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A decision-tree approach to treat platelet hyperactivity and anomalous blood clotting in acute COVID-19 patients

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ABSTRACT

The coronavirus disease 2019 (COVID-19) (SARS-Cov-2) has caused a worldwide, sudden and substantial increase in hospitalizations for pneumonia with multiorgan problems. An important issue is also that there is still no unified standard for the diagnosis and treatment of COVID-19. Substantial vascular events are significant accompaniments to lung complications in COVID-19 patients. Various papers have now also shown the significance of thromboelastrography (TEG®) as point-of-care technology to determine the levels of coagulopathy (both clotting and bleeding) in COVID-19, in managing COVID-19 patients. Here we present two treatment protocols that may used to treat thrombotic and bleeding or thrombocytopenia pathologies. Both the protocols use clinical parameters like D-dimer and CRP, as well as the TEG®, to closely follow the daily clotting propensity of COVID-19 patients. We conclude by suggesting that the treatment of COVID-19 patients, should be based on a combination of blood biomarkers, and results from point-of-care analyses like the TEG®. Such a combination approach closely follow the physiological responses of the immune system, the haematological, as well as the coagulation system, in real-time.

Keywords: COVID-19; Platelets; Blood clotting; Treatment regime

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) (SARS-Cov-2) has caused a worldwide, sudden and substantial increase in hospitalizations for pneumonia with multiorgan problems (Docherty*, et al* 2020, Wiersinga*, et al* 2020, Wynants*, et al* 2020). Severe cases of COVID-19 are almost inevitably accompanied by respiratory failure and hypoxia, and treatment includes best practices for supportive management of acute hypoxic respiratory failure (Wiersinga*, et al* 2020). Approximately 5% of patients with significant COVID-19 symptoms, experience severe symptoms necessitating intensive care (Wiersinga*, et al* 2020), where as many individuals are probably never diagnosed because of a very mild version of the disease. Depending on the clinical protocol followed by the specific hospital, among patients in the intensive care unit (ICU) with COVID-19, between 29% to 91% require invasive mechanical ventilation (Docherty*, et al* 2020, Grasselli*, et al* 2020). Some COVID-19 patients deteriorate rapidly and seemingly without warning (Ottestad*, et al* 2020). This can also be the case for relatively young patients who were previously healthy, or who had only minor underlying conditions (Ottestad*, et al* 2020). We have recently shown that in COVID-19, the clotting protein, fibrin(ogen), change to an amyloid form, that platelets are hyperactivated and that they form complexes with erythrocytes (Pretorius*, et al* 2020, Venter*, et al* 2020). In addition iron and p-selectin levels are also significantly dysregulated. It is also now accepted that coagulation pathology is central in the disease (Giannis*, et al* 2020, Kollias*, et al* 2020, Levi*, et al* 2020, Liu*, et al* 2020, Middeldorp*, et al* 2020, Miesbach and Makris 2020).

Despite the worsening trends of COVID-19 deaths, currently no drugs have been validated to have significant efficacy in large-scale studies (Jean*, et al* 2020). An important issue is also that there is still no unified standard for the diagnosis and treatment of COVID-19 (Oldenburg and Doan 2020). However, various antiviral agents, some antibiotics and anti-inflammatory agents have been explored and their efficacy debated (see Table 1 for such a list of medications).

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Table 1: Various antiviral agents, antibiotics and anti-inflammatory agents suggested to be useful in the treatment of COVID-19.

Agent	Use and comments	References (general references and those investigating use in COVID-19)
Inhibition of the RNA-dependent RNA polymerase (antivirals)		
Remdesivir	Anti-viral therapeutic. Results vary: No statistically significant clinical benefits for severe COVID-19; or improvement time to recovery (hospital discharge or no supplemental oxygen requirement) from 15 to 11 days.	(Holshue, *et al* 2020, Jean, *et al* 2020, Wang, *et al* 2020, Wiersinga, *et al* 2020)
Favipiravir	Known to be active *in vitro* against oseltamivir-resistant influenza A, B, and C viruses. Favipiravir was approved for treatment of novel influenza on February 15, 2020 in China. A 2020 study showed significantly better treatment outcomes is COVID-19	(Cai, *et al* 2020, Dong, *et al* 2020, Wang, *et al* 2019)
Protease inhibitors		
Lopinavir/ritonavir	Inhibition of papain-like protease and 3C-like protease, but in hospitalized adult patients with severe Covid-19, no benefit was observed with lopinavir–ritonavir treatment	(Cao, *et al* 2020, Dong, *et al* 2020)
Inhibition of cathepsin L and cathepsin B in host cells		
Other therapies of interest		
Monoclonal or polyclonal antibodies	Prophylactic and therapeutic tools against some viral infections, such as influenza. Large gaps exist in our understanding of the risk of immunopathology in COVID-19, the epidemiological risk factors, the mechanism and immune mediators of pathology during CoV infections. Monoclonal antibodies and hyperimmune globulin may provide additional preventive strategies.	(Beigel, *et al* 2019, de Alwis, *et al* 2020, Wiersinga, *et al* 2020)
Convalescent plasma	A rapid method to derive antiviral treatment for Covid-19 is the use of convalescent plasma derived hyperimmune globulin. No clear evidence of benefit yet and a randomized trial found that it did not shorten time to recovery.	(Bloch, *et al* 2020, Brown and McCullough 2020, de Alwis, *et al* 2020, Wiersinga, *et al* 2020)
Dexamethazone	Emerging data indicate that dexamethasone therapy reduces 28-day mortality in patients requiring supplemental oxygen compared with usual care.	(Wiersinga, *et al* 2020)
L-ergothioneine	Potent antioxidant	(Borodina, *et al* 2020, Cheah and Halliwell 2020)
Lactoferrin	Iron chelator and interferes with viral attachment to membrane receptors	(Kell, *et al* 2020)
Niclosamide	Niclosamide has been identified as a potent inhibitor of SARS-Cov-2 by Institut Pasteur Korea, with potency >40 x higher than remdesivir	https://news.cision.com/union-therapeutics/r/union-receives-approval-from-danish-medicines-agency-to-initiate-clinical-study-with-niclosamide-for,c3145312

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Presently, it is suggested that best practices for supportive management of acute hypoxic respiratory failure and acute respiratory distress syndrome (ARDS) should be followed (Alhazzani, *et al* 2020, Wiersinga, *et al* 2020). The American Thoracic Society-led international task force has also released a guidance document to help clinicians manage COVID-19. The new guidance - "COVID-19: Interim Guidance on Management Pending Empirical Evidence"- is published as an open access document on the American Thoracic Society's website (https://www.thoracic.org/covid/covid-19-guidance.pdf). These guidelines in broad terms suggest the use of ventilation in patients who have refractory hypoxemia and ARDS, to consider extracorporeal membrane oxygenation in patients who have refractory hypoxemia, COVID-19 pneumonia (i.e. ARDS), and have failed prone ventilation. Most significant is that the guidelines recognise and state that: "We believe that in urgent situations like a pandemic, we can learn while treating by collecting real-world data."

Evidence-based guideline initiatives have also been established by many countries and professional societies, e.g. in South Africa, the South African Society of Anaesthesiologists (SASA) has detailed guidelines on their website (https://sasacovid19.com/#guidance-documents). In addition, guidelines are updated regularly by the National Institutes of Health (https://www.covid19treatmentguidelines.nih.gov/).

