi:10.3390/v13030526

14. Mahase E. Omicron: South Africa says fourth wave peak has passed as it lifts curfew. *BMJ*. 2022:o7. doi:10.1136/bmj.o7

15. Jassat W, Abdool Karim SS, Mudara C, et al. Clinical severity of COVID-19 patients admitted to hospitals during the Omicron wave in South Africa. Cold Spring Harbor Laboratory; 2022.

16. Maxmen A. Are new Omicron subvariants a threat? Here’s how scientists are keeping watch. News. *Nature*. 2022-04-15 2022;604(7907):605-606. doi:doi:10.1038/d41586-022-01069-4

17. Sguazzin A. South Africa Had Fifth Covid Wave Despite 97% Antibody Protection. 2022. <https://www.bloomberg.com/news/articles/2022-05-30/s-africa-had-fifth-covid-wave-despite-97-antibody-protection>

18. Iba T, Levy JH, Levi M, et al. Coagulopathy in COVID-19. *J Thromb Haemost*. Sep 2020;18(9):2103-2109. doi:10.1111/jth.14975

19. Gómez-Mesa JE, Galindo-Coral S, Montes MC, et al. Thrombosis and Coagulopathy in COVID-19. *Curr Probl Cardiol*. Mar 2021;46(3):100742. doi:10.1016/j.cpcardiol.2020.100742

20. Hadid T, Kafri Z, Al-Katib A. Coagulation and anticoagulation in COVID-19. *Blood Rev*. May 2021;47:100761. doi:10.1016/j.blre.2020.100761

21. Nägele MP, Haubner B, Tanner FC, et al. Endothelial dysfunction in COVID-19: Current findings and therapeutic implications. *Atherosclerosis*. Dec 2020;314:58-62. doi:10.1016/j.atherosclerosis.2020.10.014

22. Choudhary S, Sharma K, Singh PK. Von Willebrand factor: A key glycoprotein involved in thrombo-inflammatory complications of COVID-19. *Chem Biol Interact*. Sep 10 2021;348:109657. doi:10.1016/j.cbi.2021.109657

23. Cremer S, Jakob C, Berkowitsch A, et al. Elevated markers of thrombo-inflammatory activation predict outcome in patients with cardiovascular comorbidities and COVID-19 disease: insights from the LEOSS registry. *Clin Res Cardiol*. Jul 2021;110(7):1029-1040. doi:10.1007/s00392-020-01769-9

24. Gerotziafas GT, Catalano M, Colgan MP, et al. Guidance for the Management of Patients with Vascular Disease or Cardiovascular Risk Factors and COVID-19: Position Paper from VAS-European Independent Foundation in Angiology/Vascular Medicine. *Thromb Haemost*. Dec 2020;120(12):1597-1628. doi:10.1055/s-0040-1715798

25. Giannis D, Ziogas IA, Gianni P. Coagulation disorders in coronavirus infected patients: COVID-19, SARS-CoV-1, MERS-CoV and lessons from the past. *J Clin Virol*. Jun 2020;127:104362. doi:10.1016/j.jcv.2020.104362

26. Grobler C, Maphumulo SC, Grobbelaar LM, et al. Covid-19: The Rollercoaster of Fibrin(Ogen), D-Dimer, Von Willebrand Factor, P-Selectin and Their Interactions with Endothelial Cells, Platelets and Erythrocytes. *Int J Mol Sci*. Jul 21 2020;21(14)doi:10.3390/ijms21145168

27. Kell DB, Laubscher GJ, Pretorius E. A central role for amyloid fibrin microclots in long COVID/PASC: origins and therapeutic implications. *Biochemical Journal*. 2022;479(4):537-559. doi:10.1042/bcj20220016

28. Kollias A, Kyriakoulis KG, Dimakakos E, et al. Thromboembolic risk and anticoagulant therapy in COVID-19 patients: emerging evidence and call for action. *Br J Haematol*. Jun 2020;189(5):846-847. doi:10.1111/bjh.16727

29. Laubscher GJ, Lourens PJ, Venter C, et al. TEG®, Microclot and Platelet Mapping for Guiding Early Management of Severe COVID-19 Coagulopathy. *Journal of Clinical Medicine*. 2021;10(22):5381. doi:10.3390/jcm10225381

