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Cellular and molecular mechanisms in COVID-19 coagulopathy: role of inflammation and endotheliopathy

Rossella Cacciola[1] · Elio Gentilini Cacciola[2] · Veronica Vecchio[3] · Emma Cacciola[4]

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Abstract

Introduction Coronavirus 2 (CoV-2) infection or coronavirus disease 2019 (COVID-19) is frequently associated with microvascular thrombosis.The microthrombosis in COVID-19 is the result of the interplay between inflammation and endotheliopathy. Elevated interleukin-6 (IL-6) characterizes COVID-19 inflammation resulting in endotheliopathy and coagulopathy marked by elevated D-dimer (DD). Aim of this study is to identify and to describe the coagulation changes in 100 moderate COVID-19 patients having lung involvement and to determine the association of coagulopathy with the severity and prognosis.

Methods Inflammation, endothelial and coagulation molecules were measured in moderate and mild disease.

Results IL-6 and tumor necrosis factor-α (TNF-α) and tissue factor (TF), von Willebrand factor (VWF), and tissue factor pathway inhibitor (TFPI) significantly increased in moderate disease as well as D-dimer, thrombin antithrombin complex (TAT), Fibrinogen (Fib), platelet factor-4 (PF4), β-thromboglobulin (β-TG), P-selectin, and platelet adhesion. Shortened clotting time (CT) and clot formation time (CFT), high maximum clot firmness (MCF) and low LY at 30 min were present in 100% of moderate COVID-19 patients compared with mild COVID-19 patients.

Conclusions These findings demonstrate that moderate COVID-19 has a profound inflammation associated with severee ndotheliopathy and intense coagulation activation uncontrolled by TFPI.

Attention should be paid to coagulopathy in COVID-19. Closely monitoring of coagulation and application of appropriate anticoagulation may improve the prognosis of moderate COVID-19 and to prevent the progression to severe COVID-19 disease.

Keywords Cytokines · Endothelial molecules · Coagulative molecules · Platelet activity

Highlights

- Inflammation and endotheliopathy characterize the vascular disease.
- Coagulopathy and piastrinopathy characterize the haemostasis vascular.
- Hypofibrinolysis characterizes the vascular thrombosis.
- TFPI is a prognostic marker of severe vascular disease.

Introduction

The hypercoagulability characterizes COVID-19 [1, 2]. Postmortem thrombotic microangiopathy, pulmonary thrombi and multiorgan failure have been observed [3–8].

✉ Rossella Cacciola
rcacciol@unict.it

1 Hemostasis Unit, Department of Clinical and Experimental Medicine, University of Catania, Via S. Sofia 78, 95123 Catania, Italy

2 Infectious Diseases Unit, Sapienza University of Rome, Rome, Italy

3 Medical School of Catania, University of Catania, Catania, Italy

4 Hemostasis Unit, Department of Medical, Surgical Sciences and Advanced Technologies "G.F. Ingrassia", University of Catania, Catania, Italy

Inflammation and vascular damage cause thrombi [4]. D-dimer (DD), von Willebrand antigen and PF4 are elevated in COVID-19 and DD correlates with PF4 and monocyte TF predicting mortality [9–13]. Pathogens-derived polyphosphates stimulate inflammation and monocytes and macrophages TF realizing thromboinflammation [14–20].

TF is normally present in the circulation at very low levels and its increase causes coagulation activation and thrombin generation inducing leukocyte, platelet and endothelial activation amplifying TF expression, cytokines release and inflammation [21–28]. IL-6 induces platelet reactivity and high Fib linking inflammation and thrombosis [29, 30]. Activated protein C (APC), thrombomodulin (TM) and TFPI, a endogenous serine protease producted by the endothelium, can limit microthrombosis, inflammation and organ injury [30–36]. The mechanisms underlying coagulation changes in COVID-19 are linked to inflammation [14]. It has been reported profound inflammation ("cytokine storm") characterized by high levels of inteleukin-1 (IL-1), IL-6, and TNF-α [14, 37]. The International Society of Thrombosis and Hemostasis (ISTH) developed criteria of coagulopathy called disseminated intravascular coagulopathy (DIC) and sepsis-induced coagulopathy (SIC) where SIC is a coagulopathy less severe than DIC but progressing to DIC [14, 38–40], ISTH DIC and SIC have been used in China to study the COVID-19 coagulation changes and it has been observed that COVID-19 coagulopathy is a SIC without bleeding [14, 41–44].