Although current practice in the treatment of patients with severe COVID-19 suggests mechanical ventilation, many patients might have normal lung compliance, yet they remain hypoxic in spite of high inspiratory fractions. An important question to ask is therefore why is the survival rate for patients in ICUs still so low? We note that most COVID-19 deaths are because of ARDS. Recently it was reported that the mortality rate of patients on ventilators in China is 80% (Yang, *et al* 2020). In general, the mortality rate of patients with ARDS is proportionate to the severity of the disease, with 27%, 32%, and 45% for mild, moderate, and severe disease, respectively (Diamond 2020). It was also noted that the pooled mortality rate for all ARDS from 1994 to 2006 in the studies that were evaluated was 43% (Diamond 2020). The question that now comes to mind is whether there might there be a pathophysiology link that is not considered in the current best-practice protocols.

Significant evidence for hypercoagulation during the early stages of COVID-19 disease

We have presented evidence that COVID-19 can be seen as a two-phase 'rollercoaster' of events, characterized by (i) thrombotic and (ii) bleeding or thrombocytopenia pathologies (Grobler, *et al* 2020). These substantial vascular events are significant accompaniments to ARDS and lung complications and both vascular events are seen in COVID-19 patients. (Al-Samkari, *et al* 2020, Bikdeli, *et al* 2020, Boccia, *et al* 2020, Connors and Levy 2020, Li, *et al*

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2020a, Wright, *et al* 2020, Zou, *et al* 2020). Clearly these coagulopathies might be seen as polar opposites, and it might be seen as odd if both are said to accompany COVID-19 pathology; the resolution of the apparent paradox is that these coagulopathies can be differentiated in time (Figure 1). During the progression of COVID-19, the circulating biomarkers P-selectin, von Willebrand Factor (VWF), fibrin(ogen) and D-dimer may either be within healthy levels, upregulated or eventually depleted (Pretorius, *et al* 2020, Venter, *et al* 2020). In COVID-19 patients, dysregulation, has been noted in each of them and this may lead to the extensive endotheliopathy noted in COVID-19 patients (Ackermann, *et al* 2020, Goshua, *et al* 2020b) (see Table 2).

Table 2: Dysregulation of circulating biomarkers P-selectin, von Willebrand Facto, fibrin(ogen) and D-dimer in COVID-19. See Figure 1 for levels during COVID-19.

Circulating biomarker	**Selected references**
P-selectin	(Goshua, *et al* 2020a, Neri, *et al* 2020)
Fibrinogen and D-dimer	(Al-Samkari, *et al* 2020, Favaloro and Thachil 2020, Garcia-Olivé, *et al* 2020, Li, *et al* 2020a, Li, *et al* 2020b, Lippi and Favaloro 2020, Panigada, *et al* 2020, Spiezia, *et al* 2020, Zou, *et al* 2020)
Von Willebrand Factor	(Escher, *et al* 2020, Zachariah, *et al* 2020).

Figure 1 shows the fine balance during COVID-19, between these biomarkers and the development of an initial hyperclotting and thrombosis that can be followed by thrombocytopenia and bleeding; the latter is followed by the cytokine storm (at the end stage of the disease) (Grobler, *et al* 2020). Depending on the direction (i.e, increases or decreases), dysregulation of fibrin(ogen), D-dimer, VWF and P-selectin may result in either hypercoagulation or excessive bleeding and thrombocytopenia (hypocoagulation). We suggested that patients need to be treated early in the disease progression, when hypercoagulation is clinically diagnosed (discussed later in the treatment protocol). Early in the hypercoagulation phase of the disease, high levels of VWF, P-selectin and fibrinogen are present, but there are still normal or slightly increased levels of D-dimer. If the disease is left to progress until the patient presents with VWF and fibrinogen depletion, and with high D-dimer levels (and even higher P-selectin levels), it will be indicative of a poor prognosis, an imminent cytokine storm, and ultimately death. This rollercoaster disease progression is a continuum and the progression of disease has no specific tipping point (Figure 1). In a recent JAMA editorial, the question was also asked whether the cytokine storm should be seen as significantly relevant in COVID-19, and it was referred to as “tempest in a teapot” (Sinha, *et al* 2020). The basis for this conclusion was that the presence of elevated circulating mediators in the claimed cytokine storm are likely to reflect endothelial dysfunction and systemic inflammation leading to fever, tachycardia, tachypnea, and hypotension (Sinha, *et al* 2020), rather than the more immediately lethal ARDS. The JAMA editorial concluded by suggesting

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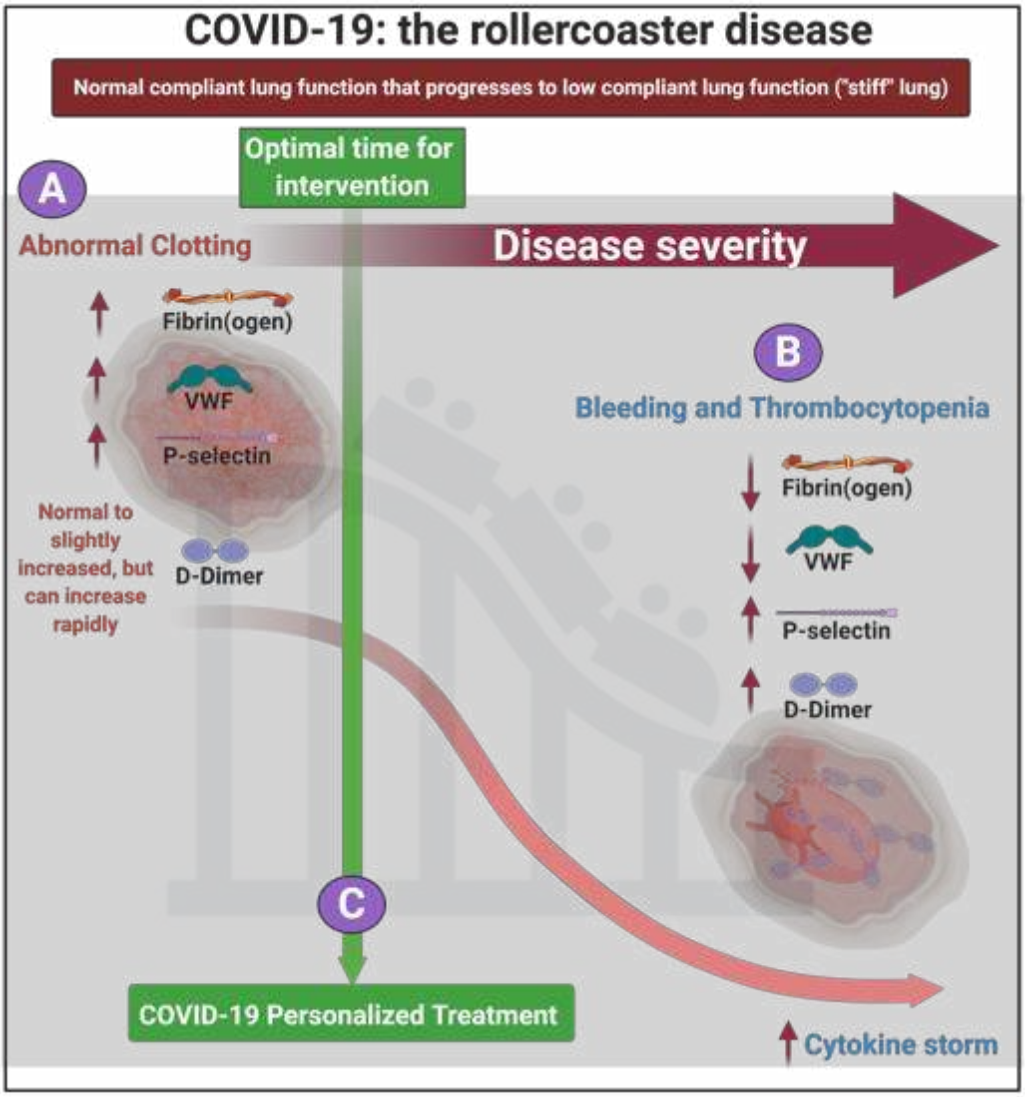
that incorporating the cytokine storm may only further increase uncertainty about how best to manage this heterogeneous population of patients (Sinha, *et al* 2020). Our rollercoaster diagram (Figure 1) also notes that increased levels of inflammatory cytokines will already start early in the disease (Grobler, *et al* 2020).

