Viscoelastic Tests in the Evaluation of Haemostatic Disorders in SARS-CoV-2 Infection

Testes Viscoelásticos na Avaliação de Alterações da Hemostase na Infeção por SARS-CoV-2

ABSTRACT

COVID-19 associated coagulopathy is a dysfunction of severe SARS-CoV-2 infection, characterized by significantly increased fibrinogen, D-dimer and C reactive protein and normal to near-normal prothrombin time, activated partial thromboplastin time and platelet count. Hypercoagulopathy and hypofibrinolysis coexist and are detected by viscoelastic tests. These features, when associated with immobilization and intrinsic risk factors (age, obesity, comorbidities, drugs) of the patient, can trigger thromboembolic events, despite thromboprophylaxis. The lungs are the first and most severely damaged organ. To date, most patients have exhibited hypercoagulability on viscoelastic tests not detected by standard coagulation tests. A high rate of thrombotic events was reported, suggesting that it should be considered as a cause of clinical deterioration in intensive care and potentially other clinical settings. In advanced stage, COVID-19 associated coagulopathy, fibrinogen and platelet count can decrease significantly, depending on the severity of clinical status resembling consumptive coagulopathy. In this stage, bleeding events can occur, especially if the patient is under extracorporeal membrane oxygenation (ECMO). Viscoelastic tests are very useful tools to assess hypercoagulability and hypofibrinolysis (not detectable by standard coagulation tests) in critically ill SARS-CoV-2 patients with COVID-19 associated coagulopathy and look like very promising tools for anticoagulation management. However, further research needs to be carried out to determine whether abnormal viscoelastic tests alone or in combination with other clinical or laboratory findings can identify patients at increased thrombotic risk. Clinical trials to evaluate hypercoagulability using viscoelastic tests and the need for personalized dosage of anticoagulation in SARS-CoV-2 patients are quickly emerging.

Keywords: Blood Coagulation Disorders; Blood Coagulation Tests; Coronavirus Infections; COVID-19; SARS-CoV-2; Thrombosis

RESUMO

A coagulopatia associada à COVID-19 é uma disfunção associada à infecção SARS-CoV-2 grave, caracterizada por aumento significativo do fibrinogênio, D-dimeros e Proteína C reativa, e por valores normais/muito pouco alterados do tempo de protrombina, tempo de tromboplastina parcial ativado, e número de plaquetas. A hipercoagulabilidade e a hipofibrinólise coexistem e são detetadas por testes viscoelásticos. Quando associados à immobilização e aos fatores de risco intrínsecos do doente (idade, obesidade, comorbididades, drogas) potenciam eventos tromboembólicos, apesar da tromboprofilaxia. Os pulmões são o órgão inicialmente e mais gravemente afetado. Até à data, a maioria dos doentes apresentou hipercoagulabilidade nos testes viscoelásticos, não detetada pelos testes de coagulação de rotina, e foi reportada uma elevada taxa de eventos trombóticos, sugerindo que esta deveria ser considerada uma das causas de deterioração clínica, não só em cuidados intensivos. Na coagulopatia associada à COVID-19 avançada, o número de plaquetas e o fibrinogênio podem diminuir significativamente, dependendo da gravidade clínica da infecção, assemelhando-se o quadro a uma coagulopatia de consumo. Nesta fase pode haver hemorragia, especialmente se o doente estiver sob extracorporeal membrane oxygenation. Os testes viscoelásticos afligiram-se muito úteis para avaliar a hipercoagulabilidade e a hipofibrinólise em doentes críticos SARS-CoV-2 com coagulopatia associada à COVID-19, parecendo também promissores para a gestão da anticoagulação. No entanto, é necessária mais investigação para determinar se testes viscoelásticos alterados, individualmente ou quando combinados com outros resultados clínicos/laboratoriais, podem identificar os doentes com risco trombótico acrescido. Estão a emergir rapidamente ensaios clínicos para avaliação da hipercoagulabilidade por testes viscoelásticos e da necessidade de personalização da anticoagulação em doentes SARS-CoV-2.

Palavras-chave: COVID-19; Infecções por Coronavirus; Perturbações da Coagulação Sanguínea; SARS-CoV-2; Testes de Coagulação Sanguínea; Trombose

INTRODUCTION

The major challenge associated with COVID-19 is severe, often fatal, interstitial pneumonia.1 COVID-19 mortality burden is mainly attributable to a progressive bilateral pneumonia that can progress to acute respiratory distress syndrome (ARDS), requiring intensive care support.2 While the pulmonary pathophysiology is not fully understood, severe COVID-19 infection is associated with an alveolar inflammatory cell infiltrate, and a systemic cytokine storm.1 Pro-inflammatory cytokines are modulators of coagulation and fibrinolysis activation and might constitute another trigger to explain the procoagulant imbalance in these patients.3 Endothelial injury may play an additional role.3 Post-mortem studies corroborate that explanation, highlighting marked pathological changes involving lung microvasculature, disseminated micro-thrombi and haemorrhagic necrosis.4 Severe COVID-19 is also associated with an increased risk of deep vein thrombosis (DVT) and pulmonary embolism (PE).3


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Several studies reported a COVID-19 associated coagulopathy (CAC), which is one of the most significant poor prognostic features. CAC is characterized by significantly increased fibrinogen, D-dimer and C reactive protein (CRP) and normal to near-normal prothrombin time (PT), activated partial thromboplastin time (APTT) and platelet count. Hypercoagulopathy and hypofibrinolysis coexist and are more easily detected by viscoelastic tests (VET), since conventional coagulation and fibrinolytic tests only reflect a part of the coagulation system.