The link between CoV-2 and endothelial ACE-2 receptor causes death of endothelial cells, distruption of the antithrombotic properties, and thrombotic microangiopathy [14, 20, 45–47]. Therefore, an endotheliopathy contributes to the coagulation changes in COVID-19 causing micro- and macrovascular thrombosis [47–49].

Tang et al. [11] and Ranucci et al. [50] reported high Fib associated with high IL-6 confirming the interplay between inflammation and coagulation.

We investigated biomarkers and measures that have been published previously as representing risk predictors in severe COVID-19. This study differs for having studied these biomarkers and measures in moderate COVID-19.

Moderate COVID-19 exhibits increased inflammation including IL-6 and TNF-α and endotheliopathy including TF, VWF and TFPI and that profound inflammation and endothelial activation are determinant to coagulation activation and platelet activation through mechanisms involving D-dimer, TAT, and Fib and PF4 and β-TG, and P-selectin, respectively. Our data shed new light on pathogenesis of the COVID-19 coagulopathy involving whole haemostatic system as documented by blood viscoelastic properties measurement. Of note that the associations observed between inflammation, endothelium, coagulation and platelet correlated with pulmonary embolism suggest a biologically plausible hypothesis.

There is no scientific basis to conclude that TFPI is responsible for worse outcome in moderate COVID-19 but the finding that TFPI does not attenuate the coagulopathy is a biologically plausible basis and the association between TFPI and dilute Prothrombin time (dPT) is a proper focus.

Materials and methods

We prospectively studied 100 moderate COVID-19 patients on reverse transcription polymerase chain reaction (RT-PCR) within 72 h of hospitalization. The comparator groups were 16 presenting mild disease and 16 asymptomatic patients and 40 healthy subjects age- and sex-matched SARS-CoV-2^{-}on RT-PCR (Table 1). The definition of moderate and mild was chosen in according to the diagnosis and treatment protocol novel coronavirus pneumonia (trial version 7) [1, 51] published by the National Health Commission that has defined moderate cases showing fever and respiratory symtoms with radiological findings of pneumonia and mild cases showing mild clinical symptoms and no sign of pneumonia on imaging. The outcome of moderate cases was characterized by deterioration of disease defined as pulmonary embolism (PE) on computed tomography (CT) angiography, without mortality.Our management protocol included antithrombotic prophylaxis with 40–60 mg of enoxaparin per day and antimicrobial treatment (amoxifloxacin and/or cephalosporin) and antiviral therapy (oseltamivir and/or ganciclovir). In addition, all moderate cases were administered corticosteroid (methylprednisolone) during the course of hospitalization. The controls were not under anti-inflammatory or antiplatelet drugs for at least 2 weeks.

The justification of many biomarkers studied in this study is to have collected all the biomarkers at the time investigated in COVID-19.

Inflammatory and endothelial mediators

Interleukin-6 (IL-6) and TNF-α were measured by multiplex bead array (Millipore Sigma) on Luminex 200 machine, and TF, VWF and TFPI were measured by ELISA kits (R&D Systems; Abcam and American Diagnostica Inc., Greenwich, CT).

Coagulation activation mediators

DD and TAT were measured by ELISA kit (Diagnostic Stago, Boehringer Mannheim, Mannheim, Germany; Dade Berhing Marburg GmbH, Marburg, Germany), and Fib was measured by Clauss method (Giesse Diagnostics, Italy).