30. Miesbach W, Makris M. COVID-19: Coagulopathy, Risk of Thrombosis, and the Rationale for Anticoagulation. *Clin Appl Thromb Hemost*. Jan-Dec 2020;26:1076029620938149. doi:10.1177/1076029620938149

31. Moriarty PM, Gorby LK, Stroes ES, et al. Lipoprotein(a) and Its Potential Association with Thrombosis and Inflammation in COVID-19: a Testable Hypothesis. *Curr Atheroscler Rep*. Jul 25 2020;22(9):48. doi:10.1007/s11883-020-00867-3

32. Pretorius E, Vlok M, Venter C, et al. Persistent clotting protein pathology in Long COVID/Post-Acute Sequelae of COVID-19 (PASC) is accompanied by increased levels of antiplasmin. *Cardiovascular Diabetology*. 2021;20(1)doi:10.1186/s12933-021-01359-7

33. Smadja DM, Mentzer SJ, Fontenay M, et al. COVID-19 is a systemic vascular hemopathy: insight for mechanistic and clinical aspects. *Angiogenesis*. Jun 28 2021:1-34. doi:10.1007/s10456-021-09805-6

34. Smolarz A, McCarthy P, Shmookler A, et al. Utilization of Thromboelastogram and Inflammatory Markers in the Management of Hypercoagulable State in Patients with COVID-19 Requiring ECMO Support. *Case Rep Crit Care*. 2021;2021:8824531. doi:10.1155/2021/8824531

35. Townsend L, Fogarty H, Dyer A, et al. Prolonged elevation of D-dimer levels in convalescent COVID-19 patients is independent of the acute phase response. *J Thromb Haemost*. Apr 2021;19(4):1064-1070. doi:10.1111/jth.15267

36. Pretorius E, Venter C, Laubscher GJ, et al. Prevalence of readily detected amyloid blood clots in ‘unclotted’ Type 2 Diabetes Mellitus and COVID-19 plasma: a preliminary report. *Cardiovascular Diabetology*. 2020;19(1)doi:10.1186/s12933-020-01165-7

37. Hong Q, Han W, Li J, et al. Molecular basis of receptor binding and antibody neutralization of Omicron. *Nature*. 2022;604(7906):546-552. doi:10.1038/s41586-022-04581-9

38. McCallum M, Czudnochowski N, Rosen LE, et al. Structural basis of SARS-CoV-2 Omicron immune evasion and receptor engagement. *Science*. 2022;375(6583):864-868. doi:doi:10.1126/science.abn8652

39. Wadman M. New Omicron begins to take over, despite late start. *Science*. Feb 4 2022;375(6580):480-481. doi:10.1126/science.ada0852

40. Lacobucci G. Covid-19: Runny nose, headache, and fatigue are commonest symptoms of omicron, early data show. *Bmj*. Dec 16 2021;375:n3103. doi:10.1136/bmj.n3103

41. Malhotra S, Mani K, Lodha R, et al. COVID-19 infection, and reinfection, and vaccine effectiveness against symptomatic infection among health care workers in the setting of omicron variant transmission in New Delhi, India. *The Lancet Regional Health - Southeast Asia*. 2022:100023. doi:10.1016/j.lansea.2022.100023

42. Collie S, Champion J, Moultrie H, et al. Effectiveness of BNT162b2 Vaccine against Omicron Variant in South Africa. *New England Journal of Medicine*. 2022;386(5):494-496. doi:10.1056/nejmc2119270

43. Tseng HF, Ackerson BK, Luo Y, et al. Effectiveness of mRNA-1273 against SARS-CoV-2 Omicron and Delta variants. *Nature Medicine*. 2022;28(5):1063-1071. doi:10.1038/s41591-022-01753-y

44. Accorsi EK, Britton A, Fleming-Dutra KE, et al. Association Between 3 Doses of mRNA COVID-19 Vaccine and Symptomatic Infection Caused by the SARS-CoV-2 Omicron and Delta Variants. *JAMA*. 2022;327(7):639. doi:10.1001/jama.2022.0470

45. Hartmann J, Ergang A, Mason D, et al. The Role of TEG Analysis in Patients with COVID-19-Associated Coagulopathy: A Systematic Review. *Diagnostics*. 2021;11(2):172. doi:10.3390/diagnostics11020172

46. Pretorius E, Page MJ, Hendricks L, et al. Both lipopolysaccharide and lipoteichoic acids potently induce anomalous fibrin amyloid formation: assessment with novel Amytracker™ stains. *J R Soc Interface*. Feb 2018;15(139)doi:10.1098/rsif.2017.0941

