



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

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Abstract

Earlier variants of SARS-CoV-2 have been associated with hypercoagulability 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 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 with those harboring 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 may play an important role in reflecting the severity of symptoms observed in COVID-19.

Keywords

- ▶ COVID-19
- ▶ variants
- ▶ Omicron
- ▶ coagulation
- ▶ fluorescence microscopy
- ▶ microclots

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 June 23, 2022) worldwide.¹ Adaptive mutations in the SARS-CoV-2 viral genome can alter its pathogenic potential by affecting 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, which was determined to have 10 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 sublineages: 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 sublineages 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 sublineages to determine the potential these sublineages 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 available only 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 fibrin (“fibrinoid”) with higher than normal resistance to fibrinolysis, and a size range from 1 to 200 μm when measured on the longest axis.²⁷ Our group has 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 sublineage of the omicron variant.

Materials and Methods**Blood Sample Preparation**

Citrated blood samples were collected and centrifuged at 3,000 × g for 15 minutes where after the platelet-poor

Table 1 WHO list of SARS-CoV-2 variants of concern and variants of interest

WHO label	PANGO lineage	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

Source: Modified from WHO.⁹

plasma (PPP) was stored at -80°C , until analyzed later, with the hematocrit being analyzed on the day the sample was received.

Participant Demographics

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 includes individuals with the beta and delta COVID-19 variant and will be denoted in this article as β/Δ . Six of the patients were hospitalized.

Participants with COVID-19: Omicron Infection (January 2022 Onward)

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 mid-night protocol on the GridION device (Oxford Nanopore Technologies, Oxford, United Kingdom).⁵⁴ Of the 10 samples, 6 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 COVID-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) to 10 (dead). Scores 1 to 3 represent ambulatory mild disease ranging from asymptomatic to symptomatic needing assistance. Scores 4 to 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 to 9 categorize hospitalized patients with severe disease: oxygen by high-flow or noninvasive ventilation (score 6), intubation and mechanical ventilation with partial pressure oxygen (pO_2)/fraction of inspired oxygen (FiO_2) ≥ 150 or oxygen saturation (SpO_2)/ $\text{FiO}_2 \geq 200$ (score 7), intubation and mechanical ventilation with pO_2/FiO_2 mm Hg < 150 or $\text{SpO}_2/\text{FiO}_2$ mm Hg < 200 (score 8), or extracorporeal membrane oxygenation (ECMO; score 9).⁵⁵

Thromboelastography of Whole Blood

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 naive 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 20 μL 0.2 M calcium chloride (CaCl_2) (7003; Haemonetics, Niles, IL) to a

Table 2 TEG parameters for whole blood^{29,56}

Thromboelastography	
TEG parameters	Explanation
Reaction time (R)	Time of latency from start of test to initial fibrin formation (amplitude of 2 mm); i.e., initiation time
Kinetics (K)	Time taken to achieve a certain level of clot strength (amplitude of 20 mm); 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	Reflects the ultimate strength of the contracted platelet-fibrin clot

disposable TEG cup (HAEM 6211; Haemonetics, Niles, IL), followed by the addition of 340 μ L-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 the TEG 5000 Hemostasis Analyzer System (07–033; Haemonetics, Boston, MA) and allowed to run until the maximum amplitude (MA) was reached. All analyses were performed at 37 °C.

Quantitative Assessment of Anomalous Fibrin Amyloid Clotting by Fluorescent Microscopy of Platelet-Poor Plasma

To detect microclot presence in PPP, thioflavin T (ThT) 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} ThT is a fluorescent probe frequently used to detect amyloid fibrils at roughly 482 nm with an excitation of 450 nm.^{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 482 nm) 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), at a final exposure concentration of 5 μ M and for a period of 30 minutes. Following incubation, 3 μ L 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 63 \times /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 450 to 488 nm and the emission at 499 to 529 nm.