VET are in vitro point of care (POC) devices, capable of assessing viscoelastic properties of clotting native whole blood upon activation of hemostasis by added triggers. This technology provides a fast and dynamic assessment of haemostasis, and it is a validated device for clinicians to make an early diagnosis of the coagulopathy and to choose the most appropriate targeted treatment. VET also measure hypercoagulability in various clinical scenarios which is not detected by standard coagulation tests (SCT). Global hemostatic tests such as tromboelastography and thromboelastometry are more reliable in the early identification of a hypercoagulable state (HS) and management of thrombotic scenarios in COVID-19 infection but these are not commonly used yet. We may consider using one of the VET as a screening tool or to optimize anticoagulation therapy, particularly in acute intensive care units (ICU), although this requires further research.

The aim of this paper is to review all relevant scientific data on the use of VET in detecting the hypercoagulability profile of SARS-CoV-2 infection and its possible role in tailoring antithrombotic therapy.

MATERIAL AND METHODS


RESULTS

Viscoelastic testing

This review will address two similar technologies, thromboelastography (TEG®) and thromboelastometry (ROTEM®), and sonic estimation of elasticity via resonance (SEER) sonorheometry (Quantra®).

TEG® and ROTEM® are both established POC VET with a wide range of applications in several scenarios. Both devices measure coagulation in whole blood under static low shear stress conditions, without blood vessels or flow contribution. When a clot starts to form inside the cup, it generates a graphically transduced signal. The magnitude of displacement is referred to as ‘clot amplitude or firmness’ and relates to the strength of the clot. The viscoelastic changes that occur during coagulation are recorded, providing a graphical representation of the fibrin polymerization. This process depends on platelets, fibrinogen, and the presence of pro and anticoagulants.

These devices showed a good correlation with the assessment of coagulation factors, fibrinogen and platelet count in critically ill patients. Goal-directed transfusion therapy using this technology in different bleeding settings (trauma, perioperative and post-partum hemorrhage) are now fully studied and applied with success. This technology has been successfully applied to areas where conventional testing is inadequate, such as hypercoagulability screening, assessment of thrombotic risk and the effects of systemic anticoagulants. Its implementation requires adequate technical and interpretation training, and interdisciplinary cooperation.

All VET parameters are shown in Table 1 and ROTEM® assays and parameters are described elsewhere. ROTEM® and TEG® parameters are comparable but not interchangeable (Table 1).

The use of thromboelastometry and thromboelastography in the detection of HS has been described in several clinical conditions. A short clotting time (CT) or reaction time (R-time) has been associated with a prothrombotic state. The ‘thickness’ of the VET tracing (maximum amplitude (MA) on TEG® or maximum clot firmness (MCF) amplitude at ten minutes (A10)- INTEM or FIBTEM on ROTEM®) appears to be the most useful parameter in guiding transfusion and predicting thrombotic complications. However, no definitive definition of HS based on VET has been established.

A Chinese expert consensus, as well as other authors, recommend the use of VET to evaluate severe CAC and monitor anticoagulation therapy in critically ill patients. Direct thrombin inhibitors (DTI) (argatroban, bivalirudin) are used in COVID-19 infected patients with significantly lower antithrombin levels, or if heparin induced thrombocytopenia (HIT) occurs. DTI inhibit thrombin in the Clauss reagent, underestimating fibrinogen quantification, and making VET a valid method for monitoring fibrinogen levels in this setting.

Emerging studies using VET aim to address the hemostatic changes found in SARS-CoV-2 infection, but no prospective randomized clinical trial (RCT) data is available.

ROTEM® (rotational thromboelastometry)

Overview

ROTEM® delta is a semi-automated system that provides four independent channels. The ROTEM® sigma is a cartridge-based fully automated closed system, including four assays (Table 1, Fig. 1).

Thromboelastometry has shown to be useful in identifying hypercoagulability in various clinical settings not detected by SCT, through the presence of an accelerated clot formation with significantly lower clot formation time (decreased CFT-EXTEM/INTEM/FIBTEM) due to the increase of plasma fibrinogen and excessive thrombin generation, and/or an increase in clot strength (increased MCF-EXTEM/INTEM/FIBTEM and A10) and higher α
ARTIGO DE REVISÃO

NA

HTEG

systemic fibrinolysis (SF)

30

APTT

NA

30

with a shorter CFT and high clot firmness

26

32

CTR

Hemostatic Factors

anal and therefore a higher dose

CTH

32,33

NA

27

and decreased

Heparin presence

32,33-

R

and that robust anticoagulation

- R

Coagulation factors, Fibrinogen,

- Coagulation factors, Fibrinogen,

- Platelets, FXIII, Colloids

Fibrinogen

( inhibition of platelets to
evaluate only the contribution
of fibrinogen for clot strength)

Platelet deficit and/or
dysfunction

Platelet deficit or

Heparin presence

FVIII deficit

Hepatitis C virus (HCV)