47. Pretorius E, Page MJ, Engelbrecht L, et al. Substantial fibrin amyloidogenesis in type 2 diabetes assessed using amyloid-selective fluorescent stains. *Cardiovascular Diabetology*. 2017;16(1)doi:10.1186/s12933-017-0624-5

48. Pretorius E, Mbotwe S, Bester J, et al. Acute induction of anomalous and amyloidogenic blood clotting by molecular amplification of highly substoichiometric levels of bacterial lipopolysaccharide. *Journal of The Royal Society Interface*. 2016;13(122):20160539. doi:10.1098/rsif.2016.0539

49. Pretorius E, Bester J, Vermeulen N, et al. Poorly controlled type 2 diabetes is accompanied by significant morphological and ultrastructural changes in both erythrocytes and in thrombin-generated fibrin: implications for diagnostics. *Cardiovasc Diabetol*. Mar 8 2015;14:30. doi:10.1186/s12933-015-0192-5

50. Nunes JM, Fillis T, Page MJ, et al. Gingipain R1 and Lipopolysaccharide From Porphyromonas gingivalis Have Major Effects on Blood Clot Morphology and Mechanics. *Front Immunol*. 2020;11:1551. doi:10.3389/fimmu.2020.01551

51. De Villiers S, Swanepoel A, Bester J, et al. Novel Diagnostic and Monitoring Tools in Stroke: an Individualized Patient-Centered Precision Medicine Approach. *Journal of Atherosclerosis and Thrombosis*. 2016;23(5):493-504. doi:10.5551/jat.32748

52. Bester J, Soma P, Kell DB, et al. Viscoelastic and ultrastructural characteristics of whole blood and plasma in Alzheimer-type dementia, and the possible role of bacterial lipopolysaccharides (LPS). *Oncotarget*. Nov 3 2015;6(34):35284-303. doi:10.18632/oncotarget.6074

53. Adams B, Nunes JM, Page MJ, et al. Parkinson's Disease: A Systemic Inflammatory Disease Accompanied by Bacterial Inflammagens. *Front Aging Neurosci*. 2019;11:210. doi:10.3389/fnagi.2019.00210

54. Freed NS, Olin. SARS-CoV2 genome sequencing protocol (1200bp amplicon "midnight" primer set, using Nanopore Rapid kit) V.6.

55. Marshall JC, Murthy S, Diaz J, et al. A minimal common outcome measure set for COVID-19 clinical research. *The Lancet Infectious Diseases*. 2020;20(8):e192-e197. doi:10.1016/s1473-3099(20)30483-7

56. Pretorius E, Swanepoel AC, DeVilliers S, et al. Blood clot parameters: Thromboelastography and scanning electron microscopy in research and clinical practice. *Thromb Res*. Jun 2017;154:59-63. doi:10.1016/j.thromres.2017.04.005

57. Page MJ, Thomson GJA, Nunes JM, et al. Serum amyloid A binds to fibrin(ogen), promoting fibrin amyloid formation. *Scientific Reports*. 2019;9(1)doi:10.1038/s41598-019-39056-x

58. Naiki H, Higuchi K, Hosokawa M, et al. Fluorometric determination of amyloid fibrils in vitro using the fluorescent dye, thioflavin T1. *Anal Biochem*. Mar 1989;177(2):244-9. doi:10.1016/0003-2697(89)90046-8

59. Xue C, Lin TY, Chang D, et al. Thioflavin T as an amyloid dye: fibril quantification, optimal concentration and effect on aggregation. *Royal Society Open Science*. 2017;4(1):160696. doi:10.1098/rsos.160696

60. Voropai ES, Samtsov MP, Kaplevskii KN, et al. Spectral Properties of Thioflavin T and Its Complexes with Amyloid Fibrils. *Journal of Applied Spectroscopy*. 2003;70(6):868-874. doi:10.1023/b:japs.0000016303.37573.7e

61. Grobbelaar LM, Venter C, Vlok M, et al. SARS-CoV-2 spike protein S1 induces fibrin(ogen) resistant to fibrinolysis: implications for microclot formation in COVID-19. *Bioscience Reports*. 2021;41(8)doi:10.1042/bsr20210611

