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## **Hypercoagulability of COVID-19 patients in Intensive Care Unit. A Report of Thromboelastography Findings and other Parameters of Hemostasis**

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## ABSTRACT

**Background.** The severe inflammatory state secondary to Covid-19 leads to a severe derangement of hemostasis that has been recently described as a state of disseminated intravascular coagulation (DIC) and consumption coagulopathy, defined as decreased platelet count, increased fibrin(ogen) degradation products such as D-dimer as well as low fibrinogen.

**Aims.** Whole blood from 24 patients admitted at the intensive care unit because of Covid-19 was collected and evaluated with thromboelastography by the TEG point-of-care device on a single occasion and six underwent repeated measurements on two consecutive days for a total of 30 observations. Plasma was evaluated for the other parameters of hemostasis.

**Results.** TEG parameters are consistent with a state of hypercoagulability as shown by decreased R and K values, and increased values of K angle and MA. Platelet count was normal or increased, prothrombin time and activated partial thromboplastin time were near(normal). Fibrinogen was increased and D-dimer was dramatically increased. C-reactive protein was increased. Factor VIII and von Willebrand factor (n=11) were increased. Antithrombin (n=11) was marginally decreased and protein C (n=11) was increased.

**Conclusion.** The results of this cohort of patients with Covid-19 are not consistent with acute DIC, rather they support hypercoagulability together with a severe inflammatory state. These findings may explain the events of venous thromboembolism observed in some of these patients and support antithrombotic prophylaxis/treatment. Clinical trials are urgently needed to establish the type of drug, dosage and optimal duration of prophylaxis.

## Essentials

- Covid-19 is associated with a considerable derangement of hemostasis, described as DIC
- Covid-19 is associated with hypercoagulability as shown by whole blood thromboelastography
- Covid-19 patients are candidate to antithrombotic prophylaxis/treatment
- Clinical trials are urgently needed to establish appropriate antithrombotic regime

## **INTRODUCTION**

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the pandemic outbreak across the World [1, 2]. The clinical condition that is associated with the infection has been named Covid-19. The major clinical challenge associated with Covid-19 is severe, often fatal, interstitial pneumonia [3]. The severe inflammatory state secondary to the infection leads to a severe derangement of hemostasis typically observed in patients with sepsis and has been described as a state of acute disseminated intravascular coagulation (DIC), based on decreased platelet count, prolonged prothrombin and activated partial thromboplastin time (PT/APTT), increased fibrin(ogen) degradation products such as D dimer as well as low fibrinogen [4]. Clinical observations made on a series of patients admitted to the intensive care unit (ICU) of our hospital, showed however that a certain number of patients with Covid-19 developed clinical signs of pulmonary embolism and/or deep vein thrombosis of the lower limbs (unpublished observations). The diagnosis of pulmonary embolism in these patients has not been confirmed with imaging or autopsy in all patients, as in most cases the former was not promptly available and the latter is currently not allowed in all hospitals by health authorities. However, pulmonary embolism diagnoses are supported by the fact that most (up to 1/3) Covid-19 patients had deep vein thromboses of the lower limbs as shown by compression ultrasonography. Since a thromboelastography point of care device was available in the ICU, we decided to collect viscoelastic observations in some of the Covid-19 patients.

## **PATIENTS AND METHODS**

### **Patients**

We evaluated on a single occasion 24 intubated Covid-19 patients, randomly selected from those admitted to the ICU because of acute respiratory syndromes. Six of these patients underwent repeated measurements on two consecutive days thereafter, thus totaling 30 observations. Blood was collected from an arterial line. One mL of native whole blood was used to perform thromboelastography and 3.5 mL were collected into

vacuum tubes (Becton and Dickinson, Plymouth, UK) containing 1/10 volume of 0.109 M trisodium citrate and centrifuged for 15 minutes (controlled room temperature) at 2,500g. The supernatant plasma was used to perform tests of hemostasis.