Coagulation factors: Anticoagulants; FDP; Tissue
factor-expression on monocytes

Coagulation factors from intrinsic
pathway

Table 1 – Viscoelastic tests’ corresponding parameters and laboratory tests16,21

<table>
<thead>
<tr>
<th>Clot initiation</th>
<th>ROTEM®</th>
<th>TEG®</th>
<th>Quantra®</th>
<th>Standard Laboratory</th>
<th>Hemostatic Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CT</td>
<td>R</td>
<td>CT</td>
<td>PT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EXTEM</td>
<td>(min)</td>
<td>INTEM</td>
<td>APTT</td>
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<tr>
<td></td>
<td>A10</td>
<td>(s)</td>
<td></td>
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<td></td>
<td>MCF</td>
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<tr>
<td></td>
<td>A10 EXTEN</td>
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<tr>
<td></td>
<td>A5, A10</td>
<td></td>
<td>A30, A60</td>
<td>CS</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>(mm)</td>
<td></td>
<td>MA</td>
<td>(hPa)</td>
<td>Coagulation factors, Fibrinogen, Platelets, FXIII, Coagulation factors deficit, Fibrinogen, Platelets.</td>
</tr>
<tr>
<td></td>
<td>A10 EXTEN</td>
<td></td>
<td></td>
<td>NA</td>
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<tr>
<td></td>
<td>PCS</td>
<td></td>
<td></td>
<td>Platelet Count</td>
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<td></td>
<td>(hPa)</td>
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<td></td>
<td>(PCS = CS - FCS)</td>
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<td></td>
<td>A10 FIBTEM</td>
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<td>Difference:</td>
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<td></td>
<td>A10 EXTEN-</td>
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<tr>
<td></td>
<td>A10 FIBTEM</td>
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<td>A5, A10</td>
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<td>A30, A60</td>
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<td></td>
<td>(mm)</td>
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<td>MA</td>
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<tr>
<td></td>
<td>LI30, LI60</td>
<td>(%)</td>
<td></td>
<td>CSL</td>
<td></td>
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<tr>
<td></td>
<td>(LY30, LY60)</td>
<td></td>
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<tr>
<td></td>
<td>ML (%)</td>
<td></td>
<td>ML EXT &gt; 15%</td>
<td>Unestablished</td>
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<tr>
<td></td>
<td>ML APT</td>
<td></td>
<td>NV</td>
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<td></td>
<td>Anticoagulant (heparin)</td>
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<td></td>
<td>Assessment</td>
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<tr>
<td></td>
<td>CT</td>
<td>HTEG</td>
<td>CTH</td>
<td></td>
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<td></td>
<td>HEPTEM</td>
<td>(min)</td>
<td></td>
<td>NA</td>
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<tr>
<td></td>
<td>Ratio:</td>
<td>CT</td>
<td>IN/CT HEP</td>
<td></td>
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<tr>
<td></td>
<td>HTEG</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>CTR Ratio:</td>
<td>CT/CT</td>
<td></td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

A: amplitude; A5 / A10: amplitude at 5/10 minutes after CT; APT: APT/PT; APT: activated partial thromboplastin time; CFT: clot formation time; CS: clot stiffness; CSL: clot stiffness lysis; CT: clotting time; s: second; CTH: clot heparinase; CTR: CFT ratio; EXT: EXTEM; FCS: fibrinogen contribution to CS; FDP: fibrin degradation products; FIB: FIBTEM; FID: fibrin fdegradation products; FIBTEM, HEPTEM; hPa: hector Pascals units; INT: INTEM; K: kinetic time; LI: lysis index; LI30/LI60: lysis index, residual clot firmness, 30 and 60 minutes after CT; MCF, maximum clot firmness; ML: maximum lysis during run time in % of MCF; min: minutes; mm: millimeters; NA: not applicable; NV: normal value; PCS: platelet contribution to CS; PT: prothrombin time; R: reaction time; VET: viscoelastic tests; HTEG assay: is based on rapid TEG assay, containing heparinase to neutralize the effect of unfractionated heparin (UH). The authors suggested: shorter CFT-INTEM (s), shorter CFT-EXTEM (s), no thromboembolic (TE) or hemorrhagic events (HE) were observed and D-dimers have decreased.23 The authors suggested that VET may have a role in rapidly identifying severe COVID-19 in critically ill patients with hypercoagulability,32 not detected with SCT,32 and that robust anticoagulation might be needed to prevent TE.32,33 A study by Spieza et al.30 included 22 patients admitted to the ICU and showed evidence of hypercoagulability on ROTEM® with a shorter CFT and high clot firmness (MCF).11,30 Compared with healthy controls, the following results were observed:30: (a) significantly higher fibrinogen and D-dimer levels (p < 0.0001); (b) markedly hypercoagulability on ROTEM®: shorter CFT-INTEM (p = 0.0002) and CFT-EXTEM (p = 0.01), and higher MCF in INTEM/EXTEM/FIBTEM (p < 0.001). The authors concluded that COVID-19 patients with acute respiratory failure (ARF) presented a severe HS rather than consumptive coagulopathy (CC). Unfortunately, this data did not allow for the assessment of the impact of adequate dosages of anticoagulants on clotting parameters.11 According to Madathil et al.,34 systemic fibrinolysis (SF) was not detected on EXTEM or FIBTEM (maximum lysis-ML: 0%) in 11 critically ill COVID-19 patients receiving MV,

Figure 1 – Viscoelastic Tests (VET): normal and hypercoagulable profile

Despite increased CRP and D-dimer levels. Circulating plasminogen activator inhibitor-1 (PAI-1) was increased and SF is thus unlikely to occur in COVID-19 with cytokine storm. Very high D-dimers along with high CRP levels and A10-FIBTEM were observed, especially in the subset of patients with very high D-dimer levels (> 3245 ng/mL), but without elevated ML-EXTEM (0%), suggesting that SF is unlikely to occur. They propose that critically ill COVID-19 patients have significant elevations in D-dimer levels consistent with microvascular thrombosis, but only small fractions of fibrin seem to be locally broken down.¹⁄₄

Fibrinolysis shutdown was evidenced in other studies¹⁄₄ by raised D-dimer levels and complete failure of clot lysis at 30 minutes on thromboelastography (LY30) and thromboelastometry (LI30), which predicts and correlates with TE and the need for hemodialysis in critically ill COVID-19 patients.¹⁄₄,⁵ Authors hypothesize that the main source for elevated D-dimer levels in the presence of hypercoagulability, along with decreased fibrinolysis, could be the lungs.⁵

Another study⁶ conducted in 78 COVID-19 patients (48 with ARDS in ICU; 30 in ward), despite substantial heparin plasma levels (mean anti-Xa activity of 0.35 ± 0.20 IU/mL) and normal levels of antithrombin in 91% of patients, thrombin generation was normal. These findings, along with significantly increased fibrinogen and FVIII levels, suggested high risk of hypercoagulability, probably related to major inflammatory syndrome not controlled by heparin.⁶