62. Cantón R, De Lucas Ramos P, García-Botella A, et al. New variants of SARS-CoV-2. *Revista Española de Quimioterapia*. 2021;34(5):419-428. doi:10.37201/req/071.2021

63. Lin L, Liu Y, Tang X, et al. The Disease Severity and Clinical Outcomes of the SARS-CoV-2 Variants of Concern. Systematic Review. *Frontiers in Public Health*. 2021-November-30 2021;9doi:10.3389/fpubh.2021.775224

64. Nyberg T, Ferguson NM, Nash SG, et al. Comparative analysis of the risks of hospitalisation and death associated with SARS-CoV-2 omicron (B.1.1.529) and delta (B.1.617.2) variants in England: a cohort study. *The Lancet*. 2022;399(10332):1303-1312. doi:10.1016/s0140-6736(22)00462-7

65. Ulloa AC, Buchan SA, Daneman N, et al. Estimates of SARS-CoV-2 Omicron Variant Severity in Ontario, Canada. *JAMA*. 2022;327(13):1286. doi:10.1001/jama.2022.2274

66. Meizoso JP, Moore HB, Moore EE. Fibrinolysis Shutdown in COVID-19: Clinical Manifestations, Molecular Mechanisms, and Therapeutic Implications. *J Am Coll Surg*. Jun 2021;232(6):995-1003. doi:10.1016/j.jamcollsurg.2021.02.019

67. Chu K, Alharahsheh B, Garg N, et al. Evaluating risk stratification scoring systems to predict mortality in patients with COVID-19. *BMJ Health &amp; Care Informatics*. 2021;28(1):e100389. doi:10.1136/bmjhci-2021-100389

68. Yang Z, Hu Q, Huang F, et al. The prognostic value of the SOFA score in patients with COVID-19: A retrospective, observational study. *Medicine (Baltimore)*. Aug 13 2021;100(32):e26900. doi:10.1097/md.0000000000026900

69. Obermeyer F, Jankowiak M, Barkas N, et al. Analysis of 6.4 million SARS-CoV-2 genomes identifies mutations associated with fitness. Cold Spring Harbor Laboratory; 2021.

70. Gowda N, Dominah G, Rogers H, et al. EVALUATING APACHE AND SOFA SCORING SYSTEMS IN PATIENTS WITH COVID-19. *Chest*. 2021;160(4):A1077-A1077. doi:10.1016/j.chest.2021.07.997

71. Sehgal T, Aggarwal M, Baitha U, et al. Thromboelastography determined dynamics of blood coagulation and its correlation with complications and outcomes in patients with coronavirus disease 2019. *Research and Practice in Thrombosis and Haemostasis*. 2022;6(1)doi:10.1002/rth2.12645

72. Yuriditsky E, Horowitz JM, Merchan C, et al. Thromboelastography Profiles of Critically Ill Patients With Coronavirus Disease 2019. *Critical Care Medicine*. 2020;48(9):1319-1326. doi:10.1097/ccm.0000000000004471

73. Wolter N, Jassat W, Walaza S, et al. Early assessment of the clinical severity of the SARS-CoV-2 omicron variant in South Africa: a data linkage study. *The Lancet*. 2022;399(10323):437-446. doi:10.1016/s0140-6736(22)00017-4

74. Whyte CS, Morrow GB, Mitchell JL, et al. Fibrinolytic abnormalities in acute respiratory distress syndrome (ARDS) and versatility of thrombolytic drugs to treat COVID‐19. *Journal of Thrombosis and Haemostasis*. 2020;18(7):1548-1555. doi:10.1111/jth.14872

75. Bois MC, Boire NA, Layman AJ, et al. COVID-19–Associated Nonocclusive Fibrin Microthrombi in the Heart. *Circulation*. 2021;143(3):230-243. doi:10.1161/circulationaha.120.050754

76. Wygrecka M, Birnhuber A, Seeliger B, et al. Altered fibrin clot structure and dysregulated fibrinolysis contribute to thrombosis risk in severe COVID-19. *Blood Advances*. 2022;6(3):1074-1087. doi:10.1182/bloodadvances.2021004816

77. Zhang X, Yang X, Jiao H, et al. Coagulopathy in patients with COVID-19: a systematic review and meta-analysis. *Aging*. 2020;12(24):24535-24551. doi:10.18632/aging.104138