Figure 1: The rollercoaster vascular pathology in acute respiratory syndrome coronavirus 2 (COVID-19) [adapted from (Grobler, *et al* 2020)]. We focus on fibrin(ogen), D-Dimer, P-selectin and von Willebrand Factor dysregulation, resulting in endothelial, erythrocyte and platelet dysfunction. **A)** Early on in the disease dysregulation in clotting proteins and circulating biomarkers may occur and is suggestive of hypercoagulation. **B)** The disease may progress to bleeding and thrombocytopenia. **C)** We suggest that each patient should be treated using a personalized medicine approach in the early stages of the disease. Image created with BioRender (https://biorender.com/).



Compelling emerging clinical evidence (consistent with our rollercoaster hypothesis) shows that COVID-19 can be complicated by disseminated intravascular coagulation (DIC), which has a strongly prothrombotic character with a high risk of venous thromboembolism (Kollias, *et al* 2020). Coagulopathy is now known to occur in the majority of patients who die from

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COVID-19 (Tang, *et al* 2020b). A report from Wuhan, China, indicated that 71% of 183 individuals who died of COVID-19 met criteria for DIC (Tang, *et al* 2020b, Wiersinga, *et al* 2020). Heparin has also been found in some circumstances to be a helpful treatment for COVID-19 (Ayerbe, *et al* 2020, Menezes-Rodrigues, *et al* 2020). Patients treated with anticoagulants (such as heparin) had a higher survival rate and a **much** more favourable outcome was seen in ventilated patients were 62,7% of patients without anticoagulants died. This outcome will of course be if the patients are treated when they are still in the early stages of the disease (when hypercoagulation is present). In contrast, only 1% of patients treated with anticoagulants and who were ventilated died (Tang, *et al* 2020a). Heparin interferes with von Willebrand factor (VWF), platelet activation, and assists in the prevention of thrombotic events. It was also recently shown, in a study, of 449 patients with severe COVID-19, that anticoagulant therapy, mainly with low molecular weight heparin, appeared to be associated with lower mortality in the subpopulation meeting sepsis-induced coagulopathy criteria or with markedly elevated D-dimer (Kollias, *et al* 2020, Tang, *et al* 2020a). In this study 99 of the patients received heparin (mainly with low molecular weight heparin) for 7 days or longer (such treatment was only given in the initial stages of the disease when the patient is hypercoagulable and not during the bleeding or thrombocytopenia phase of the disease). It should be noted that the timeline of the rollercoaster disease progression can be hours and it is a continuum rather than a clear event or “flip” between hypercoagulation and bleeding. If the disease is left unabated, VWF and fibrinogen depletion, and significantly increased levels of D-dimer and P-selectin will progress on a continuum (Grobler, *et al* 2020).

Several autopsy results have also confirmed microthrombi throughout the lung and associated with right ventricular dilation of the heart. Recently, Ackermann and co-workers reported that histologic analysis of pulmonary vessels in patients with COVID-19 shows widespread thrombosis with microangiopathy (Ackermann, *et al* 2020). Furthermore, they found that alveolar capillary microthrombi were 9 times as prevalent in patients with COVID-19 as in patients with influenza ($p<0.001$). In autopsy samples of lungs from patients with COVID-19, the amount of new vessel growth - predominantly through a mechanism of intussusceptive angiogenesis (i.e. splitting of an existing vessel where the capillary wall extends into the lumen of an existing vessel) - was 2.7 times as high as that in the lungs from patients with influenza ($p<0.001$) (Ackermann, *et al* 2020). Middleton and co-workers showed the presence of neutrophil extracellular traps (NETs) in lung autopsy results, and suggested that these may be the cause of immune-thrombosis and may, in part, explain the prothrombotic clinical presentations in COVID-19 (Middleton, *et al* 2020). Menter and co-workers also showed autopsy findings from 21 COVID-19 patients, and reported that the primary cause of death was respiratory failure, with exudative diffuse alveolar damage and massive capillary

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congestion, often accompanied by microthrombi despite anticoagulation therapy (Menter*, et al* 2020). A possible reason might be because the extent of systemic hypercoagulation was too significant for the medication to have a substantial enough effect. Similarly, Spiezia and co-workers argued in most COVID-19 patients high D-dimer levels are associated with a worse prognosis (Spiezia*, et al* 2020). The authors also suggest that COVID-19 patients with acute respiratory failure represent the consequence of severe hypercoagulability, that when left untreated results in consumptive coagulopathy (end-stage DIC) and that excessive fibrin formation and polymerization may predispose to thrombosis and correlate with a worse outcome (Spiezia*, et al* 2020). Consumptive coagulopathy is characterized by abnormally increased activation of procoagulant pathways. This results in intravascular fibrin deposition and decreased levels of hemostatic components, including platelets, fibrinogen, and other clotting factors. Acute DIC results in bleeding and intravascular thrombus formation that can lead to tissue hypoxia, multiorgan dysfunction, and death (Costello and Nehring 2020). It is therefore worth to note that DIC is a thrombotic coagulopathy that eventually leads to bleeding.

Thromboelastometry (TEM), also known as rotational thromboelastography (ROTEG) or rotational thromboelastometry (ROTEM), is an established viscoelastic method for haemostasis testing. It is a modification of traditional thromboelastography® (TEG®). These techniques are crucial point-of-care techniques that we suggest that should be used in treatment of COVID-19 patients. Spiezia and colleagues also noted that COVID-19 patients with acute respiratory failure present with severe hypercoagulability due to hyperfibrinogenemia, resulting in increased fibrin formation and polymerization that may predispose the patient to thrombosis. Spiezia and co-workers also concluded that thromboelastometry is an important point-of-care test in COVID-19, as it has the advantage of providing a global assessment of whole blood's ability to clot. On the other hand, it is not able to evaluate the contribution to clot formation of each element (including endothelium, platelets, and clotting factors). In 2020, Wright and co-workers also discussed the use of clot lysis at 30 minutes (LY30) on the TEG® as point-of-care analysis method (Wright*, et al* 2020). The LY30 parameter (measured in %) is recorded at 30 minutes, measured from the point where the maximum amplitude (MA) of the clot is reached (see Figure 2). LY30 of 3% or greater defines clinically relevant hyperfibrinolysis (Chapman*, et al* 2013). The TEG® results, particularly an increased maximum amplitude (MA) and G-score (that both measures maximal clot strength) and is used to predict thromboembolic events and a poor outcome in critically ill patients with COVID-19 (Wright*, et al* 2020). MA is of great significance as it represents clot size (see Figure 2), as determined by platelet number and function, as well as fibrin cross-linking to form a stable clot.