Table 1 Characteristics of COVID-19 patients and control subjects

	Controls, n = 40	Mild/asymptomatic, n = 32	Moderate, n = 100
Age, y	50 (30–60)	30 (32–34)	55 (45–63)
Sex, male	25 (62.5)	18 (56.2)	60 (60)
Respiratory support			
Oxygen supplementation	0 (0)	0 (0)	100 (100)
Mechanical ventilation	0 (0)	0 (0)	0 (0)
28/day mortality		0 (0)	0 (0)
Comorbidities			
Obesity	0 (0)	0 (0)	0 (0)
Hypertension	0 (0)	0 (0)	0 (0)
Diabetes	0 (0)	0 (0)	0 (0)
Cancer	0 (0)	0 (0)	0 (0)
Heart disease*	0 (0)	0 (0)	0 (0)
Presenting symptoms			
Cough	0 (0)	8 (25)	92 (92)
Fever	0 (0)	0 (0)	80 (80)
Dyspnea	0 (0)	8 (25)	92 (92)
Headache	0 (0)	5 (15.6)	0 (0)
Anosmia	0 (0)	0 (0)	0 (0)

Platelet activation mediators

The rational for chosen the method of released platelet materials PF4 and β-TG by ELISA kit (R&D Systems) was the study of platelet activation function. In addition, was measured sP-Selectin by ELISA kit (R&D Systems).

Platelet adhesion

The rational for chosen the method PFA-100 System (Dade International Inc. FL, USA—American Diagnostica Inc., Greenwich, CT Systems) using Collagen/ADP (CT-ADP) and Collagen/Epinephrine (CT-EPI) cartridges was the study of vascular platelet function.

Blood viscoelastic analysis

The whole blood hemostatic activation was measured by Thromboelastometry method (Rotem delta System—Pentapharm GmbH, Germany) by parameters including CT describing the phase of coagulation extrinsic or intrinsic pathway activation, CFT describing the next phase of stable clot formation depending by activated platelets and fibrinogen, and MCF describing the firmness of the clot depending by activated platelets and fibrinogen, and clot lysis at 30 min (LY-30) describing the degree of fibrinolysis which taken place until 30 min after CT.

Coagulation functional assays

Citrated blood was analysed after centrifugation at 3000 g for 15 min at 18 °C on automated coagulation analyser (ACL 7000—Instrumentation Laboratory) to measure dPT.

Statistical analysis

The results were given as the mean ± standard deviation using the Student's t-test and correlation coefficients using the Pearson test for parametric distributions or the Spearman test for nonparametric distributions and the Fisher exact test. A p-value of < 0.05 was considered statistically significant. The log transformation was applied to the measures prior to analysis because the measures were not distributed normally. Data were analyzed using SPSS 21.0 for Windows (SPSS Inc.)

Results

The inflammation of moderate COVID-19 contributes to endothelial activation

IL-6, TNF-α, and TF and VWF were increased in moderate COVID-19 (54 ± 12 pg/ml, 45 ± 5 pg/ml, 2000 ± 500 pg/ml and 300 ± 50%) compared with mild/asymptomatic COVID-19 (4 ± 2 pg/ml, 9 ± 1 pg/ml, 20 ± 2 pg/ml and 40 ± 8%) and controls (5 ± 2 pg/ml, 10 ± 3 pg/ml, 23 ± 5 pg/ml and

60 ± 10%). IL-6 and TNF-α correlated with TF and VWF in the moderate COVID group (Table 3). TFPI was increased in moderate COVID-19 (166 ± 69 ng/ml) compared to mild/asymptomaticCOVID-19 (70 ± 10 ng/ml) and controls (81 ± 12 ng/ml) and correlated with inflammatory markers in the moderate COVID group (Table 3).

The endotheliopathy of moderate COVID-19 contributes to coagulation activation

DD, TAT and Fib were increased in moderate COVID-19 (550 ± 100 µg/l, 70 ± 10 µg/l and 600 ± 20 mg/dl) compared with mild/asymptomatic COVID-19 (60 ± 5 µg/l, 2 ± 1 µg/l and 175 ± 10 mg/dl) and controls(70 ± 5 µg/l, 3 ± 1 µg/l and