## Methods

**Thromboelastography.** Tests were carried out by TEG (Haemonetics TEG 5000, Braintree, MA), an vitro point of care device, able of assessing the viscoelastic properties of clotting native whole blood upon activation of hemostasis by exogenously added triggers (kaolin). The assay system mimics to some extent what occurs in vivo with the contribution of blood cells, platelets and plasma. TEG evaluation was performed in the presence of heparinase, meant to quench heparin as most of the patients were on prophylaxis with low molecular weight (LMWH) or unfractionated heparin. We recorded the following parameters. R (minutes), which is a measure of the clotting time from the point of coagulation ignition to the appearance of the clot; K (minutes) and K angle, which define the velocity of clot formation; MA (mm), which defines the maximal amplitude of the clot; Lys-30, which defines the percentage decrease of clot amplitude at 30 minutes post-MA. Accordingly, short R, K or Lys-30 as well as high K angle or MA denote hypercoagulability.

**Other tests of coagulation.** PT and APTT were measured by means of commercial reagents, Recombiplastin 2G, or Synthasil APTT, respectively (Werfen, Orangeburg, NY). D dimer and fibrinogen were measured by means of an immunoturbidimetric latex-particle assay or the functional clotting assay according to Clauss, respectively (Werfen). Antithrombin and protein C activities were measured by commercial kits (Werfen). Protein S was measured as the free antigen and factor VIII was measured by the one-stage clotting assay based on APTT and factor VIII-deficient plasma (Werfen). von Willebrand factor antigen and ristocetin cofactor activity were measured by means of commercial kits (Werfen). All tests were performed on the automated coagulometer ACLTop (Werfen, Bedford, MA). PT and APTT results were expressed as ratio of patient clotting time to the clotting time of a pooled normal plasma prepared and routinely used in our laboratory. Results of the other parameters were expressed as activities relative to the pooled

normal plasma to which an arbitrary potency of 100 U/dL was assigned or as concentrations.

## RESULTS AND DISCUSSION

**Thromboelastography.** Twenty-four patients were evaluated by the TEG on a single occasion and six underwent repeated measurements on two consecutive days thereafter for a total of 30 observations. Table 1 shows the viscoelastic as well as hemostasis and other biochemical results. Their values have been compared with the mean of the respective reference range, previously established in our laboratory using 40 healthy adult subjects. R and K values were shorter than the mean value of the reference population in 50% and 83% of the Covid19 population, respectively. K angle and MA values were higher than the mean value of the reference population in 72% and 83% of the Covid-19 population, respectively. Lys-30 was lower than the mean of the reference population in 100% of the Covid-19 population. Fig 1 shows that the distribution of the results for the maximal clot amplitude (i.e., MA) in the patient population was skewed towards higher than normal levels. Fig 2 shows typical TEG tracings for a patient with Covid-19 and a healthy control. Collectively, the thromboelastographic results in patients with Covid-19 denote a state of hypercoagulability.

**Other parameters of hemostasis.** The other tests of hemostasis are shown in Table 1. PT and APTT were normal or slightly prolonged; mean ratio values and range (min–max) were 1.16 (0.99-1.50) or 0.98 (0.78-1.24), respectively. Among the other parameters that are usually employed to assess for DIC, mean (min-max) fibrinogen was greatly increased 680 mg/dL (234-1,344); platelet count was normal(increased)  $348 \times 10^9/L$  (59-577); D dimer was greatly increased 4,877 ng/mL (1,197-16,954). Results for the other parameters of hemostasis were available for 11 patients and were as follows: factor VIII [297 U/dL (223-470)] and von Willebrand factor antigen [529 (210-863)] were greatly increased. Antithrombin [74 U/dL (45-120)] was slightly decreased. Protein C [122 U/dL (75-177)] was increased and the free protein S antigen [69 U/dL (33-109)] was marginally decreased.