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Recently, various papers have shown the significance of TEG® and the levels of coagulopathy in COVID-19 (an important measurement value of the TEG®) in managing COVID-19 patients is also getting more traction (Chandel, *et al* 2021, Görlinger and Levy 2021, Hranjec, *et al* 2020) (Smolarz, *et al* 2021). Hranjec and co-workers in 2020 also noted that TEG® with platelet mapping, better characterizes the spectrum of COVID-19 coagulation-related abnormalities and may guide more tailored, patient-specific therapies these patients (Hranjec, *et al* 2020). Another important test is the PFA-200 platelet test. This test may be seen as a cross between bleeding time and quick aggregation testing. See Table 3 for the various parameters for the TEG® and PFA-200.

Figure 2: TEG® traces with the various parameters discussed in Table 3, visualized. **A)** Healthy (normocotgulable) trace; **B)** Hypercoagulable trace and **C)** Hypocoagulable trace. Image created with BioRender (https://biorender.com/).

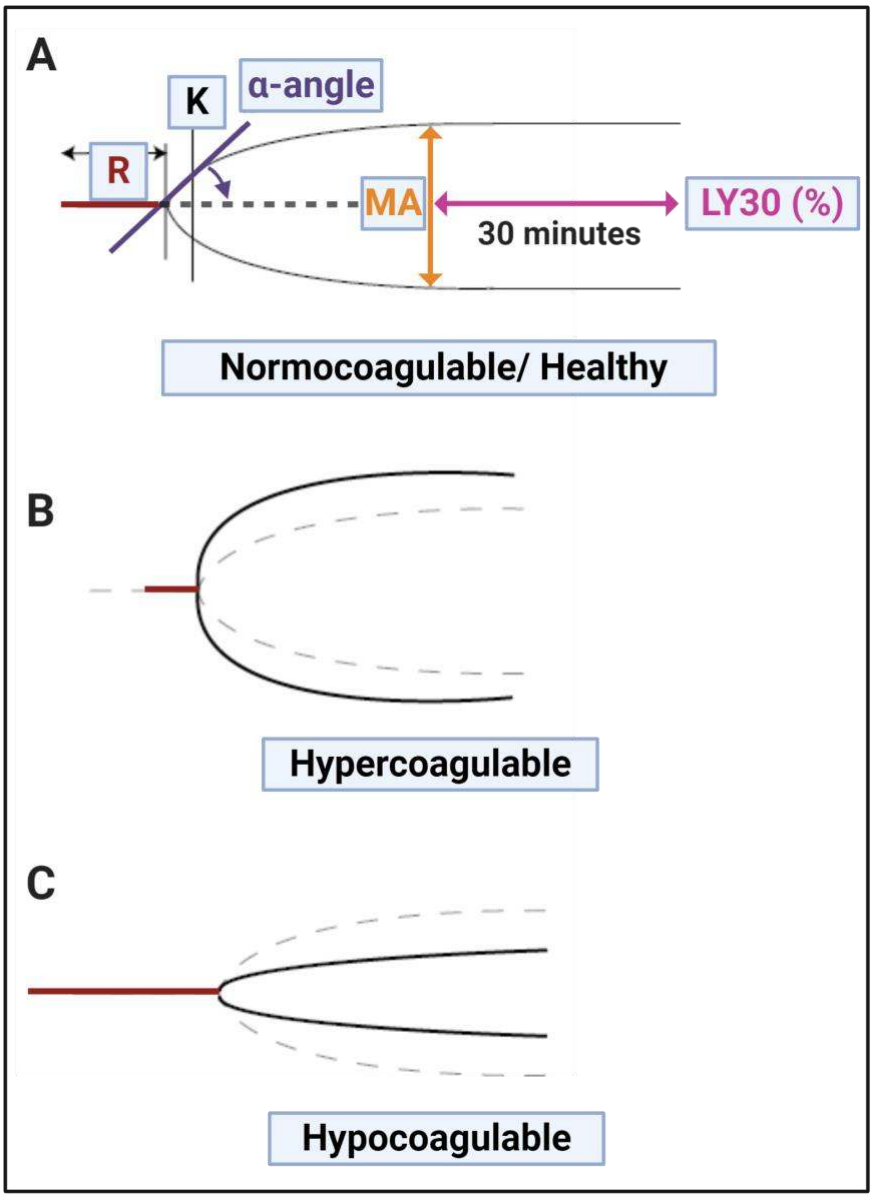

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Table 3: Thromboelastography® (TEG®) clot parameters for whole blood and PFA-200 platelet parameters.

Thromboelastography®	
TEG® parameters	**Explanation**
R value: reaction time measured in minutes	Time of latency from start of test to initial fibrin formation (amplitude of 2mm); i.e. initiation time.
K: kinetics measured in minutes	Time taken to achieve a certain level of clot strength (amplitude of 20mm); i.e. amplification.
A (Alpha): Angle (slope between the traces represented by R and K) Angle is measured in degrees	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.
MA: Maximal Amplitude measured in mm	Maximum clot size: it reflects the ultimate strength of the fibrin clot, i.e. overall stability of the clot. The larger the MA the more hypercoagulable the clot.
Maximum rate of thrombus generation (MRTG) measured in Dyn.cm^{-2}.s^{-1}	The maximum velocity of clot growth observed or maximum rate of thrombus generation using G, where G is the elastic modulus strength of the thrombus in dynes per cm^{-2}.
Time to maximum rate of thrombus generation (TMRTG) measured in minutes	The time interval observed before the maximum speed of the clot growth.
Total thrombus generation (TTG) measured in Dyn.cm^{-2}	The clot strength: the amount of total resistance (to movement of the cup and pin) generated during clot formation. This is the total area under the velocity curve during clot growth, representing the amount of clot strength generated during clot growth.
Lysis at 30 minutes (LY30) measured in %	The LY30 parameter (measured in %) is recorded at 30 minutes, measured from the point where the maximum amplitude (MA) of the clot is reached.
G value measured in Dyn.sec	G-value is a log-derivation of the MA and is meant to also represent the clot strength Elevated G-value is associated with a hypercoagulable state and therefore increases the risk for venous thromboembolic disease.
PFA-200 platelet test interpretation	
Citrated whole blood is aspirated at high shear rates through disposable cartridges. These cartridges contain an aperture within a membrane coated agonist. The agonist cartridges are Col/EPI, Col/ADP and P2Y and they report data in **closure time.** The PFA-200 test induces platelet adhesion, activation and aggregation using the three cartridges. Closure times increase progressively as the platelet counts falls below 100 x 10^9/L.	
Agonist cartridges (Favaloro and Bonar 2018)	**Test principle**	**Closure time interpretations: measured in seconds**
Collagen and epinephrine (Col/EPI): This cartridge has a collagen (2 µg equine type I) and epinephrine (10 µg)-coated membrane (C/Epi).	Co-stimulation by shear stress, collagen, epinephrine. Gives an indication of effectiveness of aspirin and GP IIβ/IIIα inhibitor dosage.	Col/EPI closure time is 82 - 150 seconds with a value >150 seconds regarded as prolonged.
Collagen and ADP (Col/ADP): This cartridge has a collagen (2 µg equine type I) and adenosine-diphosphate (50 µg)-coated membrane (Col/ADP).	Co-stimulation by shear stress, collagen, ADP. Gives an indication of effectiveness of aspirin and clopidogrel and GP IIβ/IIIα inhibitor dosage.	Col/ADP closure time is 62 - 100 seconds with a value >100 seconds regarded as prolonged.
P2Y: This cartridge has a prostaglandin E1 (5 ng) and ADP (20 µg)-coated membrane.	Co-stimulation by shear stress, ADP, PGE1 and Ca^{2+}. Gives an indication of effectiveness of clopidogrel and GP IIβ/IIIα inhibitor dosage	Shortened PFA P2Y closure times >106 seconds are viewed as prolonged.

