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, BScHons[1] Arneaux Kruger, MBChB[1] Chantelle Venter, PhD[1] Este M. Burger, MEng[2] Gert J. Laubscher, MBChB, FCP(SA)[3] Tongai G. Maponga, PhD[4] Maritha J. Kotze, PhD[5] Hau C. Kwaan, MD, PhD[6] Joseph B. Miller, MD[7] Daniel Fulkerson, MD[8] Wei Huff, MD, PhD[8] Eric Chang, BS[9] Grant Wiarda[10] Connor M. Bunch, MD[7] Mark M. Walsh, MD[9,10,11] Syed Raza, MD[12] Mahmud Zamlut, MD[12] Hunter B. Moore, MD, PhD[13] Ernest E. Moore, MD[14] Matthew D. Neal, MD[15] Douglas B. Kell, DPhil, DSc[1,16,17] Etheresia Pretorius, PhD[1,17]

[1] Department of Physiological Sciences, Faculty of Science, Stellenbosch University, Stellenbosch, Matieland, South Africa
[2] BioCODE Technologies, Stellenbosch, South Africa
[3] Mediclinic Stellenbosch, Stellenbosch, South Africa
[4] Division of Medical Virology, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, Cape Town, South Africa
[5] Division of Chemical Pathology, Department of Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University and National Health Laboratory Service, Tygerberg Hospital, Cape Town, South Africa
[6] Division of Hematology and Oncology, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois
[7] Departments of Emergency Medicine and Internal Medicine, Henry Ford Hospital, Detroit, Michigan
[8] Department of Neurosurgery, St. Joseph Regional Medical Center, Mishawaka, Indiana
[9] Indiana University School of Medicine - South Bend, Notre Dame, Indiana
[10] Department of Internal Medicine, Saint Joseph Regional Medical Center, Mishawaka, Indiana
[11] Department of Emergency Medicine, Saint Joseph Regional Medical Center, Mishawaka, Indiana
[12] Department of Critical Care Medicine, Saint Joseph Regional Medical Center, Mishawaka, Indiana
[13] Division of Transplant Surgery, Department of Surgery, Denver Health and University of Colorado Health Sciences Center, Denver, Colorado
[14] Department of Surgery, Ernest E. Moore Shock Trauma Center at Denver Health and University of Colorado Health Sciences Center, Denver, Colorado
[15] Pittsburgh Trauma Research Center, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania
[16] Department of Biochemistry and Systems Biology, Institute of Systems, Molecular and Integrative Biology, Faculty of Health and Life Sciences, University of Liverpool, England, United Kingdom
[17] The Novo Nordisk Foundation Centre for Biosustainability, Technical University of Denmark, Lyngby, Denmark

Address for correspondence Etheresia Pretorius, PhD, Department of Physiological Sciences, Stellenbosch University, Private Bag X1 Matieland, 7602, South Africa (e-mail: resiap@sun.ac.za).

Douglas B. Kell, DPhil, DSc, Department of Biochemistry and Systems Biology, Institute of Systems, Molecular and Integrative Biology, Faculty of Health and Life Sciences, University of Liverpool, L69 7ZB, United Kingdom (e-mail: dbk@liv.ac.uk).

Semin Thromb Hemost 2022;48:858–868.

published online
September 29, 2022

Issue Theme Emerging Use of Viscoelastography in Thrombosis and Hemostasis: A Challenge to Conventional Coagulation Tests?; Guest Editors: Hau C. Kwaan, MD, FRCP, Mark Walsh, MD, FACEP, Paul F. Lindholm, MD, and Maha Othman, MD, MSc, PhD

DOI https://doi.org/10.1055/s-0042-1756306.
ISSN 0094-6176.

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 ("fibrinaloid") 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 $3{,}000 \times g$ 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 midnight 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) $\geq$ 150 or oxygen saturation (SpO_2)/FiO_2 $\geq$ 200 (score 7), intubation and mechanical ventilation with pO_2/FiO_2 mm Hg $<$ 150 or SpO_2/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 µL-citrated WB. $CaCl_2$ 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 µM and for a period of 30 minutes. Following incubation, 3 µL 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 ×/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 µm (to represent infinity). A minimum of 0.5 µm 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 ± 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 (°)	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

Note: Statistical significance was established at $p < 0.05$.
Note: Parametric data are represented as the mean ± 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 variants, 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

