The Coagulation Labyrinth of Covid-19

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In the end, it's always a platoon of soldiers that save civilization

Oswald Spengler

Preface

When we were all young students in medicine, I am quite sure that our dream was to face, before the end of our carrier, a new disease to discover and fight. And of course, to defeat. Like the young officer Giovanni Drogo in Dino Buzzati's *The Tartar Steppe*, we started to practice medicine waiting for the Tartars to attack our fortress of knowledge.

At the beginning of 2020, our dreams became true, and becoming true they rapidly converted into a nightmare: the COVID-19. Italy was the first Western country hit by this hurricane of sufferance, disease, pain, and death. The landmark of the disease was the solitude: patients were alone, separated from families: sons and daughters, and wives and husbands, separated by their relatives. Doctors were alone with their burden of ignorance and impotence.

I started taking care of COVID-19 patients in March 2020, in my cardiac surgery intensive care unit, rapidly converted into a COVID-19 intensive care unit. Myself and my staff, we were all frightened, puzzled, and even fascinated by the new challenge. Being cardiac anesthesiologists, we were all involved in clinical research on bleeding and thrombosis. It was therefore natural, for my team, to observe that thromboembolic complications, namely pulmonary embolism, were unusually common in this patient population. We studied the phenomenon, and at the end of March, we sent a preliminary report to the *Journal of Thrombosis and Hemostasis.* At that time, a PubMed search on "COVID and coagulation" could produce just a dozen of studies, none of them experimental. Other Italian groups, from Milan and Padua, almost simultaneously confirmed what we called "*The procoagulant pattern of COVID-19."*

In the following months, an avalanche of studies on coagulation abnormalities associated with COVID-19 (the *COVID-19-associated coagulopathy*) were produced, and the same PubMed search, repeated on May 2021, produces about 2000 articles.

This manual has been written by a number of clinicians (anesthesiologists, intensivists, hematologists, and radiologists) directly involved in the care of COVID-19 patients. Our purpose is to provide a comprehensive overview on COVID-19-associated coagulopathy, its mechanisms, and its treatment. Far from being based on guidelines alone (that are presently limited and changing over time), we tried to collate data from the existing studies within a single scenario. It is a difficult task, given the subtle and dynamic nature of this disease. Many items remain open issues.

Many drugs have been used, and discharged, and sometimes used again. Timing of interventions is still elusive. While I write these lines, we are still in the middle of the storm. Much has been done and much has been learned, but not enough. We hope that our small contribution may help clinicians in finding the way out from the *Coagulation Labyrinth of COVID-19.*

The present large-scale scenario of vaccination has opened a new era of hope for getting rid of this disease. However, if there is really a lesson learnt, it is that we must be prepared for any possible new emergency. Zoonoses have been hitting humans since centuries, they did in the recent past, and they will in the future.

I must sincerely thank all the contributors for their wonderful and passionate work rapidly completed in difficult times.

Milan, Italy Marco Ranucci

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Contents

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General Aspects of Sepsis-Associated Coagulopathy

1

Raffaella Rossio and Armando Tripodi

1.1 Introduction

Sepsis is a life-threatening syndrome, characterized by a dysregulated inflammatory host response to infection, leading to multiorgan failure. The Sequential Organ Failure Assessment (SOFA) score is used to assess the degree of organ failure and to predict mortality. The score considers the respiratory, cardiovascular, central nervous, and hepatic system and parameters of hemostasis [1] (Table 1.1). Septic shock is a clinically defined subset of sepsis, wherein hypotension requires vasopressors to maintain a mean arterial blood pressure above 65 mmHg and concentration of serum lactate is more than 2 mmol/L [2]. The therapy is based on early use of broad-spectrum antibiotic, intravenous fluid administration, and if needed vasopressors [3].

The abnormalities of hemostasis observed in sepsis vary from thrombocytopenia to an overt disseminated intravascular coagulation (DIC) associated with consumption of platelets and coagulation factors (both pro- and anticoagulants) with a high risk of thrombotic and hemorrhagic events [4]. Up to 50–70% of patients with sepsis may have clinically symptomatic hemostasis alterations and 35% of patients may present with DIC [5]. The alterations of hemostasis associated with sepsis are not only a consequence of the disease, but they also have a pathogenetic role. In fact, the systemic inflammation leads to activation of coagulation with downregulation of the naturally occurring anticoagulants (mainly antithrombin and protein C systems) and impairment of fibrinolysis. Moreover, the coagulation system itself can enhance

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Table 1.1 The SOFA score

SOFA score	1	2	3	4
Respiration PaO_2/FiO_2, mmHg	<400	<300	<200 with respiratory support	<100 with respiratory support
Coagulation platelets × 10^3/mm^3	<150	<100	<50	<20
Liver Bilirubin, mg/dL	1.2–1.9	2.0–5.9	6.0–11.9	>12.0
Cardiovascular Hypotension	MAP <70 mmHg	Dopamine ≤5 or dobutamine (any dose)	Dopamine >5 or epinephrine ≤0.1 or norepinephrine ≤0.1[a]	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1
Central nervous system Glasgow Coma Score	13–14	10–12	6–9	<6
Renal Creatinine mg/dL or urine output	1.2–1.9	2.0–3.4	3.5–4.9 or <500 mL/day	>5.0 or <200 mL/day

[a]Adrenergic agents administered for at least 1 h (doses given are in μg/kg^{-1} min^{-1})

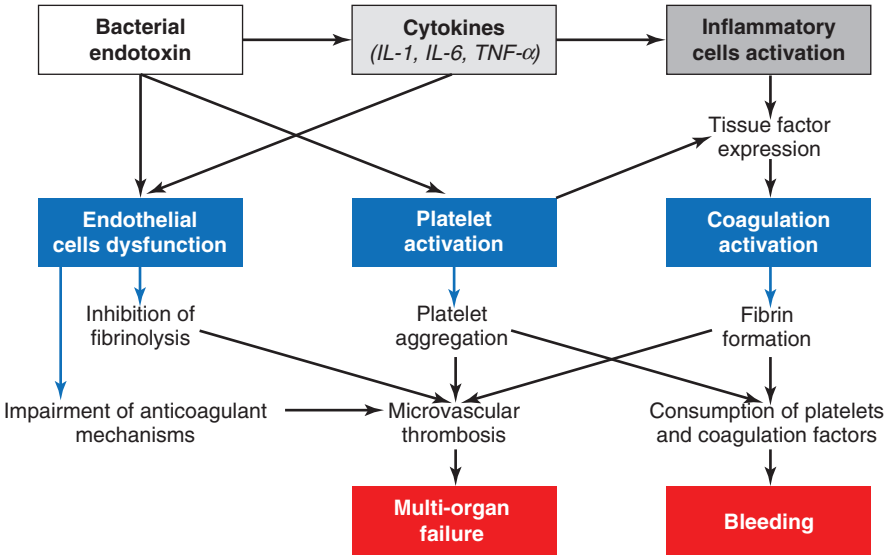

Fig. 1.1 Alterations of hemostasis in sepsis

inflammation [6]. In this process, proinflammatory cytokines, endothelial dysfunction leading to increased permeability, platelets, and coagulation activation play a key role (Fig. 1.1).

1.2 The Pathogenesis of Sepsis-Induced Coagulopathy (SIC) and Disseminated Intravascular Coagulation (DIC)

1.2.1 The Role of Platelets

The activation of platelets plays a role in the development of sepsis through their involvement both in inflammation and in thrombosis. Thrombocytopenia is defined as platelet count less than 150×10^9/L and is considered severe if platelets are less than 50×10^9/L. Thrombocytopenia is common in sepsis with a frequency ranging from 20 to 58% and the persistence of a reduced platelet count during the disease is associated with poor prognosis and correlates with the severity of sepsis [7, 8]. The mechanisms of thrombocytopenia are multifactorial and are still unknown. Reduced production, hemodilution, and increased consumption in the microcirculation can contribute to the decrease of platelet count. More complex mechanisms such as immune-mediated sequestration with autoantibodies and hemophagocytosis have been described [9]. Platelets in septic patients can be activated directly by bacterial endotoxin or by proinflammatory mediators such as platelet-activating factors and play a role in the innate immunity through interaction with leukocytes and monocytes [10, 11]. The expression of P-selectin on platelet membranes promotes the formation of platelet-leukocyte aggregates and the adhesion of platelets to endothelium. Platelets expressing P-selectin accelerate coagulation activation leading to the formation of fibrin owing to increased tissue factor (TF) on membranes of monocytes [12]. The activation of coagulation in turn promotes further activation of platelets mediated by thrombin. Interaction between platelets and neutrophils contributes to the formation of neutrophil extracellular traps (NETs) that are fragments of denatured DNA, neutrophil granule enzymes, and histones from damaged cells that play a role in the host defense, killing the pathogens and recruiting leukocytes [13]. The activation of platelets, recruitment of leukocytes and monocytes, NET formation, and activation of coagulation mediated by TF contribute to thrombosis in septic conditions [9].

1.2.2 The Endothelial Dysfunction

The endothelium plays a dynamic role in the regulation of hemostasis in septic patients through complex mechanisms. For example, some components of the bacterial cell wall can activate endothelial cell receptors through production of inflammatory mediators, including cytokines and chemokines, which are able to activate endothelial cells [14]. The involved proinflammatory cytokines are interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNFα). Other molecular species such as elastase and complement system components play a crucial role in sepsis [15]. The dysregulation of endothelium with structural and functional changes leads to increased permeability that plays a pathogenetic role and

results in tissue edema and hypovolemia. Moreover, the endothelium in normal conditions possesses antithrombotic properties that prevents an unwanted activation of coagulation on its surface, which is likely lost during sepsis [16]. Furthermore, in sepsis, activated endothelium expresses adhesion molecules with increased recruitment and adhesion of platelets that contribute to thrombosis. The disruption of the endothelial layer can expose the subendothelial collagen matrix that binds platelets through the adhesive protein von Willebrand factor (VWF) released from endothelial cells, which plays a role in platelet aggregation also [17]. Another mechanism that limits the anticoagulant properties of endothelium is the downregulation of naturally occurring coagulation inhibitors: antithrombin, tissue factor pathway inhibitor (TFPI), and protein C systems [18].