78. Zou Y, Guo H, Zhang Y, et al. Analysis of coagulation parameters in patients with COVID-19 in Shanghai, China. *Biosci Trends*. Sep 21 2020;14(4):285-289. doi:10.5582/bst.2020.03086

79. Mitra S, Ling RR, Yang IX, et al. Severe COVID-19 and coagulopathy: A systematic review and meta-analysis. *Annals of the Academy of Medicine, Singapore*. 2021;50(4):325-335. doi:10.47102/annals-acadmedsg.2020420

80. Sui J, Noubouossie DF, Gandotra S, et al. Elevated Plasma Fibrinogen Is Associated With Excessive Inflammation and Disease Severity in COVID-19 Patients. *Front Cell Infect Microbiol*. 2021;11:734005. doi:10.3389/fcimb.2021.734005

81. 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. *Thrombosis and Haemostasis*. 2020;120(10):1474-1477. doi:10.1055/s-0040-1714350

82. Bunch CM, Moore EE, Moore HB, et al. Immuno-Thrombotic Complications of COVID-19: Implications for Timing of Surgery and Anticoagulation. *Front Surg*. 2022;9:889999. doi:10.3389/fsurg.2022.889999

83. Volod O, Bunch CM, Zackariya N, et al. Viscoelastic Hemostatic Assays: A Primer on Legacy and New Generation Devices. *Journal of Clinical Medicine*. 2022;11(3):860. doi:10.3390/jcm11030860

84. Moore EE, Moore HB, Kornblith LZ, et al. Trauma-induced coagulopathy. *Nature Reviews Disease Primers*. 2021;7(1)doi:10.1038/s41572-021-00264-3

85. Barrett TJ, Bilaloglu S, Cornwell M, et al. Platelets contribute to disease severity in COVID‐19. *Journal of Thrombosis and Haemostasis*. 2021;19(12):3139-3153. doi:10.1111/jth.15534

86. 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):1330-1341. doi:10.1182/blood.2020007252

87. Althaus K, Marini I, Zlamal J, et al. Antibody-induced procoagulant platelets in severe COVID-19 infection. *Blood*. 2021;137(8):1061-1071. doi:10.1182/blood.2020008762

88. Manne BK, Denorme F, Middleton EA, et al. Platelet gene expression and function in patients with COVID-19. *Blood*. 2020;136(11):1317-1329. doi:10.1182/blood.2020007214

89. Abou-Ismail MY, Diamond A, Kapoor S, et al. The hypercoagulable state in COVID-19: Incidence, pathophysiology, and management. *Thrombosis Research*. 2020;194:101-115. doi:10.1016/j.thromres.2020.06.029

90. Zhang S, Liu Y, Wang X, et al. SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19. *Journal of Hematology &amp; Oncology*. 2020;13(1)doi:10.1186/s13045-020-00954-7

91. Len P, Iskakova G, Sautbayeva Z, et al. Meta-Analysis and Systematic Review of Coagulation Disbalances in COVID-19: 41 Studies and 17,601 Patients. Original Research. *Frontiers in Cardiovascular Medicine*. 2022-March-11 2022;9doi:10.3389/fcvm.2022.794092

92. Bashash D, Hosseini-Baharanchi FS, Rezaie-Tavirani M, et al. The Prognostic Value of Thrombocytopenia in COVID-19 Patients; a Systematic Review and Meta-Analysis. *Arch Acad Emerg Med*. 2020;8(1):e75.

93. Lippi G, Plebani M, Henry BM. Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: A meta-analysis. *Clin Chim Acta*. Jul 2020;506:145-148. doi:10.1016/j.cca.2020.03.022

94. Xu P, Zhou Q, Xu J. Mechanism of thrombocytopenia in COVID-19 patients. *Annals of Hematology*. 2020;99(6):1205-1208. doi:10.1007/s00277-020-04019-0

95. Delshad M, Safaroghli-Azar A, Pourbagheri-Sigaroodi A, et al. Platelets in the perspective of COVID-19; pathophysiology of thrombocytopenia and its implication as prognostic and therapeutic opportunity. *Int Immunopharmacol*. Oct 2021;99:107995. doi:10.1016/j.intimp.2021.107995

96. Tyagi T, Jain K, Gu SX, et al. A guide to molecular and functional investigations of platelets to bridge basic and clinical sciences. *Nature Cardiovascular Research*. 2022;1(3):223-237. doi:10.1038/s44161-022-00021-z