Table 2 Descriptive statistics

	Controls, n = 40	Mild/asymptomatic, n = 32	Moderate, n = 100
IL-6, (< 7.0 pg/ml)	5 ± 2 Q1 4, Q2 (median) 5, Q3 6, Q4 7	4 ± 2 Q1 3, Q2 (median) 4, Q3 5, Q4 6	54 ± 12* Q1 51, Q2 (median) 55, Q3 60, Q4 66
TNF-α (8–10 pg/ml)	10 ± 3 Q1 8, Q2 (median) 9, Q3 11, Q4 13	9 ± 1 Q1 8, Q2 (median) 9, Q3 9, Q4 10	45 ± 5* Q1 43, Q2 (median) 46, Q3 48, Q4 50
TF (15.6–1000 pg/ml)	23 ± 5 Q1 20, Q2 (median) 24, Q3 26, Q4 28	20 ± 2 Q1 19, Q2 (median) 20, Q3 21, Q4 22	2000 ± 500* Q1 1800, Q2 (median) 2100, Q3 2300, Q4 2500
VWF (50–160%)	60 ± 10 Q1 54, Q2 (median) 61, Q3 65, Q4 70	50 ± 8 Q1 45, Q2 (median) 50, Q3 53, Q4 58	300 ± 50* Q1 270, Q2 (median) 300, Q3 330, Q4 350
TFPI (75–120 ng/ml)	81 ± 12 Q1 77, Q2 (median) 82, Q3 87, Q4 93	75 ± 10 Q1 71, Q2 (median) 76, Q3 81, Q4 85	166 ± 69* Q1 127, Q2 (median) 167, Q3 200, Q4 235
DD (50–200 µg/L)	70 ± 5 Q1 68, Q2 median) 71, Q3 73, Q4 75	60 ± 5 Q1 57, Q2 (median) 60, Q3 62, Q4 65	550 ± 100* Q1 500, Q2 (median) 553, Q3 600, Q4 650
TAT, (1.0–4.1 µg/L)	3 ± 1 Q1 2, Q2 (median) 3, Q3 4, Q4 4	2 ± 1 Q1 1, Q2 (median) 2, Q3 3, Q4 3	70 ± 10* Q1 66, Q2 (median) 71, Q3 76, Q4 80
Fib (170–400 mg/dL)	180 ± 20 Q1 170, Q2 (median) 180, Q3 190, Q4 200	175 ± 10 Q1 170, Q2 (median) 175 Q3 180, Q4 185	600 ± 20* Q1 590, Q2 (median) 600, 3 610, Q4 620
PF4 (1–10 IU/ml)	4 ± 1 Q1 3, Q2 (median) 4, Q3 5, Q4 5	3 ± 1 Q1 2, Q2 (median) 3, Q3 4, Q4 4	158 ± 63* Q1 125, Q2 (median) 158, Q3 188, Q4 221
βTG (10–40 IU/ml)	15 ± 5 Q1 12, Q2 (median) 15, Q3 17, Q4 20	10 ± 5 Q1 7, Q2 (median) 10, Q3 12, Q4 15	245 ± 20* Q1 235, Q2 (median) 246, Q3 256, Q4 265
P-Selectin (18–40 ng/ml)	20 ± 10 Q1 15, Q2 (median) 20, Q3 25, Q4 30	30 ± 10 Q1 25, Q2 (median) 30, Q3 35, Q4 40	64 ± 10* Q1 59, Q2 (median) 64, Q3 69, Q4 74

*p values: < 0.05 compared with controls

Table 3 Markers correlations in the moderate COVID group

Markers	IL-6	TNF-α	TF	VWF	TFPI	DD	TAT	FIB	β-TG	PF4	P-selectin
IL-6			p	p	p						
TNF-α			p	p	p						
TF						p	p	p			
VWF						p	p	p			
TFPI						p	p	p			
DD									p	p	p
TAT									p	p	p
FIB									p	p	p
β-TG						p	p	p			
PF4						p	p	p			
P-selectin						p	p	p			

p values < 0.05

180 ± 20 mg/dl) (Table 2). TF, VWF, and TFPI correlated with DD and TAT and Fib in the moderate COVID group (Table 3). As TFPI can limit coagulation, we measured dPT that was high in moderate COVID-19 (59 ± 2 s) compared with mild/asymptomatic COVID-19 (25 ± 5 s) and controls (20 ± 3 s). A correlation was found between TFPI and dPT ($p < 0.05$).