1.2.3 The Coagulation System

The main activator of coagulation in sepsis is TF, a transmembrane glycoprotein that binds factor VIIa (FVIIa), thus activating factor X (FX) to FXa and forming the complex TF-FVIIa-FXa, which leads to thrombin formation and eventually to the fibrinogen-fibrin conversion [19, 20]. Moreover, decreased TFPI leads to a poor TF inhibitory activity, leading to a procoagulant imbalance [21]. There are other mechanisms that play a crucial role in maintaining the procoagulant imbalance induced by sepsis. Antithrombin in normal conditions binds the heparin-like glycosaminoglycans of the endothelial cells, thus acting as the physiological inhibitor of plasma serine proteases, including factors Xa, IXa, XIa, and thrombin. In sepsis, the anticoagulant effect of antithrombin is decreased because of defective synthesis, degradation by elastase of neutrophils, and consumption [22]. Protein C (PC), which upon activation by thrombin in complex with its endothelial receptor thrombomodulin functions as the physiological inhibitor of factor Va and FVIIIa, is low in patients with sepsis due to degradation and reduced production. Low levels of PC in sepsis are associated with high mortality [19]. Activated PC, besides its anticoagulant properties, has also anti-inflammatory and profibrinolytic properties [23]. Finally, a pro-thrombotic state leading to fibrin deposition observed in sepsis occurs and is maintained by the impairment of fibrinolysis. Plasmin, which is the key enzyme of fibrinolysis, degrades fibrin and its formation from its precursor plasminogen is regulated by a tight controlled mechanism through the balance of activators and inhibitors. Tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) regulate the conversion of plasminogen to plasmin. PAI-1 is the inhibitor of the urokinase-type plasminogen activator (uPA) and tPA. During sepsis, the activation of endothelial cells causes release of PAI-1. The thrombin-activated fibrinolysis inhibitor (TAFI), which is reduced in sepsis, contributes further to the inhibition of fibrinolysis by impeding binding of plasminogen to fibrin and its conversion to plasmin [24].

Table 1.2 Clinical conditions associated with DIC

Severe infection or sepsis
Trauma
Cancer and hematological malignancies
Obstetrical complications
Vascular malformations and aneurysms
Liver failure
Severe toxic and allergic reactions
Immunological reactions (i.e., ABO transfusion incompatibility or organ rejection)

Table 1.3 ISTH overt DIC and SIC scoring systems

		Overt DIC	SIC
Item	Score	Range	Range
Platelet count (10^9/L)	2	<50	<100
	1	≥50, <100	≥100, <150
FDP/D-dimer	3	Strong increase	–
	2	Moderate increase	–
Prothrombin time [seconds or PT (patient/normal) ratio]	2	≥6 s	>1.4
	1	≥3 s, ≤6 s	>1.2, ≤1.4
Fibrinogen (mg/dL)	1	<100	–
SOFA score	2	–	≥2
	1	–	1
Total score for DIC or SIC		≥5	≥4

ISTH International Society on Thrombosis and Haemostasis, *DIC* disseminated intravascular coagulation, *SIC* sepsis-induced coagulopathy, *SOFA* sequential organ failure assessment, SOFA score is the sum of 4 items (respiratory SOFA, cardiovascular SOFA, hepatic SOFA, renal SOFA)

1.3 Disseminated Intravascular Coagulation (DIC)

1.3.1 Definition

DIC is an acquired syndrome characterized by systemic intravascular activation of coagulation with loss of localization arising from different causes (Table 1.2). It can originate from sepsis and causes damage to the microvasculature, which if sufficiently severe can produce organ dysfunction [25]. The diagnosis of DIC is based on a diagnostic scoring system developed by the International Society on Thrombosis and Haemostasis (ISTH) that includes laboratory parameters (Table 1.3). The ISTH DIC criteria are not specific for sepsis-associated DIC, which is characterized by activation of coagulation, fibrinolysis impairment, and high incidence of organ dysfunction [26].

1.3.2 Diagnostic Criteria of Disseminated Intravascular Coagulation (DIC) and Sepsis-Induced Coagulopathy (SIC)

Diagnostic criteria of DIC have been first proposed by the Japanese Ministry of Health and Labor and included both clinical and laboratory features such as platelet count, prothrombin time (PT) ratio, fibrin/fibrinogen degradation products (FDP) or D-dimer, and fibrinogen [27]. Subsequently, the ISTH and the Japanese Association of Acute Medicine (JAAM) proposed new criteria for diagnosis of DIC [28, 29]. The ISTH criteria (Table 1.3) are based on a scoring system, which includes results of hemostasis tests. The score correlates with the disease severity also [30]. In 2017 ISTH developed SIC criteria to categorize patients with sepsis and coagulation disorders [31]. The SIC criteria were developed after the new definition of sepsis and they considered both hemostasis parameters and clinical features expressed by SOFA score [2]. The SIC score identifies patients with organ dysfunction and coagulopathy and is more sensitive than the score system for overt DIC to detect coagulopathy that could benefit from treatment.

1.3.3 Clinical Features

The clinical manifestations of DIC can be both thrombotic and hemorrhagic and can be defined according to the following clinical phenotypes:

- Asymptomatic DIC, characterized only by laboratory abnormalities
- Bleeding-type or hyperfibrinolysis DIC (i.e., as observed in hematological malignancies)
- Organ failure type or hypofibrinolysis type that is typical of sepsis, which may result in widespread microthrombosis leading to multiorgan failure

Thrombotic events in DIC are mainly due to occlusion of small- and medium-size vessels and can lead to organ dysfunction [32, 33]. Bleeding events are less common, being observed in 10% of patients with sepsis-associated DIC and are characterized by platelets and coagulation factor consumption [34]. Thrombocytopenia plays a role in the bleeding risk, especially when platelet count is less than 50×10^9/L [35].

1.3.4 Hemostasis Tests in DIC

Thrombocytopenia is an important hallmark of DIC, but its sensitivity and specificity are limited. The consumption of coagulation factors leads to the prolongation of PT and activated partial thromboplastin time (APTT) [36]. Fibrinogen levels, being often reduced in DIC, are included in the laboratory diagnostic

criteria, but fibrinogen is also an acute-phase reactant and plasma levels may remain within normal range or even increased, except for very severe DIC. Plasma levels of fibrin split products are incorporated in the diagnostic criteria of DIC; they reflect the coagulation activation and fibrin deposition within the vasculature that occur in sepsis. Most of the assays for fibrin degradation products (FDPs) do not distinguish between degradation products of cross-linked fibrin and fibrinogen [37]. D-dimer, which is the specific split product derived from cross-linked fibrin, is preferred as a laboratory tool for diagnosis of DIC over FDPs because of the relative easier procedure and availability in most clinical laboratories.

Among viscoelastic testing procedures, thromboelastography (TEG®) and rotational thromboelastometry (ROTEM®) have been used to detect sepsis-associated coagulopathy and good sensitivity and prognostic value have been reported [38]. These systems provide graphical and numerical indicators about coagulation, leading to the formation of clot and its dissolution that are variably associated with the severity of sepsis [39].

1.3.5 Treatment

A comprehensive and detailed description of the treatment of DIC is outside the scope of this chapter. However, the cornerstone of treatment of DIC associated with sepsis is the management of the underlying infection with antimicrobial therapy and support of vital functions. Supportive hemostatic therapy may also be indicated (Table 1.4).

Table 1.4 Treatment of DIC

Agent	Indications	Rationale
Transfusion (platelets/ plasma/fibrinogen)	Patients with active bleeding or high risk of bleeding	Bleeding control Shortening of prolonged PT, aPTT, and increase of fibrinogen
UFH, LMWH	Prophylaxis of VTE Therapeutic dose if VTE is confirmed	In patients with DIC often other risk factors for VTE are present
Anticoagulant factor concentrates (antithrombin, recombinant thrombomodulin)	If available and required in specific situations	Possess both anticoagulant and anti-inflammatory properties. They seem to increase survival, but not demonstrated in RCTs
Antifibrinolytic therapy (acid tranexamic)	In patients with hyperfibrinolysis	Hyperfibrinolysis is usually not present in DIC and sepsis It can be used if bleeding is present

aPTT activated partial thromboplastin time, *DIC* disseminated intravascular coagulation, *LMWH* low-molecular-weight heparin, *PT* prothrombin time, *UFH* unfractionated heparin, *VET* viscoelastic tests

1.3.6 Transfusion of Blood Components

Although no evidence-based thresholds for transfusion in patients with DIC are established, platelets and/or plasma are commonly and empirically transfused in patients with active bleeding or in patients, who require high-risk surgical procedures. In such situations, the transfusion of platelets may be considered when platelet count is less than 50×10^9/L. In patients without active bleeding a lower platelet count ($20–30 \times 10^9$/L) can be used as cutoff and transfusion could be considered if there is a high risk of bleeding. In bleeding patients with DIC and prolonged PT and APTT or with decreased fibrinogen (less than 150 mg/dL) the administration of plasma may be useful, and decision should be made on an individual basis. Initial doses of 15 mL/kg of fresh frozen plasma (FFP) are suggested. If fluid overload is present, coagulation factor concentrates (i.e., prothrombin complex concentrate) could be the alternative but they do not contain all coagulation factors as plasma. The persistence of hypofibrinogenemia despite FFP replacement may be treated with fibrinogen concentrate or cryoprecipitate [40].

1.3.7 Anticoagulant Drugs

There are no randomized clinical trials demonstrating that the use of heparins in patients with DIC results in an improvement of clinically relevant outcomes. Unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH) are recommended for prophylaxis of VTE in patients with DIC, especially when other risk factors favoring thrombosis such as immobilization are present. Therapeutic doses of UHF or LMWH are indicated in patients with clinically overt VTE, purpura fulminans, or acral ischemia [41, 42]. Intravenous UHF requires laboratory control for dose adjustment and APTT or anti-FXa assays are the tests of choice. LMWH does not require laboratory monitoring in most cases and whenever testing is needed the anti-FXa assay is the test of choice.

1.3.8 Anticoagulant Factor Concentrates

There are several drugs that, based on their principle of action, deserve close attention as potential treatment of DIC. Retrospective studies showed that treatment with antithrombin could reduce mortality in patients with DIC [43]. Based on these studies antithrombin concentrates are used in Japan, while the global sepsis guidelines do not recommend their use, since they may increase the risk of bleeding, especially when used in combination with heparins [44].

The recombinant activated PC preparation (drotrecogin α) was developed and approved to treat sepsis [45]. Subsequent studies did not confirm the reduction of mortality rate and an increased risk of bleeding was showed. The drug is no longer on the market.

Recombinant human soluble thrombomodulin (ART-123) binds thrombin to form a complex that activates PC and hence downregulates thrombin generation. The efficacy of recombinant thrombomodulin has been investigated in Japan. Phase III trial reported better resolution of DIC compared to heparins and is currently approved for use in Japan. Studies are currently ongoing to better characterize subsets of patients, who can benefit from the administration of soluble thrombomodulin [46].

As mentioned earlier TF is the main activator of coagulation in sepsis-associated DIC and therefore its inhibition could have a therapeutic role. In this respect, TFPI being the physiological inhibitor of the complex TF-FVIIa could represent an interesting therapeutic approach. Recombinant TFPI (tifacogin) was investigated in patients with sepsis, but it did not reduce the mortality rate. Its use is therefore not recommended [47].