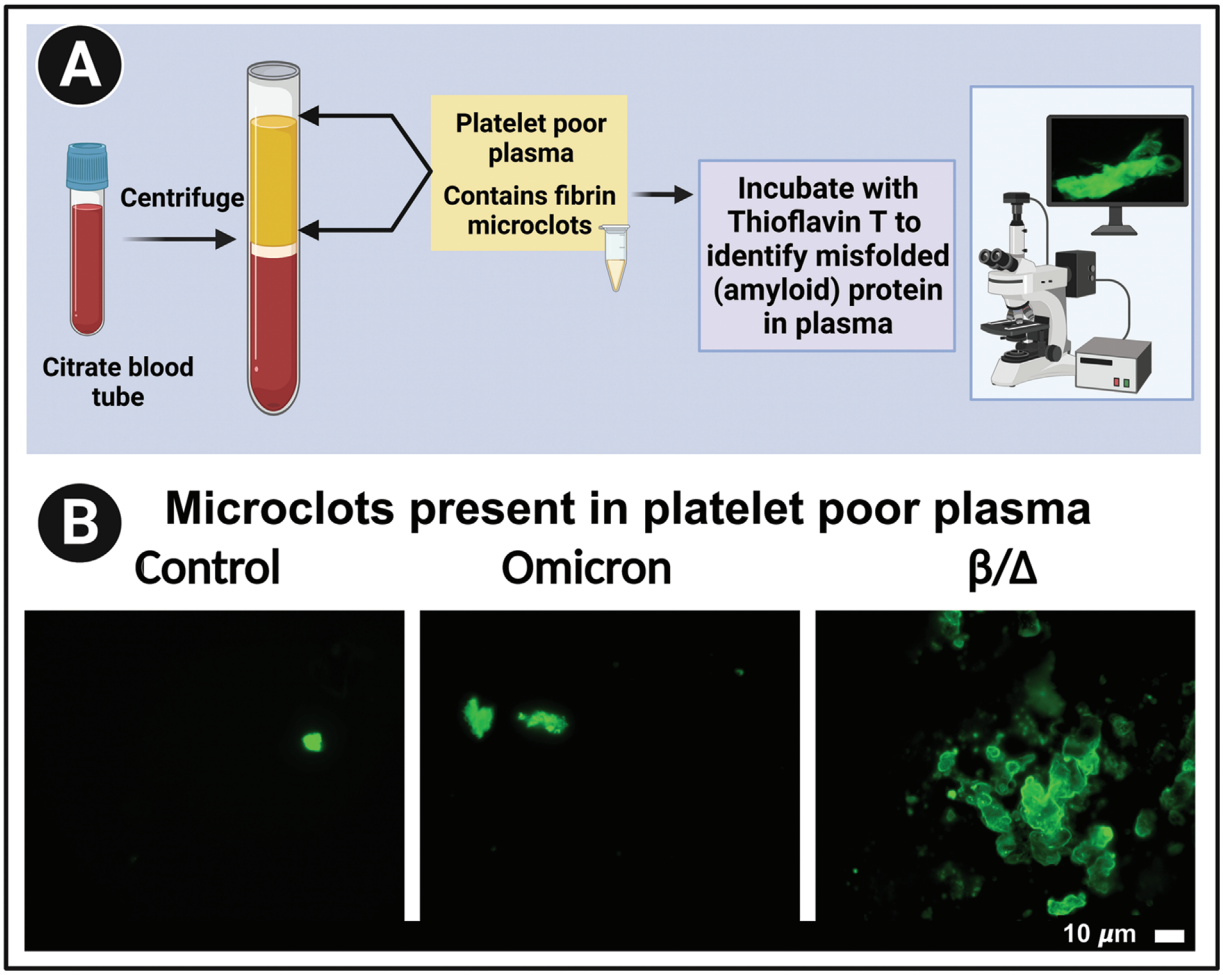

Fig. 1 (**A**) Cartoon to explain the preparation and detection of fibrin microclots in platelet-poor plasma (PPP). (**B**) Representative micrographs of microclots in PPP, after addition of the amyloid protein stain, thioflavin T (ThT).

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 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 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.

Funding

D.B.K.: Novo Nordisk Foundation for support (grant NNF20CC0035580). M.J.K. and T.G.M.: Research reported in this article was supported by the South African Medical Research Council with funds received from the Department of Science and Innovation (Project Code 96825). E.P.: Laboratory research supported by NRF of South Africa (grant number 142142) and SA MRC (self-initiated research (SIR) grant). The content and findings reported and illustrated are the sole deduction, view, and responsibility of the researchers and do not reflect the official position and sentiments of the funders.