A TEM-IPA assay was used to detect both hypercoagulability and hypofibrinolysis simultaneously, and is a promising biomarker of thrombosis risk.⁶
<table>
<thead>
<tr>
<th>Clinical trial 2020</th>
<th>n</th>
<th>Age (mean)</th>
<th>Sex M/F</th>
<th>Study local</th>
<th>Clinical status</th>
<th>Standard laboratory</th>
<th>ROTEM profile</th>
<th>Study conclusion, AC therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raval et al 32</td>
<td>1</td>
<td>63 years</td>
<td>M</td>
<td>USA</td>
<td>ARDS, ICU Shock, MV; vasopressor support (VS)</td>
<td>-↑ DD</td>
<td>Hypercoagulable profile: -↑ MCF-EXT, INT, FIB</td>
<td>- 7500 IU UFH 8th for prophylaxis - No TE or HE; JD. - VET may have a role in rapidly identifying severe SARS-CoV-2.</td>
</tr>
<tr>
<td>Iwasaki et al 23</td>
<td>1</td>
<td>53 years</td>
<td>F</td>
<td>Japan</td>
<td>ARDS, ICU Severe pneumonia</td>
<td>-↑ DD, CRP -NV: PT, APTT, Platelet.</td>
<td>Hypercoagulability: -↑ MCF-INT, FIB</td>
<td>- UFH (10,000IU/day) Hypercoagulability not seen with SCT but detected with Rotem. Robust anticoagulation might be needed to prevent TE in a subset of COVID-19 pts.</td>
</tr>
<tr>
<td>Speia et al 20</td>
<td>22</td>
<td>68 ± 8 years</td>
<td>M: 20</td>
<td>Padova Univ. Hospital Italy</td>
<td>Acute respiratory failure (ARF) ICU</td>
<td>Significantly: -↑ DD -↑ Fibrinogen Both: p&lt;0,0001</td>
<td>Marked hypercoagulability (vs healthy controls): ↓CFT-INT (p = 0.0002) ↓CFT-EXT (p = 0.0011)</td>
<td>- SARS-CoV-2 with ARF present a severe hypercoagulability rather than a CC. - Fibrin formation/polymerization may predispose to TE and correlate with worse outcome.</td>
</tr>
<tr>
<td>Madathil et al 34</td>
<td>11</td>
<td>53 (45.5 - 65.5) years</td>
<td>M: 64%</td>
<td>Maryland USA</td>
<td>ARDS, ICU MV AH: 54.5% Diab: 45.5%</td>
<td>Significantly (sig.): ↑ DD ↑ Fibrinogen ↑ ORP</td>
<td>Despite significantly high CRP+ DD, systemic fibrinolysis (SF) was not seen on EXT or FIB (ML = 0%). SF is thus unlikely to occur in SARS-CoV-2 with cytokine storm</td>
<td>Critically ill SARS-CoV-2 pts demonstrate significantly ↑ in DD consistent with microvascular thrombosis, but only small fractions of fibrin seem to be locally broken down, and no SF was observed. - Fibrinolysis shutdown</td>
</tr>
<tr>
<td>Ibáñez et al 25</td>
<td>19</td>
<td>61 (55 - 73) years</td>
<td>M: 10</td>
<td>Hospital Clinic Barcelona, Spain</td>
<td>ARDS, ICU MV AH: 47% Diab: 19% DI/SC: 1:1.8</td>
<td>SCT, DD plus ROTEM 24-48h after ICU admission:</td>
<td>- Hypercoagulability: ↓ clot firmness: MCF-EXT/FIB - Fibrinolysis shutdown: ↓ clot lysis:Li30/ Li60-EXT/FIB:100% - 99% - No sig. correlation between Rotem, DD and SOFA score</td>
<td>- All pts under thromboprophylaxis Conclusion: ROTEM showed hypercoagulability with decreased fibrinolytic capacity despite increased DD, of which main source could be the lungs</td>
</tr>
<tr>
<td>Nougier et al 36</td>
<td>78</td>
<td>60.2 ± 14.4 years</td>
<td>M: 51</td>
<td>Hospital Edouard Herriot Lyon, France</td>
<td>ICU: ARDS MV - 66.7% KRT - 14.6%</td>
<td>Significantly (sig.): ↑ DD, (+ICU) ↑ Fibrinogen ↑ Peak Thrombin (+ICU) ↑ FVIII, ETP ↑ α2antiplamin ↑ 4-PA, PAI-1 ↑ TAFI la/AT</td>
<td>- Hypercoagulability: ↑ MCF ↑ TEM-1/PA-MCF ↑ TEM-1 α angle - Fibrinolysis shutdown: ↓ clot lysis Li30 (+ICU)</td>
<td>- All under thromboprophylaxis 29%/ICU: thrombosis-8 PE, 5 DVT,1 aortic Conclusion: ↑ thrombin generation capacity which remained within NV despite heparin, and hypofibrinolysis mainly associated with ↑PAI-1 levels. Both contribute to thrombosis risk despite adequate AC therapy. Modified ROTEM (TEM-4 PA) is able to detect HS+ hypofibrinolysis at the same time in COVID-19 with thrombosis</td>
</tr>
<tr>
<td>Pavoni et al 2 6</td>
<td>40</td>
<td>61 ± 13 years</td>
<td>M: 60%</td>
<td>Florence, Italy</td>
<td>ARDS, ICU Severe pneumonia</td>
<td>SCT, DD + ROTEM tests performed at admission (T0) and 5 (T5) and 10 (T10) days after hospital admission</td>
<td>Hypercoagulability: -↑ CCT, INT; 40-50% EXT-50%pts, - Sig. higher CS: MCF-INT-50%, EXT-70%, FIB-72.5% pts - This HS persist in the first 5 days, but it ↓ 10 days after, without returning to NV</td>
<td>- ROTEM analysis showed that an inflammatory state was associated with a severe hypercoagulability profile, rather than a CC, that persisted over time. Hypofibrinolysis not found on ROTEM or SCI - SCT fail to highlight the severity of prothrombotic profile, where ROTEM can help be very useful</td>
</tr>
</tbody>
</table>

Table 2 – Hypercoagulability evaluation on SARS-CoV-2 infection using viscoelastic testing (section 1 of 4)
Hypercoagulable profile: clinical case
- ↑ Fibrinogen
- No TE or HE; ↓ DD.
- ↑ α angle EXTEM identifying severe SARS-CoV-2.
- UFH (10 000 IU/day)

Severe pneumonia
- ↑↑ DD, CRP
- ↑↑ Fibrinogen
- ↑↑ TEM t-PA α angle

Hypercoagulability versus years
- ↑↑ DD
- ↑↑ Fibrinogen
- ↓ CFT-FIBTEM

Hypercoagulability rather than a CC.
- ↑↑ Fibrinogen
- ↓ CFT-INT
- ↑↑ A10, MCF-EXT/INT/FIB

Signs of organ dysfunction.