1.3.9 Antifibrinolytic Treatment

Patients with DIC should not generally be treated with antifibrinolytic agents. The use of antifibrinolytic therapy (i.e., tranexamic acid) could have a role in patients with DIC and hyperfibrinolysis. This is sometimes present in acute promyelocytic leukemia (AML-M3) and in some cases of malignancies (e.g., prostate carcinoma). Although a randomized controlled clinical trial has shown a beneficial effect of antifibrinolytic agents in AML-M3 [48], more recent studies did not confirm these results [49].

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Standard Coagulation Tests in COVID-19

2

Marco Ranucci and Tommaso Aloisio

2.1 Prothrombin Time (PT) and International Normalized Ratio (INR)

2.1.1 General Aspects

PT is one of the commonest coagulation tests. Its clinical use is basically to diagnose bleeding disorders, liver function, and effects of anticoagulants (warfarin). Technically, it is performed on platelet-poor plasma, adding calcium and thromboplastin (tissue factor, TF), and phospholipids, and measuring the coagulation time. Due to a high interlaboratory variability (basically due to differences in the nature and quantity of the activator), PT is generally associated to the INR (patient PT/mean normal PT according to the manufacturer of the test). From the pathophysiological point of view, the PT and INR explore the extrinsic pathway of coagulation, detecting disturbances of vitamin K-dependent coagulation factors (II, VII, IX, X, proteins C and S). Heparin usually does not prolong PT, unless in high doses. Other factors prolonging the PT are lipemia, hyperbilirubinemia, and dysfibrinogenemia.

2.1.2 Prolonged PT

Apart from the effects of oral anticoagulants, other clinical conditions may prolong the PT and increase the INR. Among these, acute or chronic liver failure with the consequent decrease in vitamin K-dependent coagulation factors is one of the most relevant.

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Disseminated intravascular coagulation (DIC) is a complex syndrome triggered by many different clinical conditions (trauma; central nervous system injuries; burns; various neoplasia; shock of different nature; infections; and others). Initiation of DIC is characterized by a thrombin burst (mainly triggered by tissue factor [TF] release) which activates platelets and fibrinogen. When the physiological inhibitors of thrombin generation (tissue factor pathway inhibitor; antithrombin) are overwhelmed, thrombin generation becomes pathological and DIC initiates. Uncontrolled clot formation triggers fibrinolysis, thrombocytopenia, and coagulation factor consumption. Therefore, on a clinical basis, and within the context of time progression, DIC may be characterized by thrombosis, bleeding, or both. Within this setting, a prolonged PT with thrombocytopenia, increased D-dimer levels, and decreased fibrinogen are diagnostic criteria.

Sepsis and septic shock are common triggers of this pattern. Early identification of coagulation derangement is of paramount importance in septic patients. In a series of 66 septic patients admitted to the intensive care unit (ICU), Liu and associates [1] found significantly higher INR values at the admission in the ICU in non-survivors, with an odds ratio of 2.0 for mortality in a multivariable model. A similar study, based on coagulation parameter in septic patients at the admission in the ICU [2], showed that prolonged PT values were associated with the development of acute kidney injury. In a retrospective study on 647 patients with sepsis or septic shock, Benediktsson and associates [3] could demonstrate that prolonged PT was associated with mortality, with a hazard ratio of 1.422. Overall, prolonged PT is a marker of severity and predictive for mortality in septic patients, likely anticipating the onset of a sepsis-related DIC.

2.1.3 Shortened PT

A shortened PT is associated with a number of clinical conditions characterized by a high risk for thrombotic complications, or overt thrombosis. These include diabetes, obesity, acute coronary syndrome, and stroke [4–6]. However, PT may be normal in a number of thrombophilic conditions, like congenital/acquired antithrombin deficiency, congenital/acquired protein C-S deficiency, factor V Leiden, and others.

2.1.4 PT in COVID-19

Despite the recognized pro-thrombotic pattern of COVID-19, PT is usually normal at least in the early phases of the disease [7, 8]. The first Chinese reports on COVID-19 patients are quite concordant in showing initially normal PT values with no difference between survivors and non-survivors [9–12]. However, two studies showed longer PT in patients with acute respiratory distress syndrome (ARDS) [9, 10], a finding not confirmed in another series [11]. A meta-analysis confirmed that in COVID-19 no clear changes of PT are evident, unless for a slightly prolonged value in more severe cases [13].

Pathologically prolonged PT and higher INR have been demonstrated in late stages of the disease, and are associated with the onset of a DIC pattern [14].

2.2 Activated Partial Thromboplastin Time (aPTT)

The aPTT measures the components of the intrinsic and common pathway of coagulation. The test is performed on plasma incubated with a reagent containing phospholipids and kaolin or ellagic acid. The time to clot formation is measured and compared with the reference value, producing a time (seconds) and an aPTT ratio.

The aPTT is sensitive to deficiency of the clotting factors II, V, VIII, IX, X, XI, and XII, and even to anti-factor VIII antibodies (in congenital or acquired hemophilia).

2.2.1 Prolonged aPTT

Congenital or acquired deficiencies of the above-listed coagulation factors prolong the aPTT. Hemophilia is one of the most common conditions. Lupus anticoagulant and antiphospholipid syndrome, even being pro-thrombotic conditions, prolong the aPTT acting on the reactant phospholipid component. Other clinical conditions leading to a prolonged aPTT are liver disease, decreased fibrinogen levels, and DIC. In septic patients, early prolongation of aPTT has been associated with bad outcomes [2, 3].

The main cause for a prolonged aPTT is unfractionated heparin (UFH) therapy, and aPTT can be used to monitor the anticoagulant effects of UFH (i.e., during extracorporeal membrane oxygenation). Conversely, the response of the aPTT to low-molecular-weight heparin (LMWH) and fondaparinux is variable and monitoring their effects requires titrated anti-FXa measure. aPTT is sensitive to the effects of dabigatran, but not in a dose-dependent fashion. Consequently, a normal aPTT makes unlikely the presence of high levels of dabigatran [15], but dabigatran monitoring requires specific tests (diluted thrombin time or ecarin time). Finally, aPTT is sensitive to intravenous direct inhibitors of thrombin, like bivalirudin and argatroban, and is commonly used to monitor the effects of these drugs.

2.2.2 Shortened aPTT

Differently from the PT, a shortened aPTT is considered a strong predictive marker for hypercoagulation [15]. Shortening of the aPTT is associated with a high level of coagulation factors (namely FVIII and fibrinogen) and is often found in the setting of an acute-phase reaction during inflammation and in sepsis. A short aPTT has been associated with deep venous thrombosis (DVT) [16] and arterial thrombosis [5].

2.2.3 aPTT and COVID-19

The first reports on COVID-19 patients from China offer a number of interesting data on aPTT that at first sight were probably underestimated. The aPTT has a tendency toward short values in a series of 99 cases, with 16% of the patients showing values shorter than the lower limit of normal range [10]. In a series of 201 COVID-19 patients [9], there was a trend ($P = 0.13$) toward shorter values in ARDS (26 s, interquartile range 22.5–35) than in non-ARDS (29.7 s, interquartile range 25.6–32.8) patients. Within ARDS patients, non-survivors had significantly ($P = 0.04$) shorter values (24.1 s, interquartile range 22.2–28.3) than survivors (29.6 s, interquartile range 24–35.7). This difference reached a *P*-value of 0.06 in a multivariable analysis. Finally, there was a trend ($P = 0.09$) toward shorter aPTT values in ICU patients in a series of 138 COVID-19 patients [11].

In a wide meta-analysis, no difference was found for aPTT values between severe and non-severe cases [13]. However, it should always be considered that some patients, and namely the most severe cases, could have been treated with UFH, which prolongs the aPTT. This is certainly a strong potential confounder.

2.3 Platelet Count

Platelet count and function are extensively treated in Chap. 6. Basically, the existing literature reports variable patterns ranging from thrombocytosis to normal platelet count to thrombocytopenia. The main player, in this setting, is likely to be the time course of the disease.

2.3.1 Thrombocytosis

Thrombocytosis in COVID-19 ARDS patients has been reported by some authors [12, 17–19]. In a series of 30 ICU patients followed for 14 days, Correa and associates [20] showed a progressive, significant increase of platelet count from admission to day 14. This behavior was more pronounced in less severe cases, with a median value of 469,000 cells/μL on day 14. Similar results were found in a study from our group [21], where viscoelastic tests demonstrated a platelet contribution to clot strength higher than the upper limit of normal range in 62% of the patients. There are different mechanisms that could induce thrombocytosis in COVID-19 patients [22]. Cytokine storm may be a major player, since various cytokines (IL-3, IL-6, IL-9, IL-11) can stimulate the production of megakaryocytes and IL-6 directly stimulates thrombopoiesis. The endothelial damage may induce a release of von Willebrand factor which may interact with megakaryocytes increasing platelet production. Finally, thrombopoietin production by the liver is directly stimulated by IL-6. The role of thrombocytosis in the determinism of thromboembolic events in COVID-19 patients is unclear, but it cannot be excluded. This introduces the

hypothesis that in the presence of thrombocytosis, specific antiplatelet therapies, and namely P2 Y_{12} inhibitors, may be useful [21, 22].

2.3.2 Thrombocytopenia

More focus exists on thrombocytopenia and its link to bad outcomes. There is in fact a consistent body of literature showing an association between thrombocytopenia, COVID-19 severity, and bad outcomes. In a meta-analysis including 1779 patients, Lippi and associates could find that platelet count was significantly lower in patients with severe patterns of COVID-19 and non-survivors [23]. The odds ratio for severe patterns of COVID-19 was 5.1 for patients with a low platelet count. Various studies showed a lower platelet count in non-survivors [2, 24–27]. However, other authors could not confirm this finding [9, 11, 28].

The mechanism(s) for thrombocytopenia in COVID-19 remain unclear. The cytokine storm could be involved in decreasing platelet synthesis; the development of autoantibodies could accelerate platelet destruction; finally, the injured endothelial layer may promote platelet activation, adhesion, and aggregation, and at the level of lung vasculature, megakaryocytes could be entrapped [22]. These last hypotheses, and namely platelet sequestration inside the newly formed thrombi, appear the most suggestive. Of notice, hemorrhagic complications in thrombocytopenic patients remain rare, and anticoagulation in this setting plays a confounding role.

2.4 Fibrinogen

2.4.1 General Aspects

Fibrinogen (coagulation factor I) is the most widely represented plasma protein coagulation factor. It is synthesized in liver hepatocytes and its plasma concentration range is 2.0–4.5 g/L [29].

Fibrinogen is a 340 kDa glycoprotein composed of two sets, each one containing three peptide chains: Aα, Bβ, and γ, linked by disulfide bridges [29, 30].