The coagulopathy of moderate COVID-19 contributes to platelet activation

PF4 and β-TG and P-Selectin were increased in moderate COVID-19 (158.1 ± 63 IU/ml and 245 ± 20 IU/ml and 62 ± 10 ng/ml) compared with mild/asymptomatic COVID-19 (3 ± 1 IU/ml and 10 ± 5 IU/ml and 30 ± 10 ng/ml) and controls (4 ± 1 IU/ml and 15 ± 5 IU/ml and 20 ± 10 ng/ml) (Table 2). PF4 and β-TG and P-Selectin correlated with DD and TAT and Fib in the moderate COVID group (Table 3).

We also found increased platelet adhesion in moderate COVID-19 on C/ADP and C/EPI (C/ADP 48 ± 10 s and C/EPI 42 ± 5 s) compared with mild/asymptomatic COVID-19 (C/ADP 70 ± 10 s and 90 ± 10 s) and controls (75 ± 15 s and 100 ± 20 s) (Table 4).

The inflammation and endotheliopathy of moderate COVID-19 contribute to whole haemostatic activation

Shortened CT (CT, unit: s. n.v. 100–240 s) (45 ± 20 s), shortened CFT (CFT, unit: s, n.v. 30–160 s (15 ± 10 s), increased MCF (MCF, unit: mm, n.v. 50–72 mm (120 ± 10 mm) and lower LY-30 (LY-30, %: v.n. 15% (0.8%) were in moderate COVID-19 compared with mild/asymptomatic COVID-19 (CT 100 ± 10 s and CFT 40 ± 5 s and MCF 70 ± 10 mm and LY-30 (15%) and controls (CT 110 ± 10 s and CFT 30 ± 5 and MCF 60 ± 10 mm and LY-30 (15%) (Table 5).

Discussion

Thrombosis in COVID-19 is an important part of the clinical picture that needs to be considered [14].

Table 4 PFA-100 parameters of COVID-19 patients and control subjects

	Controls, n = 40	Mild/asymptomatic, n = 32	Moderate, n = 100
C/ADP, s[a]	75 ± 15	70 ± 10	48 ± 10*
C/EPI, s[a]	100 ± 20	90 ± 10	42 ± 5*

*p values: < .05 compared with controls

[a]Reference values of C(ADP (68–121 s), C/EPI (84–160 s)

Table 5 ROTEM parameters of COVID-19 patients and control subjects

	Control, n = 40	Mild/asymptomatic, n = 32	Moderate, n = 100
CT, s[a]	110 ± 10	100 ± 10	45 ± 20*
CFT, s[a]	30 ± 5	40 ± 5	15 ± 10*
MCF, mm[a]	60 ± 10	70 ± 10	120 ± 10*
LY, %[a]	15	15	0.8*

*p values: < .05 compared with controls

[a]Reference values of CT (100–240 s), CFT (30–160 s), MCF (50–72 mm), LY (15%)

Histopathologic studies reveal diffuse alveolar damage with profound inflammation and thrombotic microangiopathy of lung vessels [52] and an autopsy series of 11 patients showed thrombosis of small and midsized pulmonary arteries in all patients [53].

Microvascular thrombosis have also been reported in extrapulmonary organs, which may explain the acute onset of multiorgan failure [54]. COVID-19-associated coagulopathy (CAC) is emerged as prominent feature of severe or critically ill patients [55]. It has been reported that COVID-19 is highly thrombotic. Cui et al. [56] reported a 25% incidence of deep vein thrombosis in patients with severe coronavirus pneumonia in Wuhan [26]. Klok et al. [57] found a 31% combined incidence of deep vein thrombosis, pulmonary embolism, and arterial thrombosis in critically ill patients with coronavirus [27]. Of these events 81% were pulmonary thromboemboli.

The hallmark of COVID-19 is a profound inflammation, described as "cytokine storm", characterized by high levels of interleukin-1 (IL-1), IL-6, tumor necrosis factor, and other inflammatory cytokines [37].

Inflammation promotes thrombosis through activation of endothelial cells, platelets, monocytes, and tissue factor, and by altering fibrinolysis and natural anticoagulant pathways (eg, through changes in levels of thrombomodulin, Proteins C and S, and TFPI) [58, 59].

One mechanism of microvascula rthrombosis that may be specific to COVID-19 is the affinity of virus for angiotensin-converting enzyme2, which is expressed on alveolar epithelial type II cells and various extrapulmonary tissues, including endothelial cells. SARS-CoV-2 has been shown to directly invade the endothelial cell [47]. Endothelial cell activation may be a unique mechanism of COVID-19-mediated microvascular injury, thrombosis, and subsequent multi system organ failure and is believed to be a major contributor to coagulopathy, morbidity and mortality [47].