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An important consideration is that TEG® can be used to study the clotting parameters of both whole blood (WB) and platelet poor plasma (PPP). Whole blood TEG® gives information on the clotting potential affected by the presence of both platelets and fibrinogen, while PPP TEG® only presents evidence of the clotting potential of the plasma proteins (Nielsen 2008, Nielsen 2017, Nielsen*, et al* 2007, Nielsen 2007). Reasons for a hypercoagulable TEG® trace when using PPP, may be indicative of the presence of dysregulated inflammatory biomarkers, including P-selectin, inflammatory cytokines and increased levels of fibrinogen (Bester*, et al* 2018, Bester and Pretorius 2016, Bester*, et al* 2015, Pretorius*, et al* 2017b, Pretorius*, et al* 2018, Randeria*, et al* 2019). Our research group recently found that during the presence of systemic inflammation, and the increased presence of inflammagens, the biochemistry of the fibrin(ogen) molecule changes its folding characteristics (Figure 3). We could visualize these changes using fluorescent markers (Kell and Pretorius 2015, Kell and Pretorius 2017, Page*, et al* 2019, Pretorius*, et al* 2016, Pretorius*, et al* 2017a). The fluorescent markers we use to show these structural changes in the fibrin(ogen) biochemistry was thioflavin T (ThT) and amytracker. These fluorescent markers are typically used to show amyloid changes to proteins (Kell and Pretorius 2015, Kell and Pretorius 2017, Page*, et al* 2019, Pretorius*, et al* 2016, Pretorius*, et al* 2017a), suggesting the misfolding seen in fibrin(ogen) during the presence of inflammagens in the blood, could also be described as amyloid. We showed, that when fibrin(ogen) is exposed to increased levels of inflammatory biomarkers and bacterial (viral) inflammagens, either in the laboratory or in patients with increased levels, TEG® of PPP was significantly hypercoagulable (Kell and Pretorius 2017, Nunes*, et al* 2020, Page*, et al* 2019, Pretorius*, et al* 2016). Figure 3A shows the uncoiling of the fibrin(ogen) molecule where it caused plasma and WB to become hypercoagulable. Figure 3B and C shows scanning electron micrographs of representative examples of a healthy clot and a clot from a Type 2 Diabetes (T2DM) individual [taken from (Randeria*, et al* 2019)]. The value of using both the TEG® and fluorescent markers was again seen in our recent studies on the effects that COVID-19 has on the coagulation system, where an increased clot strength and amyloid formations were previously seen (Pretorius*, et al* 2020, Venter*, et al* 2020).

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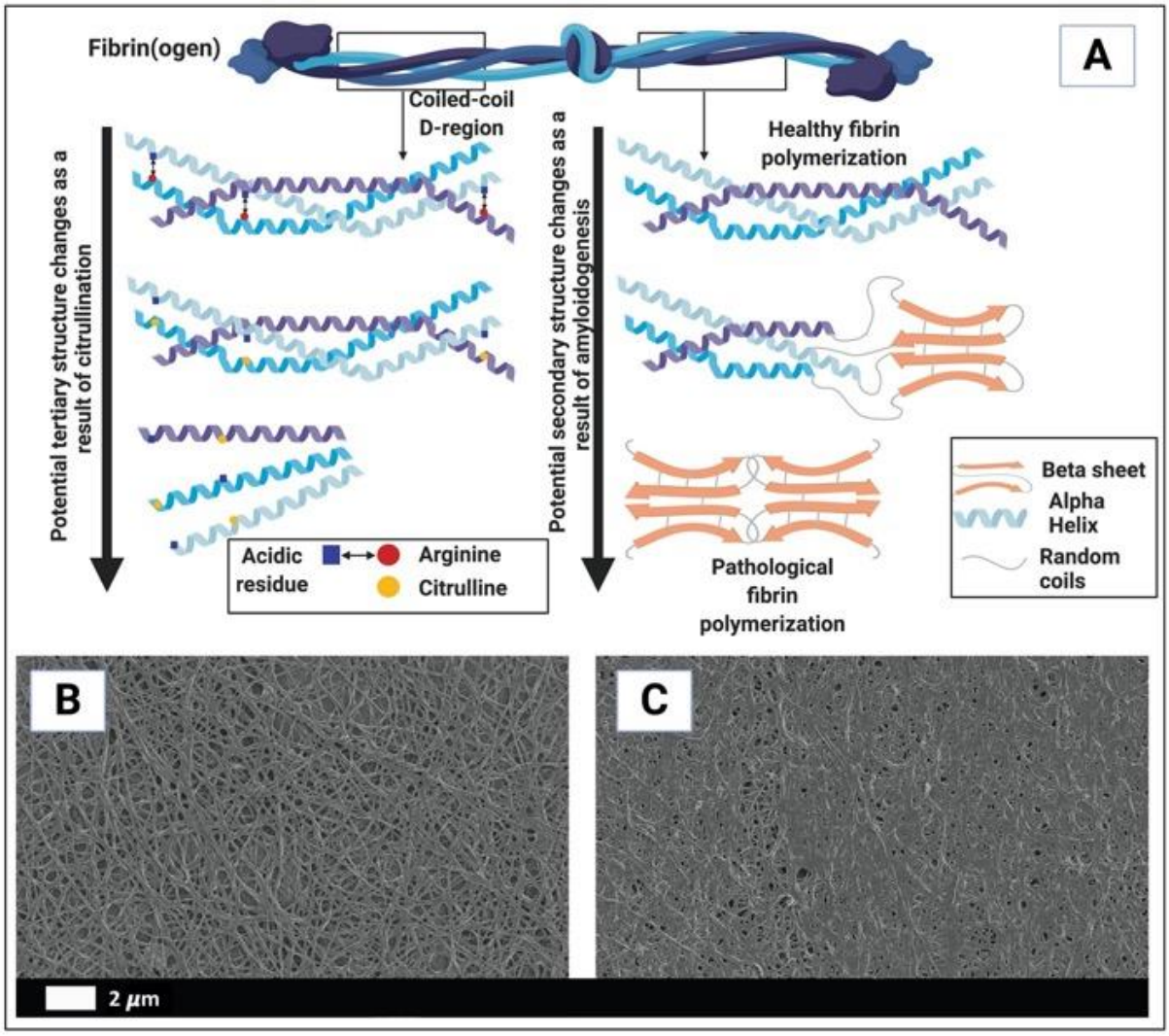
Figure 3: A) The uncoiling of the fibrin(ogen) protein (in part) resulting in whole blood and plasma hypercoagulability. **B and C**) are examples of scanning electron microscopy micorgraphs of **B)** fibrin clot from a healthy individual (created with platelet poor plasma with added thrombin); **C)** fibrin clot from a diabetes individual (created with platelet poor plasma with added thrombin) [B and C taken from (Randeria*, et al* 2019)]. Image created with BioRender (https://biorender.com/).