Together with platelets, fibrinogen and its derivate fibrin (FIa) are the components of a stable clot. However, fibrinogen has a double action in promoting clot formation.

The first is the development of a fibrin network: thrombin (FIIa) is the trigger of fibrinogen conversion to fibrin. Characteristics of thrombin generation are addressed in Chap. 4. Thrombin cleaves the fibrinopeptides A and B from the Aα and Bβ chains. Through this cleavage, fibrinogen is converted into fibrin monomers [31]. Subsequently, with the action of coagulation factor XIIIa (coagulation factor XIII activated by thrombin), the fibrin monomer is polymerized by a cross-link process based on reactions between two γ chains or one γ and one α chain [32]. The markers of fibrin formation are the fibrinopeptides A and B. The two principal fibrinogen

forms are high-molecular-weight (HMWF) and low-molecular-weight (LMWF) fibrinogen. HMWF promotes a fibrin network characterized by low-density thick fibers, while LMWF forms a high-density, thin-fiber fibrin network [33]. The first type of fibrin network is more efficient for angiogenesis and wound healing than the second.

The second very important role of fibrinogen is its ability to cross-link. Silent platelets may be activated through a number of different receptors and pathways; however, one of the most important activation pathways is again triggered by thrombin, which acts on the family of protease-activating receptors. Once activated, platelets express the integrin αIIb β3 (better known by clinicians as the GP IIb/IIIa receptor) on their surface. The GP IIb/IIIa receptor binds fibrinogen producing a cross-link between platelets (platelet aggregation).

Therefore, both these reactions see thrombin as the main player for fibrinogen contribution to clot formation; unless under very peculiar conditions (like a reptile bit injecting reptilase or botropase in the systemic circulation), without thrombin no conversion of fibrinogen to fibrin is elicited.

Fibrin network is destroyed by the fibrinolytic process that is addressed in Chap. 5.

Unlike for thrombin, where a number of drugs are available to directly or indirectly antagonize its action, controlling high levels of fibrinogen is a less common pharmacological intervention. Platelet aggregation through fibrinogen cross-link is blunted by GP IIb/IIIa inhibitors and this is commonly used in clinical practice. In the treatment of thrombotic complications, and namely pulmonary embolism and stroke, fibrinolytic (thrombolytic) drugs are commonly employed. A direct reduction of high levels of fibrinogen (hyperfibrinogenemia) is still outside the clinical practice.

2.4.2 Fibrinogen and Inflammation

The interaction between coagulation and inflammation is well known. The main player of this interaction is again thrombin generation elicited by blood-borne tissue factor (see Chap. 4). However, fibrinogen is another pivotal molecule, basically linked to inflammation by the complement system, through a common ancestral pathway [34].

Factor XIII is responsible for the generation of complement C5a during plasma clotting. Fibrinogen enhances the activity of the lectin complement pathway [35]. These (and other) mechanisms are inflammation triggers of coagulation. Inflammation, in turn, is able to not only trigger thrombin generation, but also elicit fibrinogen-dependent processes.

Fibrinogen synthesis is strongly enhanced by inflammation and fibrinogen is an acute-phase protein. Basically, the three genes producing the fibrinogen chains show an enhanced transcription in the early phases of inflammation. This is mainly triggered by elevated levels of interleukin-6 C-reactive protein [36].

2.4.3 Hyperfibrinogenemia

Hyperfibrinogenemia may result from genetic factors, but is more commonly associated with concomitant inflammatory diseases, sepsis, chronic kidney disease, lifestyle (smoking), and other physiological conditions (pregnancy, acute exercise). Elderly subjects and females have higher fibrinogen values [37]; seasonal variations are reported [38].

Increased fibrinogen levels are associated with an increased cardiovascular risk. Many studies demonstrated an association between elevated plasma fibrinogen levels and cardiovascular risk [39–41]. Even venous thromboembolism is associated with high fibrinogen levels. Therapies targeted to reduce the cardiovascular risk, like ACE inhibitors, result in a reduction of fibrinogen levels. However, it is not totally demonstrated that the link between high fibrinogen levels and cardiovascular events is causative rather than associative. Conceptually, elevated fibrinogen levels could trigger cardiovascular events (acute myocardial infarction, stroke, mesenteric infarction …) through a number of pathways. These mainly pertain the role of fibrinogen in the context of an unstable arterial plaque. Within this context, fibrinogen may certainly contribute to thrombus formation. In animal models, high fibrinogen levels shorten the time to vessel occlusion, generating a thick, stable, and lysis-resistant clot. Even in case of stable, chronic atherosclerosis plaques contain fibrin deposit that contributes to plaque growth and possible evolution to instability [33].

However, as already mentioned, the type of fibrinogen incorporated in the clot determines different degrees of firmness and resistance to lysis. Basically, clots characterized by an increased fibrin fiber density (produced by LMWF) are more likely to be associated with cardiovascular events, as demonstrated in young subjects with acute coronary syndrome [33].

2.4.4 Fibrinogen and COVID-19

High levels of fibrinogen are almost invariably reported in patients with COVID-19, both in less or more severe cases [20, 21, 42–48]. Values in the range of 6–7 g/L are not unusual. With respect to the time course and the role of anticoagulation, data in literature are concordant.

In critically ill patients aggressively treated with steroids and anticoagulation, there is a significant progressive decrease of fibrinogen levels [20, 21]; however, no differences were found between patients receiving low- and high-dose anticoagulation [44].

Discordant reports exist with respect to the severity of the disease and the presence of thrombotic complications. Fibrinogen levels have been found higher in patients with thrombotic complications in some studies [46] but not in others [47]. In a large series of patients, Li and associates demonstrated significantly lower fibrinogen levels in patients with venous thromboembolism vs. patients without

[49]. The severity of the disease is associated with higher levels of fibrinogen in some studies [42, 45, 46], but others could not confirm this finding [20, 50, 51].

Interestingly, the inflammatory trigger for fibrinogen formation was clearly highlighted in a study from our group, where a significant association between IL-6 values and fibrinogen levels was found (Fig. 2.1) [21].

The link between fibrinogen levels and outcome in COVID-19 patients reflects the same uncertainties of the link between fibrinogen levels and cardiovascular events. In a nice overview of this issue, Thachil introduced the concept of the potential protective role of fibrinogen in the setting of infective diseases [52]. In the presence of a microbial aggression, fibrinogen acts as an acute-phase protein targeted to defend the host. Fibrinogen is a ligand of leukocyte integrin regulating the inflammatory response; additionally, thrombus formation itself may limit the spread of the invading pathogens, mainly at the level of lungs. The author hypothesizes a multiple-step mechanism for hyperfibrinogenemia in COVID-19 patients. At the initial stage, the main role is to limit the exaggerated inflammatory reaction, and this could be a beneficial effect. Lately, thrombus formation becomes predominant, occurring at a low level, and with moderately increased D-dimer. Finally, massive thrombus formation induces a reduction in fibrinogen levels, with a concomitant increase in D-dimer. According to this theory, the ratio between fibrinogen and D-dimer could be more suggestive of the time course of the disease and of its progression toward severe patterns. In the final stage, low levels of fibrinogen (and platelets) create the environment for hemorrhagic complications.

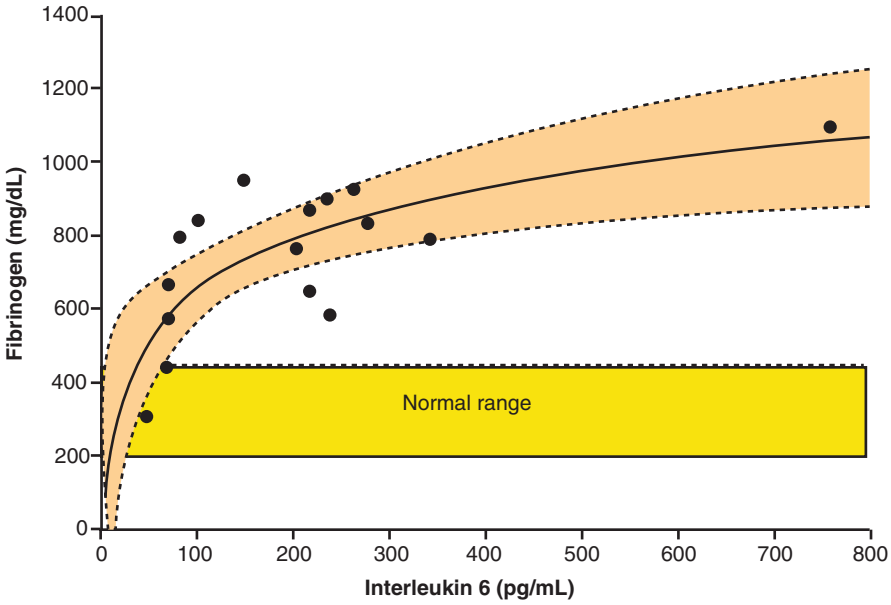

Fig. 2.1 Association between IL-6 and fibrinogen levels. Data from Ref. [21]

2.5 D-Dimer

2.5.1 General Aspects

D-dimer is a term defining multiple peptide fragments derived from plasmin degradation of fibrin polymer. Plasmin cleaves fibrin polymer at specific sites, producing fibrin degradation products (FDP) that, in this first step, are large [53]. Subsequently, the breakdown process generates the fragment D-dimer/fragment E complex (DD/E complex) that is a small (228 kDa) compound [53]. The DD/E is not the only degradation product included in the definition of D-dimer, which includes larger (over 10,000 kDa) FDP [54]. Small amounts of D-dimer are detectable in healthy subjects and derive from the spontaneous conversion of fibrinogen into fibrin and the consequent low-degree fibrinolysis.

Fibrinolysis is extensively treated in Chap. 5. For the purposes of the present sub-chapter, it is worthwhile to highlight that the products derived from fibrinogenolysis and fibrinolysis (both in the domain of FDP) are different. Only stable fibrin polymers, obtained through the action of FXIII, will produce D-dimer once plasmin activates their degradation. Monoclonal antibodies of the currently available immunoassays do not detect other FDP and are specific for D-dimer [55].

There are various factors that may affect a laboratory measure of D-dimer. Preanalytical variables include the size and length of the needle; the tube material; and the amount and quality of anticoagulant (recommended: sodium citrate; allowed: heparin, EDTA). The great majority of the assays use plasma, but whole-blood tests are available.

There are two units of measure for D-dimer: the FEU and the DDU. The FEU compares the mass of D-dimer to that of fibrinogen and the DDU determines the mass of the estimated weight of D-dimer [53]. The conversion factor between FEU and DDU is 2 (FEU=DDU × 2). Regardless of this, the final measure units are expressed in ng/mL, mg/L, μg/mL, and others. So, there are more than ten combinations of D-dimer measure depending on FEU vs. DDU and on the final measure unit. This is certainly a challenging condition for clinicians, and clinical laboratories should be very active in communicating to the clinicians any change in analytical practice, unit of measure, and normal range.