**Table 1:** WHO list of SARS-CoV-2 Variants of Concern and Variants of Interest (Modified from: 9).

|  |  |  |  |
| --- | --- | --- | --- |
| **WHO label** | **PANGO linage** | **Earliest documented****samples** | **Date of designation** |
| **Variants of Concern (VOC)** |
| Omicron | B.1.1.529 | Multiple countries, Nov-2021 (SA 4th wave) | 26-Nov-2021 |
| **Previously circulating VOC** |
| Alpha | B.1.1.7 | United Kingdom, Sep-2020 (SA 1st wave) | 18-Dec-2020 |
| Beta | B.1.351 | South Africa, May-2020 (SA 2nd wave) | 18-Dec-2020 |
| Gamma | P.1 | Brazil, Nov-2020 | 11-Jan-2021 |
| Delta | B.1.617.2 | India, Oct-2020 (SA 3rd wave) | 11-May-2021 |
| **Variants of Interest (VOI)** |
| Epsilon | B.1.427/B.1.429 | United States of America,Mar-2020 | 5-Mar-2021 |
| Zeta | P.2 | Brazil, Apr-2020 | 17-Mar-2021 |
| Eta | B.1.525 | Multiple countries, Dec-2020 | 17-Mar-2021 |
| Theta | P.3 | Philippines, Jan-2021 | 24-Mar-2021 |
| Iota | B.1.526 | United States of America,  Nov-2020 | 24-Mar-2021 |
| Kappa | B.1.617.1 | India, Oct-2020 | 4-Apr-2021 |
| Lambda | C.37 | Peru, Aug-2020 | 14-Jun-2021 |
| Mu | B.1.621 | Colombia, Jan-2021 | 30-Aug-2021 |

**Table 2.** TEG® parameters for whole blood29,56.

|  |
| --- |
| **Thromboelastography**® |
| **TEG**® **parameters**  | **Explanation** |
| **Reaction time (R)** | Time of latency from start of test to initial fibrin formation (amplitude of 2mm); i.e., initiation time.  |
| **Kinetics (K)** | Time taken to achieve a certain level of clot strength (amplitude of 20mm); i.e., amplification.  |
| **Alpha angle (A)** | The angle measures the speed at which fibrin build up and cross linking takes place, hence assesses the rate of clot formation, i.e., thrombin burst.  |
| **Maximum amplitude (MA)** | Reflects the ultimate strength of the contracted platelet-fibrin clot.  |

**Table 3.** Participant demographics and COVID-19 positive WHO clinical progression scores. Statistical significance was established at p<0.05 (\* = p<0, 05; \*\* = p<0,01; \*\*\* = p<0,001; \*\*\*\* = p<0,0001). Parametric data are expressed as mean ± standard deviation, and nonparametric data are expressed as median and [Q1 – Q3 interquartile range].

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Demographics** | **Healthy Participants (n = 10)** | **Omicron (n = 10)** | **β/Δ (n = 10)** | **P-value** |
| Age | 49.5 [29.8 – 53.0] | 32.5 [27.0 – 41.0] | 57.0 [52.5 – 62.5] | **0.003 (\*\*)** |
|  | **Frequency** | **Percentage** | **Frequency** | **Percentage** | **Frequency** | **Percentage** |  |
| **Gender**FemaleMale | 64 | 6040 | 73 | 7030 | 73 | 7030 |  |
| **Comorbidities** |  |  |  |  |  |  |  |
| Obesity |  |  |  |  | 3 | 30 |
| Dyslipidaemia |  |  |  |  | 4 | 40 |
| Hypertension |  |  | 1 | 10 | 4 | 40 |
| Type II Diabetes Miletus |  |  |  |  | 3 | 30 |
| Ischemic Heart disease |  |  |  |  | 1 | 10 |
| Familial Hypercholesterolaemia |  |  | 1 | 10 |  |  |
| Anxiety/Depression |  |  | 3 | 30 |  |  |
| Hypothyroidism |  |  | 1 | 10 |  |  |
| Previous cancer diagnosis |  |  | 1 | 10 | 1 | 10 |  |
| WHO Clinical Progression Score |  | 2 (± 0) | 4.9 (± 2.56) | **0.002 (\*\*)** |