The rate of 87.7% positivity for lupus anticoagulant in patients with COVID-19 reported by Helmes et al. [60] is striking and needs to be verified, but it supports the idea that

endothelial injury is a key mechanism of multiorgan failure and coagulopathy in this disease.

The characteristic laboratoriy findings of CAC are significantly elevated levels of D-dimer and fibrinogen, indicating a highly thrombotic state with high fibrin turnover [11]. However, other markers of disseminated intravascular coagulation remain relatively unchanged [42]. The prothrombin time and activated partial thromboplastin time are normal or only mildly prolonged, if at all, and platelet counts are usually normal or marginal reduced [42]. Abnormalities in coagulation parameters have been shown to correlate directly with inflammatory cytokines, severity of illness and adverse outcomes [59].

Many studies have studied the thrombo-inflammatory markers that are associated with the worst prognosis in COVID-19 patients [11] and several reports and meta-analysis have evaluated the link between elevated D-dimer levels and severity and mortality in COVID-19 [41]. Anyway, there is strong criticism about the use of D-dimer test as marker in COVID-19 infection. Infact, it has been reported poor specificity while high levels in clinical conditions other than COVID-19 such as old age, African and American ethnicity, females, malignancies, trauma from surgey, state of pregnancy, physical immobility, drug use, connective tissue inflammation, severe kidney failure, and thromboembolic syndrome [61]. In addition, the D-dimer characterizes an advanced coagulation stage and it results from degradation of clot by fibrinolytic system. The traditional laboratory assays such as PT and APTT are used to measure the plasma coagulation but they do not provide any information about the platelets and the fibrinolysis. In addition, the number of platelets and the fibrinogen levels are fixed parameters that do not give any functional information [61]. Hottz et al. [10] report that activated platelets or products from platelet activation infiltrate to the airways of patients with severe COVID-19. They quantified the levels of TXB2 and the proteins from platelet α-granules PF4/CXCL4 and PDGF in tracheal aspirates from patients with COVID-19 under mechanical ventilation and showed the presence of platelet-derived factors in tracheal aspirates from COVID-19 patients, suggesting that platelet activation and platelet-secretory products gain access to the airways in severe COVID-19 syndrome [10].

As discussed has been reported in severe or critically ill patients with coronavirus. Little attention has been paid to those with mild or moderate infection.

In this study, we show that increased inflammation and hypercoagulability predict moderate COVID-19 patients'poor outcomes, including the requirement of antiviral and eparin therapy and in-hospital admission.

Table 6 COVID-19-associated coagulopathy

Summary of findings
1. Inflammation is manifest as elevated IL-6 and TNF-α
2. Endotheliopathy is manifest as elevated TF, VWF, and TFPI
3. Coagulopathy is manifest as elevated DD, TAT, and Fib
4. Platelet activation is manifest as elevated PF4 and βTG
5. IL-6 and TNF-α levels are correlated with TF, VWF, and TFPI
6. TF, VWF, and TFPI levels are correlated with DD, TAT, and Fib
7. DD, TAT, and Fib levels are correlated with PF4 and βTG
8. Whole blood viscoelastic analysis sustains the coagulopathy
9. Coagulopathy appears to be related to inflammation and endotheliopathy and not intrinsic viral activity

Our data show profound inflammation and endoteliopathy that confer coagulation activation and platelet activation in moderate COVID-19 patients compared with mild COVID-19 patients. First, we measured IL-6 and TNF-α levels that reflect a higher inflammatory state in moderate COVID-19 patients than mild COVID-19 patients. Second, TF and VWF are released by activated and damaged endothelium, which likely occurs early in COVID-19. Of note, our plasma very high TFPI levels measured later in the disease process during hospitalization did not attenuate inflammation and coagulation. Third, plasma D-dimer, TAT and Fg originated from thrombin-activated fibrinolysis positively correlated with TF, VWF and TFPI reflecting a endothelial hypercoagulability state (see Table 6).