From this perspective there is a conventional cutoff at 500 μg/L FEU (250 μg/L DDU) [53–55], but the clinicians should be aware that age is a strong physiological determinant of D-dimer production, and that age-adjusted cutoffs are logical. A simple age-adjusted cutoff value (FEU) is age (years) × 10 [53].

The clinical applications of D-dimer measure belong to the scenarios directly or indirectly related to thrombogenesis and fibrinolysis. These include cerebral venous thrombosis, acute aortic dissection, acute mesenteric ischemia, venous thromboembolism (namely, pulmonary embolism), and DIC. Diagnostic use of D-dimer in the setting of venous thromboembolism has been widely addressed. D-dimer measure is a highly nonspecific test, since D-dimer values increase for

Table 2.1 Principal clinical conditions leading to increased D-dimer

Physiological and paraphysiological
Gender male
Advanced age
Neonatal period
Pregnancy/puerperium
Poor mobility
Prolonged hospitalization
Chronic diseases
Chronic inflammation
Atrial fibrillation (with left atrium thrombi)
Cancer
Heart failure
Ischemic cardiopathy
Liver disease
Renal disease
Aortic aneurysm
Deep venous thrombosis
Acute diseases
Systemic/localized infections
Aortic dissection
Burns
Hemorrhage
Pancreatitis
Trauma
Disseminated intravascular coagulation
Cyanotic heart disease with polycythemia
Others
Recent surgery
Thrombolytic therapy
Extracorporeal membrane oxygenation
Ventricular assist devices

any condition where fibrin production/breakdown is triggered. In an unselected hospital patient population, almost 80% have abnormal values of D-dimer [56]. Table 2.1 reports a list of the most common conditions leading to an increased D-dimer. However, D-dimer is highly sensitive to thromboembolic events, with a sensitivity of about 95% for acute mesenteric ischemia, cerebral venous thrombosis, acute aortic dissection, and pulmonary embolism. Therefore, its measure has a high negative predictive value, and on a clinical basis it should be used to exclude (when in normal range) rather than to diagnose (when increased) a specific thromboembolic event. Together with other diagnostic procedures (and namely imaging) D-dimer remains a cornerstone of the diagnostic process of suspected thromboembolism. Within this setting, the diagnosis of pulmonary embolism is paradigmatic,

and different algorithms like the Wells Score and the Revised Geneva Score combine clinical prediction rules with D-dimer measure.

2.5.2 D-Dimer in COVID-19

Since the early reports of COVID-19 series from China, elevated levels of D-dimer were a common finding. In the series of Wu and associates [9] patients with ARDS had a D-dimer double than patients without ARDS (1.16 μg/mL vs. 0.52 μg/mL) and non-survivors an eightfold higher value than survivors (3.95 μg/mL vs. 0.49 μg/mL). Wang and associates report D-dimer values to be significantly (P = 0.001) higher in ICU (414 mg/L) than in non-ICU patients (166 mg/L) [11] and similar differences were noticed by Huang and associates [12]. Chen and associates found abnormally elevated D-dimer levels in 36% of their patient population [10]. After these early reports, the finding of elevated values of D-dimer in COVID-19 patients, and of higher values in more severe cases, was confirmed by numerous reports from Western countries [21, 42–47, 51, 57, 58]. Significantly higher values of D-dimer were found in the most severe cases [42, 43, 45], in patients with thrombosis [45–47, 58], and in non-survivors [45]. The large body of literature on D-dimer in COVID-19 generated numerous meta-analyses pooling together different studies having, as dependent variable, the severity of the disease, and/or the survival. However, a critical appraisal of these pooled data is needed, given the heterogeneous modality of D-dimer value expression.

In a nice overview, Favaloro and Thachil pointed out the possible confounders in pooling together D-dimer values from different studies. Most publications did not identify the manufacturer and the assay used; most publications failed to report if DDU or FEU units were used; half the publications did not report the cutoff value; some publications did not report units of measure of D-dimer [59].

Given these limitations, the different meta-analyses offer a generally concordant scenario.

The existing meta-analyses express the D-dimer value as a continuous variable or as a binary (normal vs. elevated) variable. In the first case, given the different units of measure, the standardized mean difference (SMD) is used.

Shi and associates [60] analyzed 21 studies (3657 patients) and found that patients with a severe pattern of COVID-19 had a higher mean standardized value of D-dimer (SMD: 0.97, 95% confidence interval [CI] 0.77–1.17). The relative risk for severe pattern was 3.3 (95% CI 1.6–6.5) and for mortality 3.9 (95% CI 2.0–7.8) in patients with elevated D-dimer.

Sakka and associates [61] analyzed six studies (1355 patients) and found that non-survivors had an SMD of D-dimer values of 3.6 (95% CI 2.8–4.4). Simadibrata and associates [62] analyzed nine studies (2911 patients) and found a relative risk for mortality of 4.8 (95% CI 3.0–7.5) in patients with elevated D-dimer.

In a large series of 29 studies (4328 patients), Nugroho and associates [63] found that patients with severe patterns of the disease had an SMD of D-dimer values of 0.95 (95% CI 0.61–1.28) and those who did not survive had an SMD of 5.54 (95% CI 3.40–7.67). Shah and associates [64] analyzed 18 studies (3682 patients) and found that patients with severe patterns of the disease had an SMD of D-dimer values of 0.5 (95% CI 0.2–0.8) and those who did not survive had an SMD of 6.1 (95% CI 4.1–8.1). When D-dimers were expressed as binary variables, the relative risks for severe pattern and mortality were 2.0 (95% CI 1.3–3.1) and 4.1 (95% CI 2.5–6.8), respectively, in patients with elevated D-dimer.

Lima and associates [65] analyzed three studies only (648 patients) and found again that non-survivors had a significantly high SMD of D-dimers (3.37, 95% CI 1.53–5.02). Finally, Bansal and associates [66] used a composite outcome (mortality or severe patterns) analyzing six studies (1338 patients) and finding that the SMD of D-dimer values was 1.67 (95% CI 0.72–2.62) in those who fulfilled the composite outcome definition.

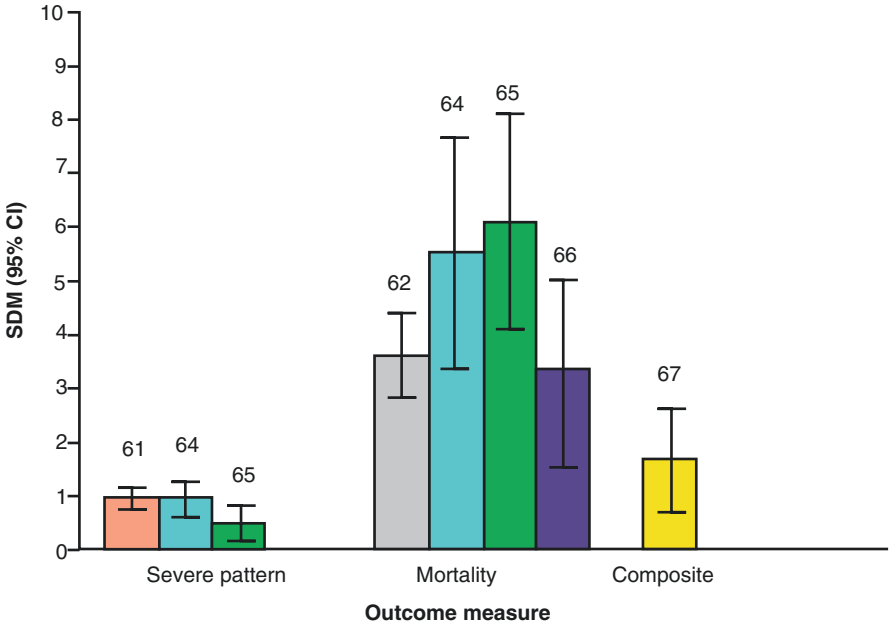

Fig. 2.2 Meta-analytic results of the association between D-dimer levels and severity of the disease, mortality, and composite outcome of severity + mortality. Numbers above the bars are the reference. *CI* confidence interval; *SDM* standardized mean difference

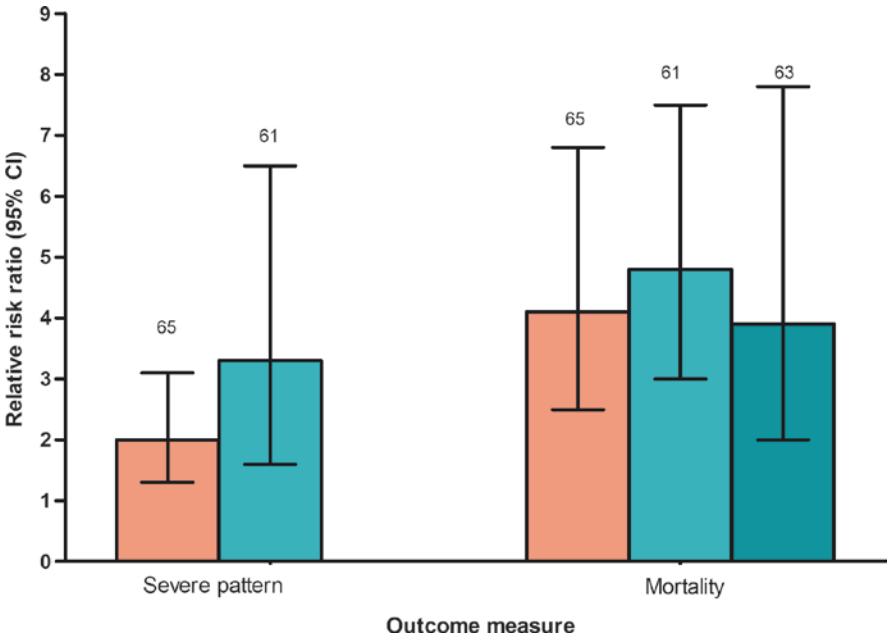

Fig. 2.3 Meta-analytic results of the association between elevated D-dimer and severity of the disease and mortality. Numbers above the bars are the reference. *CI* confidence interval

The data from the main existing meta-analyses are reported in Figs. 2.2 and 2.3. Overall the information is concordant in the finding that patients with severe patterns of the disease and moreover those who did not survive have higher values of D-dimer and that when considering D-dimer as a binary variable, patients with values above the cutoff have a double relative risk for severe patterns of the disease and a fourfold relative risk for mortality.

2.6 Conclusions

Even if routine coagulation tests have a low specificity for many infective coagulation-related disturbances, they are not useless in the setting of COVID-19, especially for the risk stratification of patients. Table 2.2 offers a summarized overview of the most common changes in routine coagulation tests in COVID-19 patients.