Overall, the results of this study support the concept that the hypercoagulability displayed by TEG is due to the profound derangement of hemostasis and is the likely contributor to pulmonary embolism and/or deep vein thrombosis of the lower limbs observed in patients with Covid-19. Other contributions may stem from the endothelial dysfunction (as shown by the greatly increased levels of von Willebrand factor, both antigen and ristocetin co-factor) and sepsis subsequent to the massive infection. There are few studies dealing with hemostasis derangement and Covid-19. Tang et al [5] reported results compatible with a state of DIC, which were however based on such biochemical markers as high fibrin degradation products (e.g., D dimer), prolonged PT/APTT and low platelet counts. Part of the above results are confirmed in this report (e.g., increased D dimer), but other signs of DIC such as prolonged PT/APTT are not confirmed. Furthermore, reduced platelet counts and low fibrinogen clotting activity, which are pathognomonic signs of DIC are normal or even increased in our cohort and do not support the consumption coagulopathy, which is the hallmark of acute DIC. The reasons for the discrepancy is unclear. It cannot be excluded that the two populations, although both admitted to the ICU, were at different time points during the course of the disease progression. Cumulatively, our results suggest that patients with Covid-19 may develop a state of hypercoagulability as shown by the TEG parameters, increased factor VIII, von Willebrand factor and fibrinogen. This hypercoagulability could contribute (in addition to other causes) to the development of pulmonary embolism and/or deep vein thrombosis of the lower limbs. The reasons for the observed hypercoagulability are unknown. Plasma hypercoagulability may be commonly due to increased levels of pro-coagulant factors, decreased levels of the naturally occurring anti-coagulant factors or both. In this cohort factor VIII, which is one of the most potent triggers of hypercoagulability, was strongly increased (up to 460 U/dL, Table1) and the main naturally occurring anticoagulants are (near)normal (i.e., antithrombin) or even increased (i.e., protein C). Additional explanations for the hypercoagulability may be the presence of high numbers of circulating microvesicles. These moieties are cytoplasmic microparticles stemming from platelets or monocytes, which carry the procoagulant asset of the parent cells (i.e., tissue factor from monocytes and phosphatidyl-serine from platelets). Microvesicles are known determinants of venous thromboembolism [6]. Indeed, increased numbers of circulating microvesicles have been reported in septic patients [7] and it is therefore possible that

they are also increased in patients with Covid-19. Neutrophil external traps (NET), which are released from activated neutrophils, constitute a mixture of nucleic DNA, histones and nucleosomes [8], which may add procoagulant substances associated with plasma hypercoagulability and increased risk of thrombosis in animal models and in humans [9, 10]. It is well known that inflammation is closely associated with thrombosis. Pro-inflammatory cytokines are established modulators of coagulation and fibrinolysis activation [11] and might constitute another formidable trigger to explain the procoagulant imbalance in patients with Covid-19. Finally, endothelial derangement may play an additional role. Elevated von Willebrand factor levels found in this study (up to 863 U/dL, Table 1) may be taken as surrogate markers of endothelial derangement in Covid-19. Whatever the reasons, the signs of hypercoagulability observed in this study would support the use of antithrombotic drugs in patients with Covid-19 to quench the procoagulant imbalance and possibly venous thromboembolism (either deep vein thrombosis of the lower limbs or pulmonary embolism).

Some limitations of this study should be recognized. First, blood collection and laboratory tests were not performed for all consecutive patients seen at the ICU in a pre-specified period (i.e., 13-24<sup>th</sup> March 2020). It is therefore possible that there may be a selection bias and that the sample size is not large enough to represent the whole population of Covid-19 patients admitted to ICU. Second, the direct signs of hypercoagulability observed in this cohort rest mainly on the TEG parameters. This device is used to guide transfusion in critically ill hemorrhagic patients [12] and more rarely to detect hypercoagulability, although there are observations showing that TEG is also useful to detect hypercoagulability [13-15]. These limitations notwithstanding, it is of interest to note that a state of hypercoagulability has been shown in these patients. Although, a recent study showed that the antithrombotic prophylaxis with LMWH or unfractionated heparin is associated with decreased mortality in severe Covid-19 patients [16], the benefit/risk ratio of using other anticoagulants, as well as the timing of starting anticoagulation at which dose and how long for, should be evaluated by appropriate randomized clinical trials.

In conclusion, the results observed in our cohort of patient with Covid-19 are not consistent with DIC, rather they support hypercoagulability together with a severe inflammatory state. The hypercoagulability in addition to the clinical finding of pulmonary



embolism and/or deep vein thromboses of the lower limbs observed in some of the Covid-19 patients support antithrombotic prophylaxis with low molecular weight or unfractionated heparin. Escalating the dose from prophylaxis to treatment needs careful consideration based on the benefit/ratio risk at least until clinical trials will inform clinicians on decision making.

### **Author Contribution**

MP and AT conceived the study. NB, PT, GG performed thromboelastography, CN performed hemostasis tests. VC data collection and analysis. AT wrote the manuscript. AP, FP and all authors reviewed data and manuscript.