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

honorarium from Haemonetics Corporation for speaking engagements, as well as research support from Janssen Pharmaceuticals (Beerse, Belgium) and Noveome Biotherapeutics (Pittsburgh, PA) outside the submitted work. He has served as a consultant to Janssen and CSL Behring (King of Prussia, PA) and serves on the Scientific Advisory Board of Haima Therapeutics (Cleveland, OH). M.M.W. has received honoraria from Alexion Pharmaceuticals (Boston, MA).

References

1 WHO. WHO Coronavirus (COVID-19) Dashboard. Accessed 23 June, 2022 at: 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 LLC; 2022
3 Meo SA, Meo AS, Al-Jassir FF, Klonoff DC. Omicron SARS-CoV-2 new variant: global prevalence and biological and clinical characteristics. Eur Rev Med Pharmacol Sci 2021;25(24):8012–8018
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 2021:ciab721. 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
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
7 Tegally H, Wilkinson E, Lessells RJ, et al. Sixteen novel lineages of SARS-CoV-2 in South Africa. Nat Med 2021;27(03):440–446
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 June 25, 2022 at 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. Lancet 2021;397(10281):1265–1275
11 Jassat W, Mudara C, Ozougwu L, et al; DATCOV Author Group. Difference in mortality among individuals admitted to hospital with COVID-19 during the first and second waves in South Africa: a cohort study. Lancet Glob Health 2021;9(09):e1216–e1225
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(03):526
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. 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. Nature 2022;604(7907):605–606
17 Sguazzin A. South Africa Had Fifth COVID Wave Despite 97% Antibody Protection. 2022. Accessed July 28, 2022 at: 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, Thachil J. Coagulopathy in COVID-19. J Thromb Haemost 2020;18(09):2103–2109
19 Gómez-Mesa JE, Galindo-Coral S, Montes MC, Muñoz Martin AJ. Thrombosis and coagulopathy in COVID-19. Curr Probl Cardiol 2021;46(03):100742
20 Hadid T, Kafri Z, Al-Katib A. Coagulation and anticoagulation in COVID-19. Blood Rev 2021;47:100761
21 Nägele MP, Haubner B, Tanner FC, Ruschitzka F, Flammer AJ. Endothelial dysfunction in COVID-19: current findings and therapeutic implications. Atherosclerosis 2020;314:58–62
22 Choudhary S, Sharma K, Singh PK. Von Willebrand factor: a key glycoprotein involved in thrombo-inflammatory complications of COVID-19. Chem Biol Interact 2021;348:109657
23 Cremer S, Jakob C, Berkowitsch A, et al; LEOSS Study Group. 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 2021;110(07):1029–1040
24 Gerotziafas GT, Catalano M, Colgan MP, et al; Scientific Reviewer Committee. 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 2020;120(12):1597–1628
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 2020;127:104362
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 2020;21(14):E5168
27 Kell DB, Laubscher GJ, Pretorius E. A central role for amyloid fibrin microclots in long COVID/PASC: origins and therapeutic implications. Biochem J 2022;479(04):537–559
28 Kollias A, Kyriakoulis KG, Dimakakos E, Poulakou G, Stergiou GS, Syrigos K. Thromboembolic risk and anticoagulant therapy in COVID-19 patients: emerging evidence and call for action. Br J Haematol 2020;189(05):846–847
29 Laubscher GJ, Lourens PJ, Venter C, Kell DB, Pretorius E. TEG®, Microclot and platelet mapping for guiding early management of severe COVID-19 coagulopathy. J Clin Med 2021;10(22):5381
30 Miesbach W, Makris M. COVID-19: coagulopathy, risk of thrombosis, and the rationale for anticoagulation. Clin Appl Thromb Hemost 2020;26:1076029620938149
31 Moriarty PM, Gorby LK, Stroes ES, Kastelein JP, Davidson M, Tsimikas S. Lipoprotein(a) and its potential association with thrombosis and inflammation in COVID-19: a testable hypothesis. Curr Atheroscler Rep 2020;22(09):48
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. Cardiovasc Diabetol 2021;20(01):172
33 Smadja DM, Mentzer SJ, Fontenay M, et al. COVID-19 is a systemic vascular hemopathy: insight for mechanistic and clinical aspects. Angiogenesis 2021;24(04):755–788
34 Smolarz A, McCarthy P, Shmookler A, Badhwar V, Hayanga AJ, Sakhuja A. 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
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 2021;19(04): 1064–1070
36 Pretorius E, Venter C, Laubscher GJ, Lourens PJ, Steenkamp J, Kell DB. Prevalence of readily detected amyloid blood clots in 'unclotted' type 2 diabetes mellitus and COVID-19 plasma: a preliminary report. Cardiovasc Diabetol 2020;19(01):193
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
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