Table 2.2 Standard coagulation tests in COVID-19 acute respiratory syndrome patients

TEST	General population	Severe pattern	Thrombosis	Survival
PT	Normal in early phase	Slightly prolonged Prolonged in late phase with DIC	No specific modification	No differences between survivors and non-survivors
aPTT	Normal or shortened in early phase Prolonged in UFH treatment	Normal or shortened in ICU cases Prolonged in DIC	No specific modification	Shortened in non-survivors without DIC
Platelet count	Normal or increased in early phase	Decreased in ICU cases Decreased in DIC	Decreased in pulmonary embolism and other major thrombosis	Decreased in non-survivors
Fibrinogen	Increased	Increased in severe cases in some reports Decreasing trend in less severe cases Increased in high inflammatory state	Increased in some reports Decreased values in VTE and major thrombosis	Controversial reports of higher values in non-survivors
D-dimer	Increased	Increased in ICU patients Relative risk for severe pattern around 2.0 when > cutoff	Increased	Increased in non-survivors Relative risk for mortality around 4.0 when > cutoff

aPTT activated partial thromboplastin time, *DIC* disseminated intravascular coagulation, *ICU* intensive care unit, *PT* prothrombin time, *UFH* unfractionated heparin, *VTE* venous thromboembolism

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Point-of-Care Coagulation Tests in COVID-19

3

Ekaterina Baryshnikova

3.1 Point-of-Care Devices and Tests

A point-of-care device is a kind of diagnostic testing placed and performed at or in proximity of the site where the patient is receiving medical care. This kind of device is normally positioned outside the central laboratory, generally in intensive care units, clinical wards, or operating rooms. POC devices allow a rapid assessment of the overall activity of the coagulation factors and of the interaction between platelets and fibrinogen as clot production and stability. Among the most known devices used in COVID-19 setting there are the thromboelastograph TEG® and rotational thromboelastometer ROTEM®, the sonorheometry-based Quantra®, and the ClotPro® based on the elastic motion thromboelastography. All the abovementioned devices work on whole blood, innate or anticoagulated with citrate, allowing a comprehensive assessment of clot properties.

TEG® and ROTEM® both employ the cup (rotating in TEG®, still in ROTEM®) and pin (still in TEG® and rotating in ROTEM®) methodology and use whole blood activated with kaolin (TEG®) or specific activators for intrinsic and extrinsic pathways (EXTEM and INTEM, respectively, ROTEM®). The growing viscoelasticity of the coagulating blood is continuously measured by electromechanical (TEG®) or optical (ROTEM®) sensors, translated into a graphical curve and visualized in real time on the monitor of a computer.

The overall activity of the coagulation factors (except fibrinogen) is defined by the R time (minutes) in TEG® and CT (clotting time, seconds) in ROTEM®, i.e., the time until the gelification given by the first fibrin polymer assembling occurs. Additional parameters are K time (minutes, TEG®) and CFT (clot formation time,

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seconds, ROTEM®)—time required until a certain clot amplitude is reached. The alpha angle is a measure of speed of clot formation; on TEG® it is defined in one of the two ways, either as the angle between baseline and a line defined by the points R and K or as the angle between baseline and a line tangential to the curve at 2 mm amplitude. On ROTEM® the alpha angle is the angle of tangent between 0 mm and the curve when the clot firmness is 20 mm. R/CT parameter is prolonged in case of inherited or acquired coagulation factor deficiencies, often associated with bleeding; otherwise it has been speculated that a shortening of the R/CT could indicate a hypercoagulable prothrombotic state. Addition of the heparinase allows ruling out heparin presence in the blood sample.

The strength and stability of the forming clot are represented by the maximum amplitude (MA, mm) on TEG® and maximum clot firmness (MCF, mm) on ROTEM®. This parameter incorporates the contribution of both platelets and fibrinogen; thus a reduced value of MA/MCF is not able to rule out the single deficiencies. The functional fibrinogen on TEG® and the FIBTEM test on ROTEM® inhibit platelet aggregation within the blood sample providing information about fibrinogen contribution to the clot.

Fully automated cartridge-based version of the devices is available for both TEG® (TEG® 6S) and ROTEM® (ROTEM® sigma). Despite technical differences between the manual and cartridge-based analyzers, correlation of the results between the analyzers of the same family has been reported [4], at least partially [5].

The Quantra® is a fully automated POC VET device based on SEER sonorheometry technology. Briefly, a sample of citrated whole blood is drawn into a multi-well cartridge and mixed with lyophilized reagent. An ultrasound pulse is sent in order to generate a shear wave, and the following deformation is measured. The frequency and amplitude of the induced deformation are directly related to the viscoelastic properties of the sample [6].

The Quantra QPlus® cartridge includes four parallel channels, each pre-filled with specific lyophilized reagents and performing simultaneous measurements. Clot coagulation time (CT and CTH, seconds) is assessed after blood activation with kaolin both with and without heparinase. The overall clot stiffness (CS, hPa) is given together with fibrinogen contribution to the overall clot stiffness (FCS, hPa, measured) and platelet contribution (PCS, hPa, calculated).

ClotPro® uses elastic motion thromboelastography with the established cup (rotating) and pin (still) semiautomated methodology and records kinetic changes in a sample of citrated whole blood. The device has been described previously [7]. The device allows performing six tests simultaneously, including the TPA test where the tissue factor-triggered extrinsic pathway is coupled to the activation of fibrinolysis by high-dose recombinant tissue plasminogen activator (tPA), reflecting the resistance to fibrinolysis.

The indisputable advantage of VETs is their capability to report different components and stages of the overall coagulation process with a single test/cartridge. Closed systems of the cartridge-based devices are particularly advantageous in a COVID working ward because of minimizing the risks of manual blood manipulations.

3.2 Thromboelastography

As early as April 2020, Panigada and associates pointed out that critically ill patients admitted to ICU showed a peculiar hypercoagulable profile [8]. Using a previously established reference healthy population, they found that COVID-19 patients have a shorter R and K time, a greater alpha angle and MA, and, most importantly, shutdown of fibrinolysis (LY30 lower than the mean of the reference cohort in 100% of cases). Interestingly, the authors observed a concomitant endothelial dysfunction indicated by the elevation of the von Willebrand factor antigen. These findings were further confirmed by similar studies [9, 10].

A typical pattern of hypercoagulability at TEG is shown in Fig. 3.1.

Cordier and colleagues compared TEG findings of critically ill patients on ICU admission to those of healthy controls [10]. The statistical significance ($P < 0.001$) was reached for all the analyzed parameters—patients affected by COVID-19 were characterized by significantly decreased R, K, and LY30, and significantly increased values of alpha angle, MA, CI (coagulation index, a composite compilation of R, K, angle, and MA), and TTG (total thrombin generation, a parameter calculated from the first derivative of the TEG waveform). With respect to TEG parameters, no differences between patients with and without obesity (body mass index, BMI, >30 kg/m^2) and between patients who survived versus who did not were found. The procoagulant profile persisted in patients who survived and had a good clinical course. There was no association between TEG values and severity of CT (computerized tomography) lesions. Anyway, a trend towards a stronger hypercoagulability in patients who developed thrombosis was noted.

On the other hand, Yuriditsky and associates reported no differences in thromboelastographic parameters between patients with venous thromboembolism (VTE) and patients without and no association between TEG and combined outcome measure (either death or confirmed VTE) [11]. Fifty percent of the analyzed population showed hypercoagulable state expressed as a CI >3 despite receiving prophylactic or therapeutic anticoagulation with heparin. Sixty percent had a maximum amplitude (MA) above the normal range, i.e., 70 mm. The median heparinase R time was below the lower limit of normal range in a significant proportion of patients (43.8%). Even if no direct association was found, the authors pointed out that the cohort was characterized by a high incidence of renal failure preceding VTE diagnosis. Wright and associates observed a complete shutdown of fibrinolysis (LY30 = 0%) in 57% of their population of 44 COVID-positive ICU patients that predicted venous thromboembolic events with an area under the receiver operating characteristic curve of 0.742 ($P = 0.021$) [12]. Patients with no fibrinolytic activity at 30 min on TEG and a D-dimer >2600 ng/mL had VTE with a rate of 50% compared to 0% of patients with neither risk factor ($P = 0.008$). Consistent with the findings of Yuriditsky [11], the hemodialysis rate in the high-risk group was 80% compared to 14% of the low-risk patients ($P = 0.004$).

Stattin and colleagues performed a prospective study following the evolution of the inflammatory and coagulation profile of the ICU patients over time (7 and more days) on the TEG 6S® platform [13]. The majority of patients maintained the

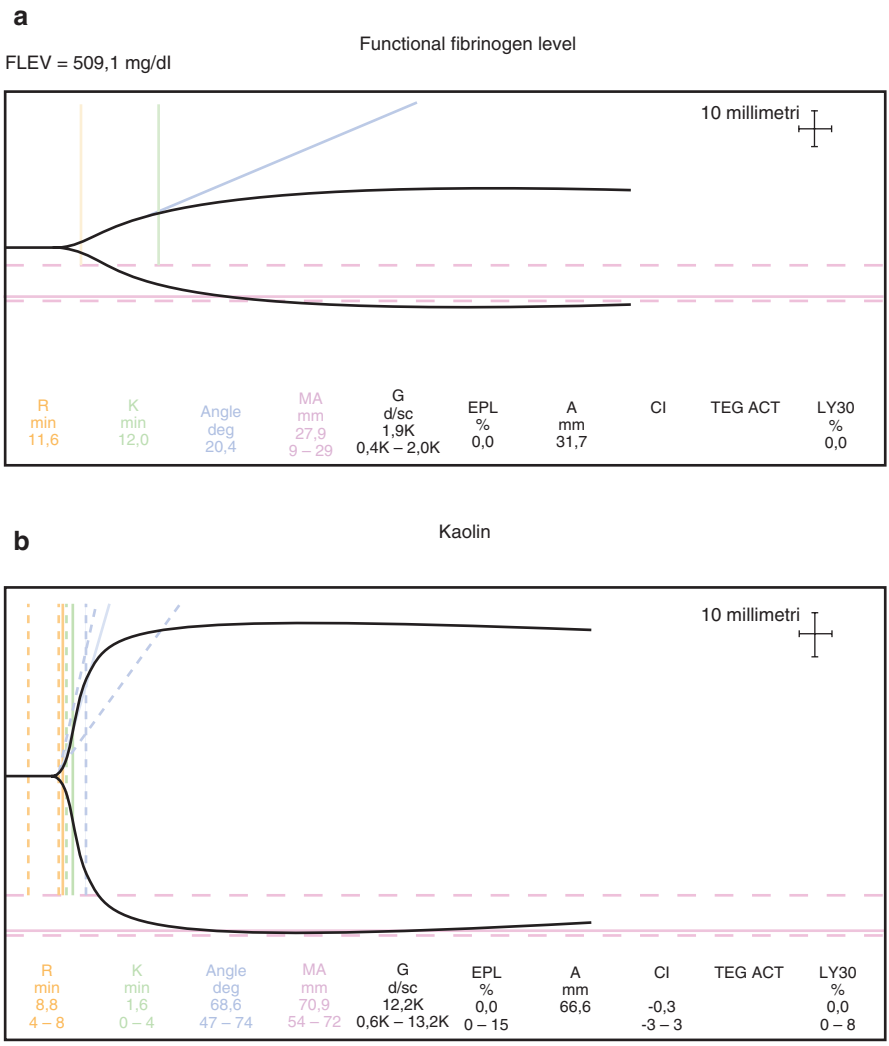

Fig. 3.1 Example of a typical TEG tracing presenting with a procoagulant pattern from a critically ill COVID-19-positive patient. A. Functional fibrinogen testing and fibrinogen level (FLEV) calculated by the software. B. Basal kaolin testing. High fibrinogen level and total absence of fibrinolysis are of note. Abbreviations: *MA* maximum amplitude, *EPL* estimated percent lysis, *CI* coagulation index, *LY30* percent lysis after 30 min

hypercoagulable profile during the hospitalization, seen as an elevation of the MA and not as a decrease in R time. Moreover, the authors pointed out the insufficient capacity of TEG® to reliably detect the effect of LMWH administration.