SCT, DD + ROTEM tests performed at admission (T0) and 5, 10 days after hospital admission (T5) and 10 (T10) days after hospital admission to NV 0.017, 0.002

Table 2 - Hypercoagulability evaluation on SARS-CoV-2 infection using viscoelastic testing (section 2 of 4)
Table 2 – Hypercoagulability evaluation on SARS-CoV-2 infection using viscoelastic testing (section 3 of 4)

<table>
<thead>
<tr>
<th>Clinical trial 2020</th>
<th>n</th>
<th>Age (mean)</th>
<th>Sex M/F</th>
<th>Study local</th>
<th>Clinical status</th>
<th>Standard laboratory</th>
<th>TEG profile</th>
<th>Study conclusion, AC therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panigada et al 1</td>
<td>24</td>
<td>NA</td>
<td>NA</td>
<td>Hospital Maggiore, Milan, Italy</td>
<td>ARDS, ICU</td>
<td>-1 Fibrinogen</td>
<td>Hypercoagulability state (HS): -1 R (50% patients) -1 K (83% patients) -1 LYS30 (100% patients) -1 K angle (72% patients) -1 MA (83% patients)</td>
<td>-Hypercoagulability along with a severe inflammatory state - may explain TE (PE/DVT) observed in some and support thromboprophylaxis. -Escalating dose from prophylaxis to treatment needs careful decision based on risk/benefit ratio, at least until clinical trials can inform about the best decision.</td>
</tr>
<tr>
<td>Wright et al 44</td>
<td>44</td>
<td>54 (42 - 59)</td>
<td>M: 63.6%</td>
<td>Univ. Colorado, Denver, USA</td>
<td>ARDS, ICU</td>
<td>-1 DD</td>
<td>Hypercoagulability state (HS): -1 MA; LYS30; ↓ R; ↑ K angle -LY30 of 0% (57% pts) predicted VTE (p = 0.021) -LY30 = 0% + DD &gt; 2.6 ng/mL: with markedly high risk: Renal failure (80%, p = 0.004), VTE and TE (50%, p = 0.008)</td>
<td>COVID-19-associated HS measured by TEG + associated fibrinolysis shutdown (defined by LY30 &lt; 0.8%). Fibrinolysis shutdown predicts TE and need for dialysis in severe COVID-19. Additional trials are required to ascertain the need of early AC and fibrinolytic therapy (t-PA).</td>
</tr>
<tr>
<td>Maatman et al 40</td>
<td>12/109</td>
<td>61 ± 16 (18 - 95)</td>
<td>M: 62%</td>
<td>Indianapolis (three Hosp.) USA</td>
<td>ARDS, ICU</td>
<td>-1 DD, Fibrinogen</td>
<td>Hypercoagulability state: ≥ 2 and ≥ 1 hypercoagulable TEG Parameters in 58% and 83% pts Respectively. -↑ R (67% pts); ↓ K; ↓ LYS30 -↑ α angle; ↓ MA; ↓ CI -CI hypercoagulable in 50%pts</td>
<td>COVID-19 results in a HS. HS and/or fibrinolysis shutdown were found to have higher rate and shorter time to VTE (40% vs 5% in pts without shutdown, p = 0.013). Routine VTE prophylaxis may be inadequate in TE prevention in severe COVID-19.</td>
</tr>
<tr>
<td>Mortus et al 40</td>
<td>21</td>
<td>68 (50 - 89)</td>
<td>M: 57%</td>
<td>Baylor College of Medicine, Houston, USA</td>
<td>ARDS, ICU</td>
<td>-1 DD</td>
<td>Hypercoagulable TEG (90%): 74% TEG defined by fibr. activity (1 Kx) + MA criteria; 26% TEG defined only by MA criteria. -Innate TEG MA: sig. greater for the high TE rate group than the low TE group (75mm vs 61mm; p = 0.01); providing 100% sensitivity and 100% negative predictive value.</td>
<td>All under thromboprophylaxis TE: 62% → therapeutic antiocoagulation -TEG may be critical in accurately identifying pts at ↑ thrombosis risk (where full heparinization is needed), avoiding unnecessary AC in low thrombosis risk group.</td>
</tr>
<tr>
<td>Eugene Fan et al 40</td>
<td>1</td>
<td>39 years</td>
<td>M (Bangladesh)</td>
<td>Hospital Tan Tock Seng, Singapore</td>
<td>Ward: no AC ↓ D + 13: Acute limb ischaemia + CAC, extensive arterial thrombosis → UFH + vascular surg. → successful endovascular Stent Graft exclusion of aortic thrombus and right lower limb embolotomy On presentation of acute ischaemic limb → HS: ↓ PT, APTT, ↓ CRP, Lupus AC (LA) ↓ Ab anticardiolipin IgG/IgM ↓ DD, ↓ Fibrinogen ↓ F: ↓ FII, FV, FVIII, ↓ FIX, WF:Ag NV: PC, PS, AT, Homocysteine DIC: score 2 (low DIC risk)</td>
<td>UFH pre-operative → successful vascular surgery → LMWH on post-operative day 1 (mean anti-Xa 0.61 UI/mL) + aspirin to prevent in stent thrombosis → warfarin ≥ 12 weeks Conclusion: TEG appears to be a useful tool in detecting HS even in the presence of heparin or anti-phospholipid syndrome in CAC. VET may be useful in the early identification of a HS and management of thrombosis in covid-19 infection.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Rodrigues A, et al. Viscoelastic tests in the evaluation of haemostasis disturbances in SARS-COV-2 infection, Willebrand factor: antigen; UFH: unfractionated heparin; α: alpha; α2-AP: alpha 2 antiplasmin; Y: years; Wards: regular wards; vWF:Ag: von versus: vs PC: protein C; PS: protein S; PT: prothrombin time; R: reaction time (= clotting time); SCT: standard coagulation tests; F: female; M: male; MA: maximal; FCS: fibrinogen contribution to the CS; Fibr: fibrinogen; GP: glycoprotein; Hosp: hospital; HS: hypercoagulable state; IL-6: interleukin 6; INR: international normalized ratio; HE: hemorrhagic event; IU: international unit; INT: INTEM; TE: TEA.