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. An ImageJ (Java 1.8.0_172) and a Python (Python 3.9.5) script were the quantitative methods used. The ImageJ methodology was adapted from as described by Grobbelaar et al.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 ImageJ script was set to analyze particles of a size of 0.5 to 200,000 μ m (to represent infinity). A minimum of 0.5 μ m was set to include smaller

particles but also account for background fluorescence. The Python script converts the images to gray scale 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 gray scale image, 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, labeled, and characterized using the skimage measure library (<https://scikit-image.org/>). The now labeled clot areas are used to calculate the total fluorescent area. The average fluorescent area measurement calculated by both the ImageJ and Python scripts is 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 \pm 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

Table 3 Participant demographics and COVID-19-positive WHO clinical progression scores

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							
Female	6	60	7	70	7	70	
Male	4	40	3	30	3	30	
Comorbidities							
Obesity					3	30	
Dyslipidemia					4	40	
Hypertension			1	10	4	40	
Type II diabetes mellitus					3	30	
Ischemic heart disease					1	10	
Familial Hypercholesterolemia			1	10			
Hypothyroidism			1	10			
Previous cancer diagnosis			1	10	1	10	
WHO Clinical Progression Score			2 (±0)		4.9 (±2.56)		0.002

Note: Statistical significance was established at $p < 0.05$.

Note: Parametric data are expressed as mean ± standard deviation, and nonparametric data are expressed as median [Q1–Q3 interquartile range].

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 (pO_2/FiO_2 mm Hg < 150 or SpO_2/FiO_2 mm Hg < 200) for WHO scores of 8. This prognostic tool indicated that the disease severity of β/Δ patients was significantly higher than that of omicron patients.

Thromboelastography of Whole Blood

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), α-angle (A), and 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 comparison of β/Δ to healthy individuals were lower than the p -values in the comparison of omicron to healthy individuals. The distribution of WB TEG parameters between all groups is shown in ► **Table 4**.

Fluorescence Microscopy to Detect Aberrant Fibrin Amyloids in Platelet-Poor Plasma Stained with Thioflavin T

Plasma from β/Δ COVID-19 samples demonstrated a significantly higher percentage area of amyloid when compared with 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 percent microclot area analysis using ImageJ, 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. ► **Fig. 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 with 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, intensive care unit (ICU) admission, morbidity, and mortality.^{62,63} The beta and delta variants posed a

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

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 ($^{\circ}$)	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 ($^{\circ}$)	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 ($^{\circ}$)	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

Note: Statistical significance was established at $p < 0.05$.

Note: Parametric data are represented as the mean \pm standard deviation and nonparametric data as the median [Q1–Q3 interquartile range].

Table 5 Percentage average amyloid area in platelet-poor plasma (PPP) of healthy individuals versus participants with β/Δ COVID-19 and participants with omicron COVID-19 versus β/Δ COVID-19 participants using different image thresholding algorithms (ImageJ and Python script)

Healthy samples ($n = 10$) vs. β/Δ samples ($n = 10$)		
Representative values	ImageJ 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%]
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%]

Note: Statistical significance was established at $p < 0.05$.

Note: Nonparametric data are represented as the median [Q1–Q3 interquartile range].

greater risk in terms of the aforementioned factors when compared with 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 with 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 who 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 var-

iants, and successive beta and delta variants, caused more hypercoagulability as documented by the incidence of venous thromboembolism, and reflected by hypercoagulopathic parameters of TEGs 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 with our β/Δ population. This study did not calculate