Analogously, the group of Bocci did not observe significant differences in thromboelastographic parameters of TEG 6S® of COVID-positive ICU patients after a 7-day follow-up with a maintenance of a hypercoagulable state, expressed as a

distribution above the normal range of the CK-MA, rTEG-MA, and FF-MA despite systemic anticoagulation [14]. TEG-ACT, a parameter calculated by the TEG 6S®, was reduced, whereas CKH-R and CKH-K stood in normal range. Seven-day-long anticoagulation therapy, either low-molecular-weight or unfractionated heparin, had no impact on TEG parameters. Shah and associates found analogous increase of alpha angle, MA, and FF-MA on TEG 6S® [15]. No difference in TEG parameters between patients with or without TE events was found in this study. Vlot and colleagues performed TEG 6S® in COVID-positive ICU-hospitalized patients on mechanical ventilation administered with high-dose LMWH in which peak Xa activity of 0.38 IU/ml was within the target range, still finding a strong procoagulant pattern despite anticoagulation with very high CFF-MA levels with overall fibrin contribution to the clot of 71% (56–85), instead of 20–25% in normal clots [16].

Chandel and associates evaluated patients on veno-venous (vv) ECMO and confirmed high values of MA (median 72.8 mm) on thromboelastography [17]. No statistically significant difference between patients with macrothrombosis versus patients without TE was found with respect to TEG-MA.

Low fibrinolytic activity, represented by the resistance to exogenously induced fibrinolysis, was specifically tested by Maier and colleagues adding tPA to platelet-poor plasma samples of 14 patients affected by acute COVID-19 disease (pooling together ICU and standard ward) and compared to 14 healthy controls [18]. Consistent with other reports, the COVID-positive patients and the controls significantly differed for the MA (43.6 ± 6.9 *vs*. 23.2 ± 5.5 mm, $P < 0.0001$). The induced fibrinolysis was 21% less in COVID patients as compared to controls at 30 min (LY30, 37.9 ± 16.5 *vs*. 58.9 ± 18.3, $P = 0.0035$). Off-label use of tPA (alteplase) in four patients for clinical evidence or suspicion of microvascular or macrovascular thromboses and hypercoagulable values on thromboelastography has been reported with benefit [19].

Attempts to stratify patients based on the definition of a hypercoagulable state (HS) have been done. Mortus and colleagues arbitrarily defined hypercoagulability as elevated fibrinogen activity greater than 73° angle or MA greater than 65 mm on TEG with heparinase correction [20]. Salem and colleagues defined HS as MA > 69 mm (upper limit of normal, ULN), alpha angle >77° (ULN), R <4.3 min (lower limit of normal, LLN), or $K < 0.8$ min (LLN) [20]. The incidence of HS varies between studies and populations from 30% [20] to up to 90% [8, 11, 20] and HS was mainly due to high MA and alpha angle. In Salem's study, HS was not associated with the occurrence of thromboembolic events; only LY30 was significantly lower in the TE group ($P = 0.041$) [21]. In the study of Mortus [20], stratifying the population based on the number of thromboembolic events (less than 2 versus 2+ TE events), elevated MA was observed in ten patients (100%) in the high-event group versus five patients (45%) in the low-event group, providing 100% sensitivity and 100% negative predictive value. On contrast, in a population of 40 ICU COVID-positive patients who developed VTE (DVT or PE) analyzed by Marvi and associates the venous thromboembolism rate was higher in patients who were not hypercoagulable for maximum amplitude ($P = 0.04$) and alpha angle ($P = 0.001$) [22].

In summary, all the studies agree on pathological increase of the clot strength (MA) attributing an important role to fibrinogen contribution, whereas contrasting findings are reported in relation to the coagulation initiation. In addition, the research for association between viscoelastic parameters, single or combined, with thromboembolic events showed different results. This could be explained by the fact that the available literature is mostly retrospective and that the protocols for TE assessment vary greatly between published reports (as variable is the reported incidence of TE in COVID-positive patients).

An overview of the published papers is given in Table 3.1.

3.3 Rotational Thromboelastometry

Similar to the TEG studies, most of the reports agree on the presence of high values of MCF in EXTEM/INTEM and especially in FIBTEM tests with higher values associated with severity of disease—from healthy controls to COVID-positive patients hospitalized in non-intensive wards to patients admitted to ICU department [23–26].

Variable observations are available on the values of CT in EXTEM and INTEM tests. Almskog found longer EXTEM CT but shorter CFT in COVID-positive patients as compared to healthy controls, and longer CT but shorter CFT values in intensive versus non-intensive patients, standing for a prolongation in clot activation but a strengthening of clot propagation and an increasing clot firmness (resistant to fibrinolysis) [23]. All the comparisons were statistically significant with a $P < 0.001$. These findings are consistent with other studies [27, 28].

A typical pattern of hypercoagulability at thromboelastometry is shown in Fig. 3.2.

Shorter CFT in EXTEM was also observed by Tsantes and colleagues in ICU-treated COVID-positive patients as compared to non-COVID-ICU patients, patients with mild COVID, and healthy controls (40.7 ± 13.0 *vs*. 63.7 ± 34.7 *vs*. 59.5 ± 24.9 *vs*. 89.2 ± 24.7 s, respectively, overall $P < 0.001$) [24]. Lower EXTEM ML was also peculiar to ICU patients with COVID (1.8 ± 2.3 *vs*. 3.2 ± 3.7 *vs*. 6.2± *vs*. 8.4 ± 4.6%, respectively, overall $P < 0.001$), as well as higher MCF (75.7 ± 5.0 *vs*. 69.4 ± 8.5 *vs*. 72.4 ± 4.0 *vs*. 59.9 mm respectively, overall $P < 0.001$).

Boss and colleagues compared thromboelastometric findings in patients with COVID-associated severe sepsis versus patients with severe sepsis but without COVID-19 disease [25]. Higher values of MCF on EXTEM (70.4 ± 10.4 *vs*. 60.6 ± 14.8 mm, $P = 0.022$) and FIBTEM (38.4 ± 10.1 *vs*. 29.6 ± 10.8 mm, $P = 0.012$) and lower level of lysis (ML 60, 0.6 ± 1.2 *vs*. 3.3 ± 3.7%, $P = 0.013$) characterized patients with COVID-19. No statistically significant differences in ROTEM® parameters between COVID-19 patients with or without thromboembolic events were found.

Spiezia and associates found similar differences between COVID ICU patients and a group of healthy age-, sex-, and body weight-matched subjects with the

Table 3.1 Overview of the papers on thromboelastography in COVID-19 setting

Author and year	Population	Study type Enrollment period	Device and tests performed
Bocci et al. (2020)	Critically ill patients admitted to ICU, *n* = 40; second assessment, *n* = 26	Observational study February–March 2020	TEG 6S®
Chandel et al. (2021)	Critically ill patients admitted to ICU and on vvECMO, *n* = 24	Retrospective study March–May 2020	TEG® 5000
Cordier et al. (2021)	Critically ill patients admitted to ICU, *n* = 24 on ICU admission, second sampling *n* = 10 Healthy control group, *n* = 20	Retrospective study March–April 2020	TEG® 5000 Citrated K, KH
Hightower et al. (2020)	Critically ill patients admitted to ICU, *n* = 5	Observational study April 2020	TEG® 5000 Citrated K, KH
Maatman et al. (2020)	Critically ill patients admitted to ICU, *n* = 12	Observational cohort study March 2020	TEG® 5000 Citrated K, KH
Marvi et al. (2021)	Critically ill patients admitted to ICU, *n* = 40	Prospective study April–July 2020	TEG® 5000 Citrated KH
Mortus et al. (2020)	Critically ill patients admitted to ICU, *n* = 21	Retrospective study March–April 2020	TEG® 5000 K, KH
Panigada et al. (2020)	Critically ill patients admitted to ICU, *n* = 24	Observational study March 2020	TEG® 5000 KH
Sadd et al. (2020)	Critically ill patients admitted to ICU, *n* = 10	Retrospective study Unknown period of enrollment	TEG 6S®
Salem et al. (2020)	Critically ill patients admitted to ICU, *n* = 52	Retrospective study April–May 2020	TEG 6S®
Shah et al. (2020)	Critically ill patients admitted to ICU, *n* = 20	Retrospective study March–May 2020	TEG 6S®
Stattin et al. (2020)	Critically ill patients admitted to ICU, *n* = 31	Prospective observational study March–April	TEG 6S®
Stillson et al. (2021)	Critically ill patients admitted to ICU, *n* = 31	Prospective study April–September 2020	TEG® 5000 Citrated K, KH
Vlot et al. (2020)	Critically ill patients admitted to ICU, *n* = 16	Observational study Unknown period of enrollment	TEG 6S®
Wright et al. (2020)	Critically ill patients admitted to ICU, *n* = 44	Observational cohort study March–April 2020	TEG® 5000 Citrated K, KH
Yuriditsky et al. (2020)	Critically ill patients admitted to ICU, *n* = 64	Retrospective cohort study April 2020	TEG® 5000 Citrated K, KH

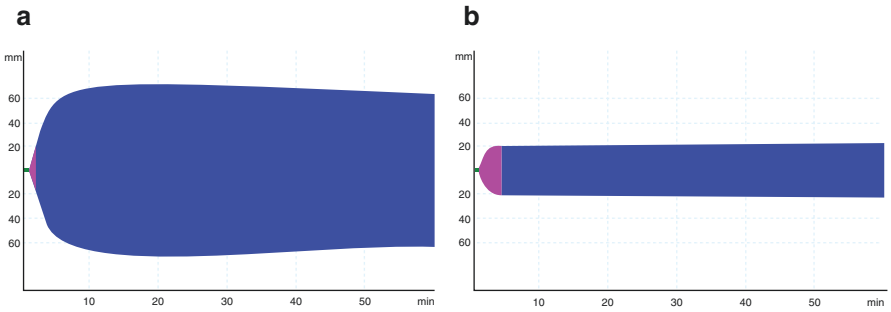

Fig. 3.2 Typical procoagulant ROTEM tracing from a representative critically ill COVID-19-positive patient. (**a**) EXTEM test. (**b**) FIBTEM test. MCF values of both the test are close to the upper limit of the normal reference range

former showing a more pronounced hypercoagulation (shorter CFT in INTEM and EXTEM and higher MCF in INTEM, EXTEM, and FIBTEM tests) [29]. In contrast to most reports, lysis parameters showed no difference between the two groups.