The main finding of this study was the increased fibrinogen contribution to the CS in SARS-COV-2 patients, mainly or more pronounced compared to non-covid-19 patients. The prothrombin time (PT) was also significantly increased in SARS-COV-2 patients, indicating a procoagulant state. The activated partial thrombin time (APTT) was also significantly increased in SARS-COV-2 patients, indicating a procoagulant state.

Compared with non-covid-19 patients, COVID-19 patients exhibited significantly increased procoagulant factors, mainly:

-↑↑ FCS (p=0.02)
-↑↑ FVIII (p=0.043)
-↑↑ FV (p=0.02)
-↑↑ Fibrinogen (p=0.02)
-↑↑ α1-anti GP (p=0.05)

No fibrinolysis shutdown seen, neither CC nor PCI.

Conclusion: The procoagulant pattern of the coagulation profile in SARS-COV-2 infection is mainly due to the increased fibrinogen contribution to the CS and the increased procoagulant factors. The procoagulant state is closely correlated with the inflammation response, associated with Th17 and Th22 cytokine production. The procoagulant state is also associated with the increased fibrinogen contribution to the CS, suggesting that the systemic inflammatory response is a major contributor to the procoagulant state in SARS-COV-2 infection.

The study was conducted in a single-center, prospective, observational study on SARS-COV-2 patients admitted to the ICU. All patients received LMWH. The study was conducted in Paris, France, in 2020. The study cohort included 42-58 patients, with a mean age of 51 years. The study was conducted in a single-center, prospective, observational study on SARS-COV-2 patients admitted to the ICU. All patients received LMWH. The study was conducted in Paris, France, in 2020. The study cohort included 42-58 patients, with a mean age of 51 years. The study was conducted in a single-center, prospective, observational study on SARS-COV-2 patients admitted to the ICU. All patients received LMWH. The study was conducted in Paris, France, in 2020. The study cohort included 42-58 patients, with a mean age of 51 years.
The study by Pavoni et al. included 40 critically ill COVID-19 patients where SCT and ROTEM® were evaluated on ICU admission day (T0), and days 5 (T5) and 10 (T10). On ICU admission, PT was slightly reduced, and increased significantly at T10 (p = 0.002), while APTT and fibrinogen values were higher at T0 than T10 (p = 0.017; p = 0.002, respectively). Platelet count was normal and increased over time. About 70% of patients had higher D-dimer levels that decreased at T10 (p = 0.392). ROTEM® profiles were consistent with hypercoagulability (lower CFT, increased MCF) which persisted in the first five days, decreasing after ten days, without returning to normal values. No signs of secondary hyperfibrinolysis or sepsis-induced coagulopathy were found. From the total, 15% and 30% of the patients had DVT and catheter-related thrombosis, respectively, despite low molecular weight heparin (LMWH) prophylaxis. In conclusion, ROTEM® showed significant evidence of a HS in severe COVID-19 that persisted over time associated with an inflammatory state, rather than CC. The improvement of fibrinogen clot firmness measurements after 10 days of illness suggests a dynamic component for the changes in coagulation that accompany the rise and fall of inflammatory parameters.

Almskog et al. evaluated whether patients with severe disease have more pronounced hypercoagulability compared with less severely ill COVID19 patients, using ROTEM® analysis. They included 60 COVID-19 patients divided into two groups depending on care level (regular wards and ICU) and were compared with 89 healthy controls. SCT and ROTEM® analysis were performed as soon as possible after admission. In total, 80% of patients received prophylaxis with LMWH. The levels of INR, APTT, and platelets were nearly normal, although markedly elevated D-dimer and fibrinogen levels were observed. There was no significant correlation between D-dimer and MCF-EXTEM (p = 0.9), but it was significant between fibrinogen and MCF-FIBTEM (p = 0.001). When compared with healthy controls, the ROTEM® variables of COVID-19 patients were significantly higher in both groups (ward/ICU) (p < 0.0001), and were higher in critically ill patients compared with the less severely ill patients in regular wards (p < 0.01 for MCF-EXTEM and p < 0.05 for FIBTEM); CT-EXTEM was significantly longer, particularly in ICU patients (p < 0.001), and CFT-EXTEM significantly shorter in COVID-19 (p < 0.001). All these ROTEM® variables determined early after admission were significantly more pronounced in patients with increased severity. This suggested prolonged hemostatic initiation, shortened clot propagation, and pronounced clot firmness, indicating hypercoagulability. The authors concluded that hypercoagulability is present in hospitalized patients at an early disease course with mild to severe COVID-19 pneumonia and ROTEM® analysis may be a potentially useful predictor of TE and mortality.