Importantly, endotheliopathy is also prevalent in patients with COVID-19 (Meizlish*, et al* 2020) (Ackermann*, et al* 2020, Goshua*, et al* 2020a). In general, endothelopathy is also known to be significantly linked to coagulopathies. Endotheliopathy activates microthrombotic pathway and initiates microthrombogenesis, leading to endotheliopathy-associated intravascular microthrombi [for a review see (Chang 2018)].

In this article, we describe a treatment regime that will address the coagulopathy in COVID-19 patients. We have found that addressing hypercoagulation and platelet hyperactivity during the early stages of the disease plays a significant role in stopping the progression of the disease and thus in lowering the high death rate in COVID-19 patients. We provide a decision-

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tree treatment protocol for the treatment of COVID-19 patients, where treatment protocols were followed as per the patient's symptoms (therefore, following a personalized patient-orientated approach), and based on oxygen saturation, TEG® and PFA-200 analysis (See Table 3).

MATERIALS AND METHODS:

Decision tree protocol development

The decision-tree treatment protocol was developed by qualified clinicians, working in private practice, based on standard clinical treatment practices. All pharmaceutical intervention suggested is within therapeutic levels and used within prescribed standard clinical protocols. All drugs suggested in the decision-tree protocol must be used as originally indicated/designed and no "off-label" treatment should done. Co-authors (EP, LMG, DBK, CV) are not medically qualified did not participate in either the development or use of the protocol. Two clinical protocols are suggested, based on clinical features of the patient:

- A prognostic indicator scoring system was developed, based on a points system, to predict which patient is most likely to develop severe disease.
- **Protocol 1:** If clinical features point to hypercoagulation [early phase on the 'rollercoaster' diagram (Figure 1A).
- **Protocol 2:** If clinical features point to bleeding (late phase on the rollercoaster diagram (Figure 1B).

RESULTS

Decision tree protocol development

The prognostic indicator

A prognostic indicator score system was developed to determine risk of developing severe disease (see Table 4). This score indicator system allows the clinician to allocate points for various parameters, including age, effort intolerance, Hypoxemia, O_2 saturation, chest Z-ray and/or CT scan carotid intima-media thickness, and other co-morbidities. In addition, a scoring based on parameters from the point-of-care TEG is also suggested.

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Table 4: Prognostic indicator based on a points system.

Prognostic indicator			
Points assigned	**0**	**1**	**2**
Age (years)	≤ 44	45-64	≥65
Effort intolerance above baseline	No	Yes	
Hypoxemia, O_2 saturation	> 95	92-95	< 92
Chest X-ray/CT scan	Normal	≤ 1 quad	≥ 1quad
Obesity (BMI)	< 26	26-36	> 36
Co-morbidities: 1 point for each comorbid condition	Type 2 Diabetes Mellitus, coronary artery disease (CAD), non-atrial fibrillation (AF) stroke, smoking, deep vein thrombosis (DVT), Hyperparathyroidism (HPT), chronic kidney disease (CKD)		
IMT (carotid intima-media thickness) increased (age adjusted)	No (0 points)	Yes (1 point)	
TEG: MA	< 69	69-75	> 75
TEG: G-score	< 10	10-15	> 15
TEG: Ly-30	> 0	None (1 point)	
Score			
Low Risk	**Moderate Risk**	**High Risk**	
0-3	4-10	≥ 11	

Proposed treatment decision-tree protocol

Protocol 1: If clinical features point to hypercoagulation [early phase on the 'rollercoaster' diagram Figure 1A]:

Figure 4A shows the decision-tree protocol that was developed by the clinical team, when a patient is admitted to the hospital with one or more possible clinical features after a positive COVID-19 nasal swab diagnosis. Low risk patients should be treated according to symptoms (Figure 4(1)). Here it is suggested that high-risk patients should be treated with a regime where anticoagulation is involved.

High-risk patients for hospitilization (Figure 4(2))). are identified based on:

- Activity-related worsening effort intolerance from your baseline (short-of breath/ dyspnoea) (according to the New York Heart Association Classification (https://manual.jointcommission.org/releases/TJC2016A/DataElem0439.html), which classifies patients in one of four categories based on their limitations during physical activity; the limitations/symptoms refers to normal breathing and varying degrees in shortness of breath and/or angina pain).
- Oxygen saturation that is **≤ 95%** room air temperature (measured with pulse oximeter to constantly monitor the patient).
- A lung CT scan suggestive of COVID-19, by using the radiology grading system, COVID-19 Reporting and Data System (CO-RADS) probability score (Prokop*, et al* 2020). The

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CO-RADS scoring is based on the CT findings, the level of suspicion of COVID-19 infection is graded from very low or CO-RADS 1 up to very high or CO-RADS 5 and the severity and stage of the disease is determined with remarks on comorbidity and a differential diagnosis (Prokop*, et al* 2020). The British Thoracic Society Severity Score (https://www.brit-thoracic.org.uk/about-us/covid-19-information-for-the-respiratory-community/) is another such lung CT scan scoring system used in COVID-19 grading.

- Once the patient is admitted to hospital, treatment should commence with loading on DAPT, dexamethasone and Fondaparinux (therapeutic levels) (See Figure 4(3)). Fondaparinux may be substituted with Low Molecular Weight heparin (LMWH) (therapeutic levels) or unfractionated heparin infusion. However, heparin infusion may be more labour intensive and heparin-induced thrombocytopenia (HIT) may come into play as a complication.
- TEG and CRP are used to monitor and guide both outpatient as well as inpatient treatment. Patients are warned of signs of bleeding, recurring fever or worsening hypoxemia. If they continue improving clinically, CRP and TEG are repeated after 4 weeks. If symptoms persist, CRP and TEG are done as needed.

The rationale for using adjuvant treatment is as follows (see Figure 4(7)):

- SSRI: platelets uses serotonin to degranulate. Blocking serotonin re-uptake by platelets has antiplatelet effects (SSRIs have different potencies to inhibit platelets. We suggest sertraline, as it is amongst the most potent SSRIs).
- Colchicine: Inhibits both IL-6 and IL-8.
- Rupanase: This a potent platelet activating factor inhibitor
- Dobutamine: Supporting a failing right ventricle (e.g. pulmonary hypertension).
- Heparin inhalation: Known to have potent anti-inflammatory, antiviral and antithrombic effects.

The protocol suggests that unfractionated heparin 5000 IU in 3 mL sterile water can be administered as inhalations every 4 to 6 hours, at any time during treatment for worsening hypoxaemia/dyspnoea. Therapeutic (not prophylactic) doses of either Fondaparinux unfractionated heparin or LMWH can be used. Therapeutic doses of Fondaparinux, unfractionated heparin or LMWH is used to control the enzymatic pathway of coagulation. The protocol also discussed the use of the potent platelet inhibitor, glycoprotein (GP) IIβ/IIIα inhibitor. This product blocks the GP IIβ/IIIα receptor on platelets. GP IIβ/IIIα inhibitor should be infused for at least 48 hours or continued for 48 hours after the administration of thrombolytic therapy (if thrombolysis is needed). High priority should be given to placement of central lines, under ultrasound guidance to prevent procedure-associated bleeding.

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An important clinical consideration is that D-dimer is only significantly elevated later in the disease (Figure 1). Therefore, our clinical observations suggest that, D-dimer levels early on (when fibrinogen levels are high), should not guide therapy, as it may falsely suggest that there is no hypercoagulation present. Therefore, D-dimer levels at presentation of the patient at the hospital should not be used to guide anticoagulation treatment, as this may only be a later sight of coagulopathy (see our rollercoaster hypothesis Figure 1). An excellent understanding and interpretation of TEG® (and PFA-200) (Table 2 and Figure 2) is imperative to applying the protocol, and de-escalation of therapy is guided by TEG® and clinical status of the patient.