39 Wadman M. New omicron begins to take over, despite late start. Science 2022;375(6580):480–481
40 Iacobucci G. COVID-19: runny nose, headache, and fatigue are commonest symptoms of omicron, early data show. BMJ 2021; 375(3103):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. Lancet Reg Health Southeast Asia 2022;100023:100023
42 Collie S, Champion J, Moultrie H, Bekker LG, Gray G. Effectiveness of BNT162b2 vaccine against omicron variant in South Africa. N Engl J Med 2022;386(05):494–496
43 Tseng HF, Ackerson BK, Luo Y, et al. Effectiveness of mRNA-1273 against SARS-CoV-2 omicron and delta variants. Nat Med 2022;28 (05):1063–1071
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(07):639–651
45 Hartmann J, Ergang A, Mason D, Dias JD. The role of TEG analysis in patients with COVID-19-associated coagulopathy: a systematic review. Diagnostics (Basel) 2021;11(02):172
46 Pretorius E, Page MJ, Hendricks L, Nkosi NB, Benson SR, Kell DB. Both lipopolysaccharide and lipoteichoic acids potently induce anomalous fibrin amyloid formation: assessment with novel Amytracker™ stains. J R Soc Interface 2018;15(139):20170941
47 Pretorius E, Page MJ, Engelbrecht L, Ellis GC, Kell DB. Substantial fibrin amyloidogenesis in type 2 diabetes assessed using amyloid-selective fluorescent stains. Cardiovasc Diabetol 2017;16(01):141
48 Pretorius E, Mbotwe S, Bester J, Robinson CJ, Kell DB. Acute induction of anomalous and amyloidogenic blood clotting by molecular amplification of highly substoichiometric levels of bacterial lipopolysaccharide. J R Soc Interface 2016;13(122): 20160539
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 2015;14:30
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
51 de Villiers S, Swanepoel A, Bester J, Pretorius E. Novel diagnostic and monitoring tools in stroke: an individualized patient-centered precision medicine approach. J Atheroscler Thromb 2016; 23(05):493–504
52 Bester J, Soma P, Kell DB, Pretorius E. Viscoelastic and ultrastructural characteristics of whole blood and plasma in Alzheimer-type dementia, and the possible role of bacterial lipopolysaccharides (LPS). Oncotarget 2015;6(34):35284–35303
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
54 Freed NSOlin. 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; WHO Working Group on the Clinical Characterisation and Management of COVID-19 Infection. A minimal common outcome measure set for COVID-19 clinical research. Lancet Infect Dis 2020;20(08):e192–e197
56 Pretorius E, Swanepoel AC, DeVilliers S, Bester J. Blood clot parameters: thromboelastography and scanning electron microscopy in research and clinical practice. Thromb Res 2017; 154:59–63
57 Page MJ, Thomson GJA, Nunes JM, et al. Serum amyloid A binds to fibrin(ogen), promoting fibrin amyloid formation. Sci Rep 2019;9 (01):3102
58 Naiki H, Higuchi K, Hosokawa M, Takeda T. Fluorometric determination of amyloid fibrils in vitro using the fluorescent dye, thioflavin T1. Anal Biochem 1989;177(02):244–249
59 Xue C, Lin TY, Chang D, Guo Z. Thioflavin T as an amyloid dye: fibril quantification, optimal concentration and effect on aggregation. R Soc Open Sci 2017;4(01):160696
60 Voropai ES, Samtsov MP, Kaplevskii KN, et al. Spectral properties of thioflavin T and its complexes with amyloid fibrils. J Appl Spectrosc 2003;70(06):868–874
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. Biosci Rep 2021;41(08): BSR20210611
62 Cantón R, De Lucas Ramos P, García-Botella A, et al. New variants of SARS-CoV-2. Rev Esp Quimioter 2021;34(05):419–428
63 Lin L, Liu Y, Tang X, He D. The disease severity and clinical outcomes of the SARS-CoV-2 variants of concern. Front Public Health 2021;9:775224
64 Nyberg T, Ferguson NM, Nash SG, et al; COVID-19 Genomics UK (COG-UK) Consortium. 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. Lancet 2022;399(10332):1303–1312
65 Ulloa AC, Buchan SA, Daneman N, Brown KA. Estimates of SARS-CoV-2 omicron variant severity in Ontario, Canada. JAMA 2022; 327(13):1286–1288
66 Meizoso JP, Moore HB, Moore EE. Fibrinolysis shutdown in COVID-19: clinical manifestations, molecular mechanisms, and therapeutic implications. J Am Coll Surg 2021;232(06):995–1003
67 Chu K, Alharahsheh B, Garg N, Guha P. Evaluating risk stratification scoring systems to predict mortality in patients with COVID-19. BMJ Health Care Inform 2021;28(01):e100389
68 Yang Z, Hu Q, Huang F, Xiong S, Sun Y. The prognostic value of the SOFA score in patients with COVID-19: a retrospective, observational study. Medicine (Baltimore) 2021;100(32):e26900
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 (04):A1077–A1077
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. Res Pract Thromb Haemost 2022;6(01):e12645
72 Yuriditsky E, Horowitz JM, Merchan C, et al. Thromboelastography profiles of critically ill patients with coronavirus disease 2019. Crit Care Med 2020;48(09):1319–1326
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. Lancet 2022;399(10323):437–446
74 Whyte CS, Morrow GB, Mitchell JL, Chowdary P, Mutch NJ. Fibrinolytic abnormalities in acute respiratory distress syndrome (ARDS) and versatility of thrombolytic drugs to treat COVID-19. J Thromb Haemost 2020;18(07):1548–1555
75 Bois MC, Boire NA, Layman AJ, et al. COVID-19-associated nonocclusive fibrin microthrombi in the heart. Circulation 2021;143 (03):230–243
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 Adv 2022;6(03): 1074–1087
77 Zhang X, Yang X, Jiao H, Liu X. Coagulopathy in patients with COVID-19: a systematic review and meta-analysis. Aging (Albany NY) 2020;12(24):24535–24551
78 Zou Y, Guo H, Zhang Y, et al. Analysis of coagulation parameters in patients with COVID-19 in Shanghai, China. Biosci Trends 2020;14 (04):285–289