Two prospective studies demonstrated that most patients admitted to ICU with severe SARS-CoV-2 infection showed a pronounced HS, characterized by increased fibrinogen and D-dimer levels, impaired endogenous anticoagulation (decreased protein S levels), decreased fibrinolysis, and increased MCF-INTTEM/EXTEM/FIBTEM. TE occurred in 20% and 33% of patients despite appropriate anticoagulation, and HE in 10%. The magnitude of coagulation abnormalities seemed to correlate with the severity of organ dysfunction according to the sequential organ failure assessment (SOFA) score being higher if SOFA was over 10. Compared with SOFA < 10, COVID-19 patients with SOFA > 10 exhibited higher fibrinogen and D-dimer levels, higher MCF-FIBTEM (p = 0.05), lower ML-INTTEM (p = 0.004), and lower antithrombin, protein C and plasminogen levels.

TEG® (thromboelastography)

Overview

Thromboelastography parameters include reaction time (R, represents the initiation phase measuring the time from the start of the test to initial fibrin formation), clot formation time (K, represents the amplification phase measuring the time until 20 mm of clot strength is achieved), angle or α (K angle, represents the propagation phase measuring the rate of clot formation), maximum amplitude (MA, represents the overall stability of the clot), and amplitude at 30 minutes (LY30, represents the fibrinolysis phase and measures the percentage of decrease in amplitude at 30 minutes post-MA). The new TEG®6s system is fully automated.

In TEG® (Table 1, Fig. 1), hypercoagulability is shown by shorter R-time and K-time, besides increased K/α angle and MA. In the Mazen et al study, hypercoagulability was defined as having at least three abnormal TEG® values. However, Maatman et al. defined a hypercoagulable profile as two or more thromboelastographic parameters beyond one Standard Deviation (SD) of the age-and gender-matched controls.

Clinical trials

Panigada et al. showed hypercoagulability along with a severe inflammatory state based on SCT and thromboelastography testing in severe SARS-CoV-2 infection. Escalating from prophylactic to therapeutic LMWH dose requires a careful approach based on the benefit/risk ratio until clinical trials are available (Table 1). Similar results were observed by Maatman et al. and Wright et al., showing COVID-19-associated hypercoagulability and fibrinolysis shutdown (LY30 < 0.8%) causing a higher rate of VTE, with both being measured by TEG® in 12 and 44 patients admitted to the ICU, respectively.

Similarly, Mortus et al. found high K/α angle or high MA on TEG® in 90.5% of patients with severe COVID-19, including increased fibrinogen activity (> 73%) plus MA (> 65 mm) in 74% of patients, and MA criteria alone in 26%. TE occurred at a 62% rate despite thromboprophylaxis in all. In comparison, innate TEG®-MA was significantly higher for the high TE rate group compared to the low TE rate group (p = 0.01). Increased MA was observed in 100% and 45% of patients in the high and low event rate group respectively. Hypercoagulable innate TEG®-MA yielded 100% sensitivity.
and 100% negative predictive value for the occurrence of multiple thrombi.42 In conclusion, in this context, TEG® may be critical in identifying patients at increased thrombotic risk, where full heparinization is beneficial, and avoiding unnecessary anticoagulation in those with low thrombosis risk.42

According to Fan et al.,10 TEG® appears to be a useful tool in detecting hypercoagulability even in the presence of heparin or antiphospholipid syndrome in CAC.

QUANTRA® (SEER sonorheometry)

**Overview**

Quantra® is a new fully automated VET closed system POC, that allows rapid whole blood global hemostasis evaluation within 15 minutes of test initiation, using self-contained cartridges (Table 1, Fig. 1).14,16,43

Quantra® uses ultrasound technology14,16 for direct measurement of physical properties of the clot, without mechanical clot disruption.14,16 The ultrasound pulses generate a shear wave in the sample and the resulting deformation is measured.16 The frequency and amplitude of the induced deformation are directly related to the sample’s viscoelastic properties.16

The following functional parameters can be analyzed14,16,43 (Table 1): clot time (CT) after blood activation with kaolin,44 provides an indication of the functional status of coagulation factors that lead to fibrin formation45; CT with heparinase I to neutralize heparin (CTH); CT ratio (CTR) (CTR over 1.4 indicates the likelihood of influence of heparin); clot stiffness (CS), measured seven minutes after clot initiation, provides information about fibrin/fibrinogen function and platelet activity in the presence of thromboplastin, and was compared with A10-EXTEM on ROTEM®.16 It represents the platelet-fibrinogen interaction through thrombin and factor XIII, but is also influenced by hematocrit and acidosis;16 fibrinogen contribution to CS (FCS) reflects only fibrinogen’s contribution to the overall CS (hPa),16,43 being compared with A10-FIBTEM on ROTEM®16; platelet contribution to CS (PCS), and also platelet activation and platelet contraction of the fibrin mesh,15,16,43 is a parameter calculated by taking into account the difference between CS and FCS15,16,43; and clot stability to lysis (CSL).

Quantra® enables a goal-directed therapy for bleeding patients in several clinical settings,43 as well as diagnosis and evaluation of hypercoagulability as seen in COVID-19. Quantra® has a good correlation with other well-established VET devices and the Clauss assay.14,16,43

**Clinical trials**

Several publications concerning COVID-19 patients have reported a procoagulant profile with increased clot strength, platelet and fibrinogen contribution to clot stiffness (PCS, FCS),11,44 which seemed to improve during ICU stay under anticoagulation.11,44,45 Besides, CT and CTH parameters can be useful to monitor anticoagulation (Table 2).44