There are data consistent with the notion that intense cytokine production may trigger hyperinflammation and haemostatic hypercoagulability [62]. Proinflammatory cytokines and procoagulant factors such as thrombin may also contribute to TF expression in monocytes [63]. We show that the proinflammatory cytokines and TAT may contribute to TF expression in our cohort of moderate COVID-19.

Four, increased platelet activation is observed in moderate COVID-19 patients, but not in patients presenting mild COVID-19 infection. The mechanisms underlying platelet activation in COVID-19 remain unknown. New studies are still necessary to better characterize the platelet activation in COVID-19. Platelets are anucleate blood cells classically known by their roles in haemostasis and patological thrombosis [10]. Haemostatic activities involve platelet aggregation and thrombus formation, platelets also coordinate vascular function and integrity by complex interactions with endothelial cells and leukocytes [10]. Activated platelets reprogram cellular functions of adjacent cells by heterotypic cellular interactions and juxtacrine signals from P-selectin

and integrins [10]. Platelets modulate critical leukocyte responses such as migration, secretion, extrusion of neutrophil extracellular traps, recruitment to growing thrombi, and monocyte expression of TF,¯an essential trigger of coagulation and thrombosis [10]. Our data show a correlation between inflammatory cytokines and platelet-secretory products suggesting that the inflammation confers platelet activation. Platelets are also known to modulate secretion of cytokines [10]. It has been shown that during viral infections, including dengue, HIV, and influenza, platelets change the monocyte cytokine profile toward a proinflammatory pattern [10]. In this study has been showed that during moderate COVID-19 infection activated platelets change the systemic cytokine profile toward a proinflammatory pattern, amplifying inflammation. Platelet hyperaggregation was assessed on COVID-19 patients who were not on aspirin or other antiplatelet agents [64]. Platelet hyperadhesion was assessed in our moderate COVID-19 patients on C/ADP in according to a recent report that report ADP as a key player in the development of microvascular thrombosis in COVID-19 patients [64]. Aspirin pretreatment of platelets from SARS-CoV-2-infected patients abolishes this hyperactivity [64]. Our findings of platelet activation further suggest that antiplatelet therapy may be warranted in treating COVID-19 patients.

In this study, each of these molecular events and cellular interactions were studied by thromboelastometry to confirm the CAC and to guide therapy.

Whole haemostatic system is easily analysed by use of thromboelastography (TEG) or rotational thromboelastometry (ROTEM), able to measure the haemostasis from the formation of the clot to its dissolution [61].

Several reports have been published describing early experience with TEG or ROTEM for patients admitted with severe or critical disease in Italy and the United States [61]. However, information on viscoelastic testing among patients with moderate COVID-19 is scarce.

Maatman et al. [65] and Wright et al. [66] reported the presence of hypercoagulability in COVID-19 by use of TEG and hypofibrinolysis. In according to these data, we noted that CT and CFT were shortened and MCF high and clot lysis at 30 min (LY-30) was low in 100% in our cohort of patients with moderate COVID-19.

In summary, our study supports moderate COVID-19 infection as a hypercoagulable and hypofibrinolytic state (Table 5). By easy test execution and its property to measure all factors involved in haemostatic system and stages of coagulation process and platelet function in the sick bed, thromboelametry might be suited to evaluate the thrombosis risk in patients with COVID-19.

In line with these results and in according to FDA Issues Guidance for Measuring Viscoelasticity Amid COVID-19 Pandemic (FDA.gov, January 28, 2021), we suggest to routinely use the whole blood viscoelastic analysis that measures the whole blood capability to make and sustain clot formation in hospitalized COVID-19 patients for the purpose of identifying the individual patient's degree of thrombotic risk. In this study the associations of menu of markers were correlated with PE suggesting the possibility to identify a specific patient population for which specific management options may be selected. Given that none of the markers are considered or recommended by societies for routine management and given that it seems highly unlikely that each test or measure would or needs to be performed in clinical practice, the clinician will interpret these results as probable harbingers of hypercoagulability and the data may suggest that one or more is sufficient for prognostic purposes.

Author contributions RC, EGC, VV, and EC equally contributed to the writing of this manuscript.

Declarations

Conflict of interest The authors declare no competing financial interests.

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