### **Disclosure**

None to declare

## REFERENCES

1. Li Q, Guan X, Wu P, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med* 2020;NEJMoa2001316.
2. Chan JF-W, Yuan S, Kok K-H, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 2020;395:514-523.
3. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020;395:1054-1062.
4. Taylor FB Jr, Toh CH, Hoots WK, et al. Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation On behalf of the Scientific Subcommittee on disseminated intravascular coagulation (DIC) of the International Society on Thrombosis and Haemostasis (ISTH). *Thromb Haemost* 2001; 86: 1327– 30.
5. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost* 2020. doi: 10.1111/jth.14768.
6. Bucciarelli P, Martinelli I, Artoni A, et al. Circulating microparticles and risk of venous thromboembolism. *Thromb Res.* 2012;129:591-7.
7. Raeven P, Zipperle J, Drechsler S. Extracellular Vesicles as Markers and Mediators in Sepsis. *Theranostics* 2018;8:3348-3365.
8. Kapoor S, Opneja A, Nayak L. The role of neutrophils in thrombosis. *Thromb Res* 2018;170:87-96.
9. Fuchs TA, Brill A, Duerschmied D, et al. Extracellular DNA traps promote thrombosis. *Proc. Natl. Acad. Sci. USA* 2010;107:15880–15885.
10. Ammollo CT, Semeraro F, Xu J, et al. Extracellular histones increase plasma thrombin generation by impairing thrombomodulin-dependent protein C activation. *J. Thromb Haemost* 2011;9:1795–1803.
11. Vazquez-Garza E, Jerjes-Sanchez C, Navarrete A, et al. Venous thromboembolism: thrombosis, inflammation, and immunothrombosis for clinicians. *J Thromb Thrombolysis* 2017;44:377-385.

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12. Thomas W, Samama CM, Greinacher A, Hunt BJ; Subcommittee on Perioperative and Critical Care. The utility of viscoelastic methods in the prevention and treatment of bleeding and hospital-associated venous thromboembolism in perioperative care: guidance from the SSC of the ISTH. *J Thromb Haemost* 2018;16:2336-2340.
  13. Kupcinskiene K, Trepenaitis D, Petereit R, et al. Monitoring of Hypercoagulability by Thromboelastography in Bariatric Surgery. *Med Sci Monit* 2017;23:1819-1826.
  14. Campello E, Spiezia L, Zabeo E, et al. Hypercoagulability detected by whole blood thromboelastometry (ROTEM®) and impedance aggregometry (MULTIPLATE®) in obese patients. *Thromb Res* 2015;135:548-53.
  15. Tripodi A, Chantarangkul V, Gianniello F, et al. Global coagulation in myeloproliferative neoplasms. *Ann Hematol* 2013;92:1633-9.
  16. Tang N, Bai H, Chen X, et al. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Thromb Haemost* 2020. doi: 10.1111/jth.14817.

**Table 1.** TEG (in the presence of heparinase), hemostasis and other biochemical parameters for the investigated population. PT ratio, prothrombin time ratio (patient/normal). APTT ratio, activated partial thromboplastin ratio (patient/normal). VWF Ag, von Willebrand factor antigen. VWF RiCof, von Willebrand factor ristocetin cofactor.