**Table 4.** Results of four viscoelastic TEG® parameters assessing coagulability of WB samples from healthy individuals, β/Δ COVID-19, and individuals diagnosed with Omicron COVID-19, respectively. Statistical significance was established at p<0.05 (\* = p<0, 05; \*\* = p<0,01; \*\*\* = p<0,001; \*\*\*\* = p<0,0001). Parametric data are represented as the mean ± standard deviation and nonparametric data as the median [Q1 – Q3 interquartile range].

|  |
| --- |
| **Healthy samples vs β/Δ samples** |
| Parameter | **Control (n =10)** | **β/Δ (n=10)** | **P-value**  |
| R (min) | 10.5 (± 4.08) | 5.7 (±1.8) | **0.003 (\*\*)** |
| K (min) | 3.38 (± 2.31) | 1.63 (±0.48) | **0.03 (\*)** |
| A (°) | 55.72 (± 13.26) | 67.56 (± 5.88) | **0.02(\*)** |
| MA (mm) | 52.3 [42.45 – 58.75] | 67.75 [61.68 – 73.93] | **0.007(\*\*)** |
| **Healthy samples vs Omicron samples** |
| Parameter | **Control (n=10)** | **Omicron (n=10)** | **P value** |
| R (min) | 8.65 [6.78 – 14.4] | 4.6 [4.1 – 5.2] | **<0.0001(\*\*\*\*)** |
| K (min) | 3.15 [1.65 – 4.23] | 1.4 [1.28 – 1.85] | **0.04 (\*)** |
| A (°) | 54.55 [43.95– 66.43] | 69.85 [65 – 71.43] | **0.01 (\*)** |
| MA (mm) | 52.12 (± 11.4) | 60.77 (± 3.96) | **0.04 (\*)** |
| **β/Δ samples vs Omicron samples** |
| Parameter | **β/Δ (n=10)** | **Omicron (n=10)** | **P value** |
| R (min) | 6.1 [4.63 – 7.1] | 4.6 [4.1 – 5.2] | 0.11 |
| K (min) | 1.55 [1.2 – 1.95] | 1.4 [1.23 – 1.85] | 0.87 |
| A (°) | 68.45 [62.05 – 72.9] | 69.85 [65 – 71.43] | 0.81 |
| MA (mm) | 67.75 [61.68 – 73.93] | 61.45 [57.83 – 63.9] | **0.05 (\*)** |

**Table 5** Percentage average amyloid area in platelet poor plasma (PPP) of healthy individuals versus participants with β/Δ COVID-19 and those participants with Omicron COVID-19 using different image thresholding algorithms (Fiji® and Python® script). Statistical significance was established at p<0.05 (\* = p<0, 05; \*\* = p<0,01; \*\*\* = p<0,001; \*\*\*\* = p<0,0001). Parametric data are represented as the mean ± standard deviation and nonparametric data as the median [Q1 – Q3 interquartile range].

|  |
| --- |
| **Healthy samples (n=10) vs β/Δ samples (n=10)** |
| **Representative values** | **Fiji® script** | **Python® script** |
| P value  | **<0.0001 (\*\*\*\*)** | **<0.0001 (\*\*\*\*)** |
| Median of healthy samples | 0.29% [0.19% - 0.4%] | 0.25% [0.18% – 0.43%] |
| Median of β/Δ samples | 3.85% [1.09% - 6.08%] | 3.15% [1.08% - 5%] |
| **Healthy samples (n=10) vs Omicron samples (n=10)** |
| P value | **0.005 (\*\*)** | **0.009 (\*\*)** |
| Mean of healthy samples  | 0.32% (± 0.16%) |  |
| Mean of Omicron samples | 1.02% (±0.67%) |  |
| Median of healthy samples |  | 0.25% [0.18% – 0.43%] |
| Mean of Omicron samples |  | 0.6% [0.38% – 1.4%] |
| **β/Δ samples (n=10) vs Omicron samples (n=10)** |
| P value | **0.007 (\*\*)** | **0.002 (\*\*)** |
| Median of β/Δ samples | 3.85% [1.09% - 6.08%] | 3.15% [1.08% - 5%] |
| Median of Omicron samples | 0.93% [0.38% - 1.68%]  | 0.6% [0.38% - 1.4%] |

**Figure 1.** **A)** Cartoon to explain the preparation and detection of fibrin microclots in platelet poor plasma (PPP) **B)** Representative micrographs of microclots in PPP, after addition of the amyloid protein stain, Thioflavin T (ThT).