If the patient is clinically stable, not oxygen dependent and TEG® normal; the clinician may consider discharge with dual antiplatelet therapy (DAPT) (e.g. Clopidogrel 75 mg/d / Aspirin 75 mg/d). To DAPT therapy a PPI is added (1 month), for gastric protection. A DOAC is added to the regime for at least 14 days. The exact duration and anticoagulation regime used for anticoagulation, post-discharge certainly needs to be carefully investigated further. The initiation of the intravenous thrombolysis protocol should be considered if, despite supplementary oxygen, the saturation remains ≤ 94%.

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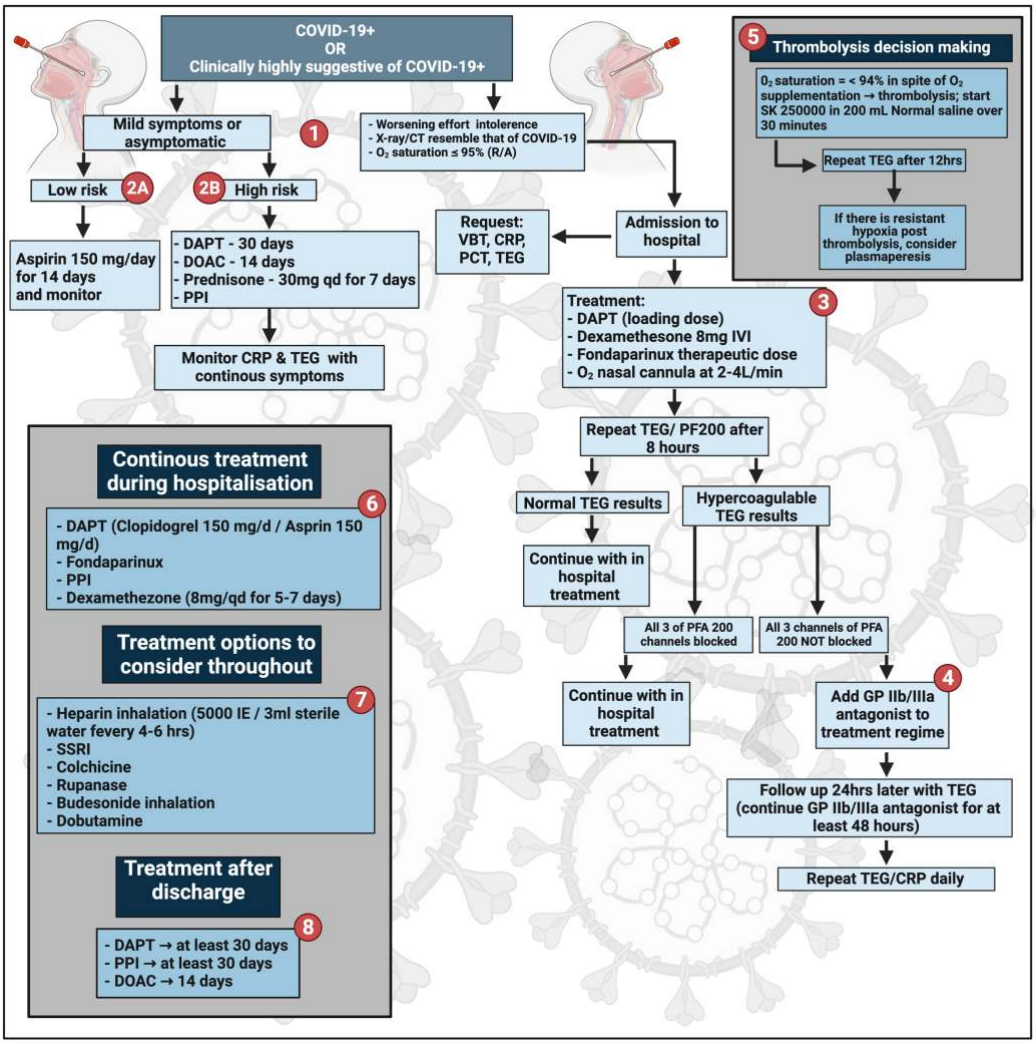
Figure 4: Clinical decision-tree protocol if clinical features point to hypercoagulation. 1) The first step would be to decide on outpatients management or if the patient should be hospitalized. **2A)** Low risk patients will require symptomatic treatment regime. **2B)** High-risk (outpatient) patients are treated with DAPT, as well as DOAC. Patients are warned of signs of bleeding, recurring fever or worsening hypoxemia. If they continue improving clinically, CRP and TEG are repeated after 4 weeks. If symptoms persist, CRP and TEG are done as needed. **3)** Once the patient is admitted to hospital, treatment should commence with loading on DAPT, dexamethasone and Fondaparinux (therapeutic levels). **4)** With a hypercoagulable TEG result and all 3 channels of the PFA200 not blocked, use (GP) IIβ/IIIα infusion for at least 48 hours, guided by the TEG results. Once the patient is on a (GP) IIβ/IIIα inhibitor, the PFA200 does not have to be repeated as that class of drug inhibits all thee platelet channels. **5)** At any time during hospitalization, the initiation of the intravenous thrombolysis protocol should be considered if, despite supplementary oxygen the saturation remails ≤ 94%. Streptokinase (250 000 IU/200 ml normal saline infused over 30 minutes. Do not use a streptokinase continuously infusion, due to potential bleeding risk. Streptokinase may be replaced with Tenecteplase or Alteplace. **6) DAPT** (Clopidogrel 150 mg/d / Aspirin 150 mg/d), Fondaparinux (therapeutic dose) and proton pump inhibitor (PPI) should be used continued throughout the hospital stay. SSRI: we suggest Sertraline 50 mg/d **7)** Adjuvant treatment may be considered. **8)** Post-discharge anticoagulation is important, however, research is still needed to determine the exact duration of the treatment. **Abbreviations:** BD: Twice daily; DAPT: Discharge with dual antiplatelet therapy; DOAC (e.g. Apixaban): Direct oral anticoagulants; IV: Intravenous; STAT: *statin* (immediately); TEG®: Thromboelastography®. Image created with BioRender (https://biorender.com/).