|                                 | Reference Range           | Observations |                           | No (%) <Lower limit or >Upper limit |                | No (%) < or > Mean Normal |                |
|---------------------------------|---------------------------|--------------|---------------------------|-------------------------------------|----------------|---------------------------|----------------|
|                                 | Mean (Lower-Upper limits) | No*          | Mean (min-max)            | <Lower limit                        | >Upper limit   | <Mean                     | >Mean          |
| Age                             |                           | 24           | 56 (23 - 71)              |                                     |                |                           |                |
| PT ratio                        | 1.02 (0.84-1.20)          | 30           | 1.16 (0.99 - 1.50)        | 0 (0)                               | 8 (27)         | 1 (3)                     | 29 (97)        |
| APTT ratio                      | 1.00 (0.86-1.20)          | 30           | 0.98 (0.78 - 1.24)        | 5 (17)                              | 2 (7)          | 19 (63)                   | 11 (37)        |
| D Dimer (ng/mL, FEU)            | <500                      | 30           | 4877 (1197 - 16954)       | 0 (0)                               | 30 (100)       |                           |                |
| Fibrinogen (mg/dL)              | 258 (165.0-350)           | 30           | 680 (234 - 1344)          | 0 (0)                               | 28 (93)        | 1 (3)                     | 29 (97)        |
| Antithrombin (U/dL)             | 102 (82-122)              | 11           | 74 (45 - 120)             | 6 (55)                              | 0 (0)          | 10 (91)                   | 1 (9)          |
| Protein C (U/dL)                | 113 (6-165)               | 11           | 122 (75 - 177)            | 0 (0)                               | 2 (18)         | 4 (36)                    | 7 (64)         |
| Protein S free antigen (U/dL)   |                           | 11           | 69 (33 - 109)             |                                     |                |                           |                |
| Factor VIII (U/dL)              | 99 (51-147)               | 11           | 297 (223 - 470)           | 0 (0)                               | 11 (100)       | 0 (0)                     | 11 (100)       |
| VWF antigen (U/dl)              | 103 (40-165)              | 11           | 529 (210 - 863)           | 0 (0)                               | 11 (100)       | 0 (0)                     | 11 (100)       |
| VWF RiCof (U/dL)                | 96 (41-151)               | 11           | 387 (195 - 550)           | 0 (0)                               | 11 (100)       | 0 (0)                     | 11 (100)       |
| <b>TEG – R (min)</b>            | <b>10.5 (4.0-8.0)</b>     | <b>30</b>    | <b>6.3 (3.0 - 11.9)</b>   | <b>4 (13)</b>                       | <b>6 (20)</b>  | <b>28 (93)</b>            | <b>2 (7)</b>   |
| <b>TEG – K (min)</b>            | <b>2.1 (0.0-4.0)</b>      | <b>30</b>    | <b>1.5 (0.8 - 2.9)</b>    | <b>0 (0)</b>                        | <b>0 (0)</b>   | <b>27 (90)</b>            | <b>3 (10)</b>  |
| <b>TEG - Angle K (degree)</b>   | <b>61.7 (47.0-74.0)</b>   | <b>30</b>    | <b>69.4 (51.1 - 78.5)</b> | <b>0 (0)</b>                        | <b>12 (40)</b> | <b>7 (23)</b>             | <b>23 (77)</b> |
| <b>TEG – MA (mm)</b>            | <b>70.8 (54.0-72.0)</b>   | <b>30</b>    | <b>79.1 (58.0 - 92.0)</b> | <b>0 (0)</b>                        | <b>25 (83)</b> | <b>4 (13)</b>             | <b>26 (87)</b> |
| <b>TEG - LY30</b>               | <b>0 (0-8)</b>            | <b>29</b>    | <b>7.8 (0 - 54.3)</b>     | <b>0 (0)</b>                        | <b>7 (23)</b>  | <b>29 (100)</b>           | <b>0 (0)</b>   |
| Platelets (x10 <sup>9</sup> /L) | 265 (130-400)             | 30           | 348 (59 - 577)            | 1 (3) *                             | 0 (0)§         | 7 (23)                    | 23 (77)        |
| C-reactive                      | <0.5                      | 30           | 16.1 (3.9 - 34.2)         | 0 (0)                               | 30 (100)       |                           |                |

|                 |  |    |                   |  |  |  |  |
|-----------------|--|----|-------------------|--|--|--|--|
| protein (mg/dL) |  |    |                   |  |  |  |  |
| Ferritin (µg/L) |  | 11 | 1485 (452 - 5792) |  |  |  |  |

\*platelets <50x10<sup>9</sup>/L

§platelets >50<100x10<sup>9</sup>/L

\*Measurements were repeated on two consecutive days in 6 patients

## LEGENDS TO FIGURES

**Fig 1.** Result distribution of the maximal clot amplitude (MA) in the patient population.

Solid and broken lines represent the univariate density estimation and the limits of the reference range, respectively.

**Fig 2.** Typical TEG tracings. Broken and solid lines represent a healthy subject and a

COVID-19 patient, respectively. The Covid-19 patient was characterized by the following

TEG parameters: R=5.5 min. K=0.9 min. Angle K=78.8°. MA=88.8 mm and by the

following hemostasis parameters: Prothrombin time ratio=1.19. Activated partial

thromboplastin time ratio=1.21. D dimer=1,829 ng/mL. Fibrinogen=849 mg/mL.

Antithrombin=87 U/dL; Protein C=116 U/dL. Protein S (free antigen)=68 U/dL. Factor

VIII=269 U/dL. von Willebrand factor (antigen)=476 U/dL. von Willebrand factor (ristocetin cofactor)=347 U/dL. Platelet count= $546 \times 10^9/L$ .