The Ranucci et al.44 study aimed to characterize the coagulation profile of COVID-19 ARDS patients, and to evaluate their changes after aggressive thromboprophylaxis. The 16 patients received a complete coagulation profile at ICU admission, as well as mechanical ventilation (MV). Ten patients were monitored in the subsequent seven days, after increased LMWH dose, antithrombin corrected if lower than 70%, and clopidogrel if platelet count above 400 x 10^9/L.44 Major thromboembolic events (TE) were not observed. At baseline, procoagulant profile was characterized by normal levels of CT, but elevation of CS, PCS, FCS, D-dimer, and fibrinogen levels, which were associated and correlated with increased interleukin-6 levels (R2 = 0.506; \( p = 0.003 \)), confirming the link between inflammation and hypercoagulability.11 After increasing thromboprophylaxis, and two weeks from the baseline, there was significant time-related decrease of fibrinogen levels (\( p = 0.001 \)), D-dimers (\( p = 0.017 \)), CS (\( p = 0.013 \)), PCS (\( p = 0.035 \)), and FCS (\( p = 0.038 \)).44

Masi et al.45 characterized the coagulation and fibrinolysis profiles of 17 COVID-19-associated ARDS patients and compared them with 11 non-COVID-19-associated ARDS patients. On admission, all received thromboprophylaxis. Pulmonary embolism (PE) was incidentally diagnosed in 17% of patients.45 Compared to non-COVID-19, COVID-19 patients exhibited: (a) significantly higher levels of procoagulant factors, mainly: fibrinogen (\( p = 0.03 \)); Factor V (FV) (\( p < 0.0001 \)); FVIII (\( p = 0.03 \)), and acute phase reactants.45 All these parameters were strongly correlated with each other (\( p < 0.05 \)); (b) significantly lower thrombin-antithrombin complex (\( p = 0.03 \)); (c) significantly higher (\( p = 0.048 \)) t-PA and PAI-1, being closely correlated (\( p < 0.001 \)); (d) fibrinolysis shutdown or CC were not observed; (e) Quantra®, exhibited twice higher levels of CS (\( p = 0.0077 \)), PCS (\( p = 0.014 \)), and FCS (\( p < 0.001 \)).45 CS was strongly correlated with fibrinogen (\( p = 0.02 \)), FV (\( p = 0.043 \)), FVIII (\( p < 0.001 \)), but not with PAI-1 levels (\( p = 0.606 \)).45 In conclusion, COVID-19-associated ARDS was correlated with significant increase in procoagulants, supporting the concept of thromboinflammation.45

**DISCUSSION**

It is essential to be familiar with the spectrum of CAC since all current studies support CAC as a hypercoagulable and hypofibrinolytic state in the ICU setting.37 Nevertheless, whether this hypercoagulability is due to the invading microorganism, individual viral load or the massive host inflammatory response, still remains unknown.27 This hypercoagulability can evolve into consumptive coagulopathy, microthrombosis and multiple organ failure.46 Post-mortem data supports hypercoagulability through the presence of micro-thrombi in several systems.4 Activation of coagulation and/or fibrinolysis occurs in COVID-19 as part of the acute inflammatory response.4 CAC may, in some way, be specific to SARS-CoV-2, representing new features that need to be clarified through further research.27

All the observational clinical studies described point to the use of VET to assess hypercoagulability and hypofibrinolysis (not detected by SCT), and probably also for
Anticoagulation monitoring shows improvements on VET parameters and patients’ clinical status were observed after introduction of tailored thromboprophylaxis. The use of TEG® or ROTEM® is recommended by some authors and advised by others for all COVID-19 patients with severe pneumonia and coagulation dysfunction.

Hypercoagulable viscoelastic profiles were identified in several clinical studies in COVID-19 patients: increased A10, MCF (INTEM/EXTEM/FIBTEM) and a angle and decreased CFT and ML for ROTEM, short R, K or LYS-30 and high α angle or MA for TEG; elevated CS, PCS and FCS for Quantra®.

Quantra® and ROTEM® have shown features of hypercoagulability in COVID-19 patients hinting towards their use in tailoring treatment, but these non-randomized small studies require confirmation. The development of prospective trials prior to their use in COVID-19 patient care is strongly recommended.

All intubated ICU patients with low bleeding risk should receive low-intensity prophylaxis with LMWH or UFH. However, due to the very high incidence of thromboembolic complications despite standard low-dose thromboprophylaxis among severe COVID-19 patients with elevated D-dimer levels, intermediate-intensity or full-dose anticoagulation is now routinely administered. VET have already been included in some algorithms to determine anticoagulation needs in COVID-19 ICU patients, although VET have not yet been validated as appropriate to manage anticoagulation.

A lower 28-day mortality rate in COVID-19 patients receiving anticoagulation with LMWH was demonstrated. Recently, the International Society of Thrombosis and Hemostasis stressed the need for implementing anticoagulation. The latest recommendations suggest that all hospitalized COVID-19 patients should receive thromboprophylaxis, or full therapeutic anticoagulation if such a prior indication exists or depending on patient’s weight and related risk factors. Other strategies could include the use of intravenous unfractionated heparin or direct thrombin inhibitors if heparin induced thrombocytopenia occurs.

Currently, data on the usefulness of VET devices in CAC is still limited. Further research is needed to investigate whether ROTEM®/TEG® are useful in identifying COVID-19 patients who might benefit from therapeutic anticoagulation, in order to guide hemostatic therapy, and to determine whether an abnormal VET alone or in combination with other findings can identify a group of patients with increased thrombotic risk.

CONCLUSION

Studies concerning the use of VET to evaluate hypercoagulability in SARS-CoV-2 patients are emerging. Prospective clinical trials, ideally RCT, could underline the additional value of VET in predicting the clinical course, guidance of anticoagulation, and the risk stratification of COVID-19 patients for CAC.

Clinical trials in hospitalized COVID-19 patients are actually underway, such as the Rotterdam cohort study using ROTEM® (ROHOCO trial) and the evaluation of hemostasis by TEG®, platelet function testing, and biomarker analysis (TARGET-COVID Study – ClinicalTrials.gov: NCT04493307).

Several studies have shown that VET are a valuable tool to assess hypercoagulability and hypofibrinolysis in critically ill COVID-19 patients with CAC, as well as an additional contributory tool in anticoagulation management of these patients. However, further research needs to be carried out in order to develop evidence-based guidelines.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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