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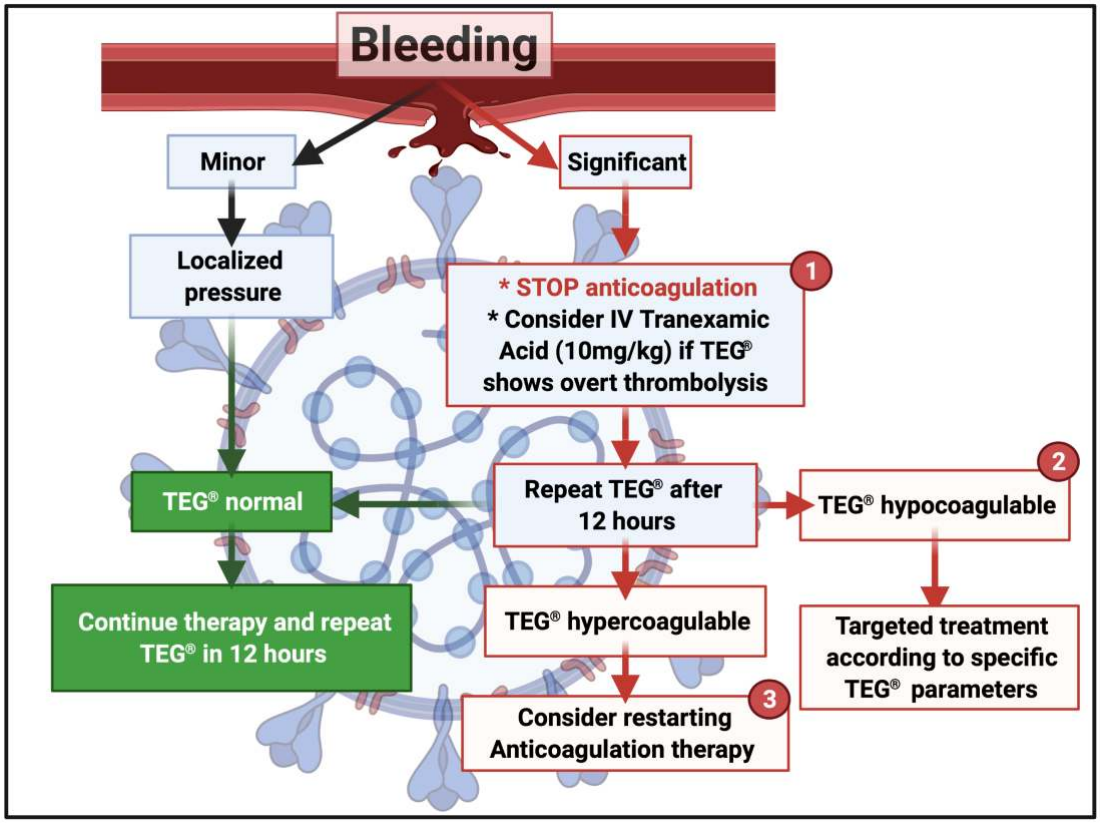
Protocol 2: if clinical features point to bleeding [late phase on the rollercoaster diagram (Figure 1B)].

Figure 5 shows the decision-tree when bleeding is diagnosed. Minor bleeds for example from puncture wounds (Central line/Arterial line) are treated by local pressure and TEG® done to evaluate further treatment if necessary. Major or significant bleeds should be treated according to area of bleeding and guided by TEG®. Blood transfusion may be deemed necessary.

When using TEG in order to treat clinical bleeding the following TEG® parameters are used as guidelines:

- Increased R time/ narrow alpha angle: stop anti-factor agents (heparines); Administer clotting factors (FFP/Cryoprecipitate).
- Decreased MA: Check platelet count; stop anti platelet agents; administer platelets, if indicated.
- Increased Lysis: Administer tranexamic acid.

Figure 5: Clinical decision-tree protocol if clinical features point to bleeding as judged by clinical observation, PFA-200 and TEG® results. Our clinical observation is that bleeding only occurs later on in the progression of the disease. **1)** Tranexamic acid may be repeated 6 – 8 hourly. **2)** TEG and full blood count will help make decision on using fresh frozen plasma, cryoprecipitate or platelet transfusion. **3)** Use TEG to decide on restarting anticoagulation. **Abbreviations:** GP IIβ/IIIα: Glycoprotein IIβ/IIIα; TEG®: Thromboelastography®. Image created with BioRender (https://biorender.com/).



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CONCLUSION

Here we suggest that when hypercoagulation is present in the early stages of the condition, coagulation parameters can be tracked very successfully with the TEG®. A close monitoring of clinical parameters of clotting, including D-dimer and TEG® parameters were crucial in managing patients. A major challenge in early response in a rapidly evolving epidemic caused by a novel pathogen is the lack of traditional randomized placebo-controlled trials (RCTs) on which to make treatment and prevention decisions (Oldenburg and Doan 2020). The identification and development of new therapeutic candidates or treatment regimes, based on emerging research and clinical evidence, require flexibility and a willingness to embrace treatment protocols that might deviate slightly from the recognized protocols. With this statement we most certainly do not imply that clinicians and researchers should lower the bar for standards of evidence. However, during this pandemic with the rapidly changing environment, traditional RCT rules may not apply (Oldenburg and Doan 2020). Because the coagulopathy changes over time (and this timeframe can be within hours), therapy should be guided by clinical parameters, including TEG® parameters, levels of fibrinogen, VWF, as well as D-dimer. Given the high stakes, the imperative for high-quality research is greater than ever. Flexible and reflective treatment protocols will be our only chance to lower the increasing death rates and eventually the outcome of this pandemic. We therefore suggest that COVID-19 is indeed (also) a true vascular disease. We conclude by suggesting that the treatment of COVID-19 patients, should be based on results from point-of-care analyses like the TEG®, that shows the physiological status of the haematological and coagulation system in real-time.

Consent for publication

All authors approved submission of the paper.

Competing interests

The authors have no competing interests to declare.

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Authors' contributions

GJL: Clinician and patient sample identification and writing of clinical workflow protocols; PSL: clinical workflow protocols; CV and LMG: technical assistance; DBK: co-corresponding author; EP: writing and editing of the paper, co-corresponding author.

TABLE AND FIGURE LEGENDS

Table 1: Various antiviral agents, antibiotics and anti-inflammatory agents suggested to be useful in the treatment of COVID-19.

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Table 2: Dysregulation of circulating biomarkers P-selectin, von Willebrand Facto, fibrin(ogen) and D-dimer in COVID-19. See Figure 1 for levels during COVID-19.

Table 3: Thromboelastography® (TEG®) clot parameters for whole blood and PFA-200 platelet parameters.

Table 4: Prognostic indicator based on a points system.

Figure 1: The rollercoaster vascular pathology in acute respiratory syndrome coronavirus 2 (COVID-19) [adapted from (Grobler*, et al* 2020)]. We focus on fibrin(ogen), D-Dimer, P-selectin and von Willebrand Factor dysregulation, resulting in endothelial, erythrocyte and platelet dysfunction. **A)** Early on in the disease dysregulation in clotting proteins and circulating biomarkers may occur and is suggestive of hypercoagulation. **B)** The disease may progress to bleeding and thrombocytopenia. **C)** We suggest that each patient should be treated using a personalized medicine approach in the early stages of the disease. Image created with BioRender (https://biorender.com/).

Figure 2: TEG® traces with the various parameters discussed in Table 3, visualized. **A)** Healthy (normocotgulable) trace; **B)** Hypercoagulable trace and **C)** Hypocoagulable trace. Image created with BioRender (https://biorender.com/).

Figure 3: A) The uncoiling of the fibrin(ogen) protein (in part) resulting in whole blood and plasma hypercoagulability. **B and C**) are examples of scanning electron microscopy micorgraphs of **B)** fibrin clot from a healthy individual (created with platelet poor plasma with added thrombin); **C)** fibrin clot from a diabetes individual (created with platelet poor plasma with added thrombin) [B and C taken from (Randeria*, et al* 2019)]. Image created with BioRender (https://biorender.com/).

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hourly. **2)** TEG and full blood count will help make decision on using fresh frozen plasma, cryoprecipitate or platelet transfusion. **3)** Use TEG to decide on restarting anticoagulation. **Abbreviations:** GP IIβ/IIIα: Glycoprotein IIβ/IIIα; TEG®: Thromboelastography®. Image created with BioRender (https://biorender.com/).

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