79 Mitra S, Ling RR, Yang IX, et al. Severe COVID-19 and coagulopathy: a systematic review and meta-analysis. Ann Acad Med Singap 2021;50(04):325–335
80 Sui J, Noubouossie DF, Gandotra S, Cao L. Elevated plasma fibrinogen is associated with excessive inflammation and disease severity in COVID-19 patients. Front Cell Infect Microbiol 2021; 11:734005
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. Thromb Haemost 2020;120(10):1474–1477
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
83 Volod O, Bunch CM, Zackariya N, et al. Viscoelastic hemostatic assays: a primer on legacy and new generation devices. J Clin Med 2022;11(03):860
84 Moore EE, Moore HB, Kornblith LZ, et al. Trauma-induced coagulopathy. Nat Rev Dis Primers 2021;7(01):30
85 Barrett TJ, Bilaloglu S, Cornwell M, et al. Platelets contribute to disease severity in COVID-19. J Thromb Haemost 2021;19(12): 3139–3153
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
87 Althaus K, Marini I, Zlamal J, et al. Antibody-induced procoagulant platelets in severe COVID-19 infection. Blood 2021;137(08): 1061–1071
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
89 Abou-Ismail MY, Diamond A, Kapoor S, Arafah Y, Nayak L. The hypercoagulable state in COVID-19: incidence, pathophysiology, and management. Thromb Res 2020;194:101–115
90 Zhang S, Liu Y, Wang X, et al. SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19. J Hematol Oncol 2020;13(01): 120
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. Front Cardiovasc Med 2022;9:794092
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(01):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 2020;506:145–148
94 Xu P, Zhou Q, Xu J. Mechanism of thrombocytopenia in COVID-19 patients. Ann Hematol 2020;99(06):1205–1208
95 Delshad M, Safaroghli-Azar A, Pourbagheri-Sigaroodi A, Poopak B, Shokouhi S, Bashash D. Platelets in the perspective of COVID-19; pathophysiology of thrombocytopenia and its implication as prognostic and therapeutic opportunity. Int Immunopharmacol 2021;99:107995
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(03):223–237