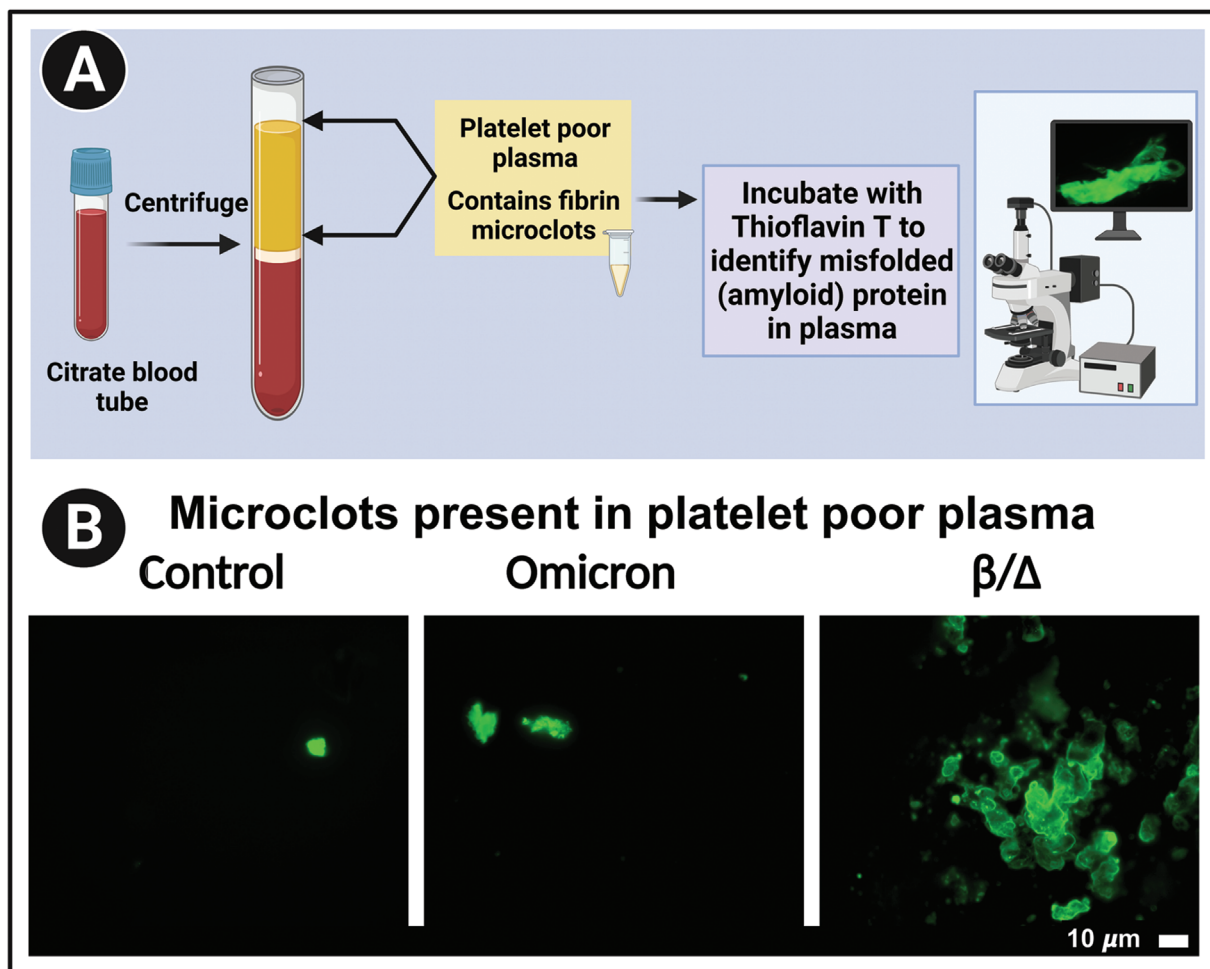


Fig. 1 (A) Cartoon to explain the preparation and detection of fibrin microclots in platelet-poor plasma (PPP). (B) Representative micrographs of microclots in PPP, after addition of the amyloid protein stain, thioflavin T (ThT).

APACHE II and IV, or SOFA scores for the COVID-19-positive population; yet, it is clear that these scores were much lower for the nonhospitalized 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 with healthy individuals versus our β/Δ population when compared with the same set of healthy individuals. A direct comparison between the omicron population and β/Δ population indicated a significantly higher MA in the β/Δ population.

Fibrinolytic abnormalities can occur in COVID-19 with fibrin deposits previously seen in the lungs⁷⁴ and hearts⁷⁵ 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 macrothrombosis 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 COVID-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 preexisting 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 with 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 macrothrombosis 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 is suggested as personalized-based medicine tools, which 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 with 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.

Ethics Statement

Ethical approval for blood collection and microclot analysis of blood samples from participants with COVID-19 and healthy individuals was given by the Health Research Ethics Committee (HREC) of Stellenbosch University (reference N19/03/043, project ID 9521; renewal 2021 and 2022). This laboratory study was performed 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. A positive COVID-19 test was confirmed before blood collection. Genomic sequencing to confirm SARS-CoV-2 is covered under Health Research Ethics Committee (HREC) of Stellenbosch University (reference #N20/04/008_COVID-19) as part of the National Genomics Surveillance Program.

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Conflict of Interest

M.J.K. is a nonexecutive director and shareholder of Gknowmix (Pty) Ltd. E.P. 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

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