In a series of 40 consecutive patients admitted to ICU, Pavoni and colleagues analyzed ROTEM parameters at three time points, at ICU admission and at days 5 and 10 [30]. They observed a hypercoagulable state analogous to the other studies (significantly higher MCF in FIBTEM, tendency to a shorter CFT and a higher MCF in INTEM and EXTEM) but also highlighted that some parameters tend to improve over time—FIBTEM MCF decreasing from 35.9 ± 5.9 mm at day 0–23 ± 3.3 mm at day 10 ($P = 0.017$), among others.

Ibanez and associates substantially confirmed what was previously found on increased clot strength and furtherly pointed out the decreased clot lysis parameters as compared to reported levels in healthy population [26]. Others [31] tried to define fibrinolysis shutdown in COVID-19 patients in a way similar to the trauma setting, as EXTEM maximum lysis of <3.5% [32]. According to this definition, 11 out of 25 (44%) of the patients in the cohort analyzed by Creel-Bulos and colleagues experienced a fibrinolysis shutdown and 8 out of 9 patients who underwent thromboembolic complications met the shutdown criterion [31].

Thromboelastometry could be modified in order to evaluate the resistance of the clot to be lysed. Nougier and colleagues added 0.625 $\mu g.mL^{-1}$ tPA and analyzed the lysis index LY30 (residual clot firmness after 30 min after coagulation time in % to MCF) in 23 patients with and without thrombotic events, and compared them to healthy controls [33]. They showed that in healthy controls the clot is almost completely lysed under stimulation with tPA, and that LY30 of ICU patients with thrombosis was significantly higher than that of other COVID-positive ICU patients with similar disease severity (63 ± 39 *vs*. 18 ± 35%, $P = 0.022$). The impaired fibrinolysis was supported by higher levels of t-PA, PAI-1, and TAFI in patients with a more severe disease.

Hoechter and colleagues compared COVID-positive intubated ICU patients with ARDS versus ICU patients with ARDS due to other bacterial/viral pneumonia [34]. They found no difference between group concerning MCF nor ML on EXTEM but a significantly higher FIBTEM MCF in COVID group (29 vs. 22 mm; $P = 0.005$) with 9 out of 11 COVID-positive patients showing MCF values above the upper limit of the normal range (9–25 mm).

Similar to thromboelastography, small patient population and inhomogeneity of TE assessing protocols do not allow solid results on association between particular hypercoagulable conditions on ROTEM and risk of developing thromboembolic complications during COVID-19 disease. More rigorous studies are required to establish such a relationship.

An overview of the published papers is given in Table 3.2.

Table 3.2 Overview of the papers on rotational thromboelastometry in COVID-19 setting

Author and year	Population	Study type Enrollment period	Device and tests performed
Almskog et al. (2021)	ICU-positive intensive and non-intensive patients, $n = 60$, vs. healthy controls, $n = 86$	Prospective study May 2020	ROTEM® sigma EXTEM, INTEM, FIBTEM, HEPTEM
Boss et al. (2021)	Patients with severe COVID-related sepsis, $n = 20$, vs. patients with severe sepsis without COVID, $n = 20$	Retrospective study March–June 2020	ROTEM® delta EXTEM, INTEM, FIBTEM, APTEM
Collett et al. (2021)	Critically ill patients admitted to ICU, $n = 6$	Retrospective study Unknown enrollment period	ROTEM® sigma EXTEM, INTEM, FIBTEM
Creel-Bulos et al. (2021)	Critically ill patients admitted to ICU, $n = 25$	Retrospective study April 2020	ROTEM® delta EXTEM, FIBTEM
Hoechter et al. (2020)	Intubated patients with ARDS: $n = 22$ COVID vs. $n = 14$ non-COVID	Retrospective study March–April 2020 (COVID group)	ROTEM® delta EXTEM, FIBTEM
Ibanez et al. (2021)	Critically ill patients admitted to ICU, $n = 19$	Prospective study April 2020	ROTEM® sigma EXTEM, INTEM, FIBTEM
Nougier et al. (2021)	COVID-positive patients admitted to ICU, $n = 48$, and internal medicine department, $n = 30$	Prospective study Unknown period of enrollment	ROTEM® delta tPA-modified EXTEM

(continued)

Table 3.2 (continued)

Author and year	Population	Study type Enrollment period	Device and tests performed
Pavoni et al. (2020)	Critically ill patients admitted to ICU, $n = 40$	Retrospective study February–April 2020	ROTEM® gamma EXTEM, INTEM, FIBTEM
Spiezia et al. (2020)	Critically ill patients admitted to ICU, $n = 22$ vs. matched healthy controls, $n = 44$	Prospective study March 2020	ROTEM® delta EXTEM, INTEM, FIBTEM
Tsantes et al. (2020)	COVID-positive patients with severe (ICU, $n = 11$) *vs*. mild ($n = 21$) disease, non-COVID ICU patients $n = 9$, healthy controls $n = 20$	Prospective study Unknown period of enrollment	Unknown ROTEM® EXTEM
Van Veenendaal et al. (2020)	Critically ill patients admitted to ICU, $n = 47$	Retrospective study April 2020	ROTEM® sigma EXTEM, INTEM, FIBTEM

3.4 ClotPro

Three studies have been carried out on critically ill COVID-19 patients.

Bachler and associates retrospectively analyzed 20 critically ill COVID-19 patients and 60 healthy controls for coagulation and fibrinolysis markers [35]. As compared to controls, COVID patients showed hypercoagulable EX test MCF (68 *vs.* 61 mm, $P < 0.01$) and FIB test MCF (34 *vs.* 17 mm, $P < 0.01$). Consistent with the findings by other viscoelastic analyzers, the disproportion between platelet and fibrinogen contribution to clot strength, normally with 75–80% of the overall stiffness ascribable to platelets and 20–25% to fibrinogen and here strongly shifted towards a fibrinogen predominance, is clear. Clotting time of EX test showed no statistical difference between groups, and IN test CT was longer for COVID patients (188 *vs.* 159 s, $P < 0.01$) but still within the reference range.

The most peculiar finding was that the fibrinolytic response as expressed by the lysis time (LT) of TPA test (fibrinolysis induced by the recombinant tissue plasminogen activator) in COVID patients was significantly longer than in controls (508 *vs.* 210 s, $P < 0.01$), as well as by maximum lysis (ML) on EX test (3 *vs.* 6%, $P < 0.01$). Overall, 70% of the patients suffered from an impairment in fibrinolysis, but no differences in thrombotic event occurrence were registered between patients with hypofibrinolysis and patients without such a condition.

Hammer and colleagues [36] also focused on fibrinolysis shutdown in a cohort of 29 patients hospitalized for COVID-19 with a diagnosis of both moderate (ward, $n = 9$) and severe (ICU, $n = 20$, 8 out of 20 were on vv-ECMO support) disease.

Blood samples from 10 healthy donors were used as reference. Severe COVID-19 patients showed a significant reduction of the spontaneous clot lysis after activation of the extrinsic coagulation pathway when compared to healthy donors (3.25% *vs.* 6.2%, $P = 0.013$), very similar to the findings of Bachler [35]. No significant difference was observed between non-ICU COVID-positive patients and healthy controls. Resistance to the fibrinolytic effect of tPA was significant in all the patients, both severe and moderate, when compared to controls (ICU COVID patients *vs.* controls, 365.7 s *vs.* 193.2 s, $P = 0.0014$; non-ICU COVID patients *vs.* controls, 354.3 s *vs.* 193.2 s, $P = 0.0005$). These data were supported by the increased concentration of plasma tPA (profibrinolytic), no increase in plasminogen (fibrinolytic), and increased PAI-1 (antifibrinolytic) in ICU COVID-19 patients indicating that, despite the profibrinolytic signaling, PAI-1 overcomes the competence of the fibrinolytic system with the final fibrinolysis slowdown effect. No association with thrombotic events or mortality was found in this cohort.

Findings of Heinz and associates [37] in a cohort of 29 COVID-positive ICU patients with ARDS (compared to 12 healthy controls) agree with those of the two previously discussed studies. In particular, the lysis time significantly differed between two groups (530 s *vs.* 211 s, $P < 0.001$), as well as EX test MCF (68 mm *vs.* 57 mm, $P < 0.001$) and FIB test MCF (37 mm *vs.* 15 mm, $P < 0.001$). Association of viscoelastic parameters with outcome and thrombotic incidence has not been investigated in this cohort.

3.5 SEER: QUANTRA

In COVID-19 setting QUANTRA analyzer was used in one study only.

Ranucci and colleagues [38] investigated a cohort of 16 COVID-positive critically ill ICU patients with a baseline coagulation assessment after 2–5 days of ICU admission, followed by a second assessment after 14 days for 9 patients. Clotting time (CT) was within the normal range and did not differ between the two assessments. The overall clot strength at baseline was higher than normal: 55 (35–63) hPa with reference range (RR) being 13–33.2 hPa. Both platelet (PCS) and fibrinogen (FCS) were above the upper limit of the reference range—43 (24–45) hPa (RR 11.9–29.8 hPa) and 12 (6–13.5) hPa (RR 1–3.7), respectively. After 2-week follow-up, a significant decrease of CS ($P = 0.013$), PCS ($P = 0.035$), and FCS ($P = 0.038$) was found. Thromboembolic prophylaxis included LMWH 6000 twice a day (8000 if BMI >35), antithrombin III correction of values <70%, clopidogrel 300 mg loading dose, and 75 mg daily maintenance if platelet count >400,000 cells/μL. No major thromboembolic events were observed in this cohort.

A Quantra screenshot of a hypercoagulable COVID-19 patient is shown in Fig. 3.3.

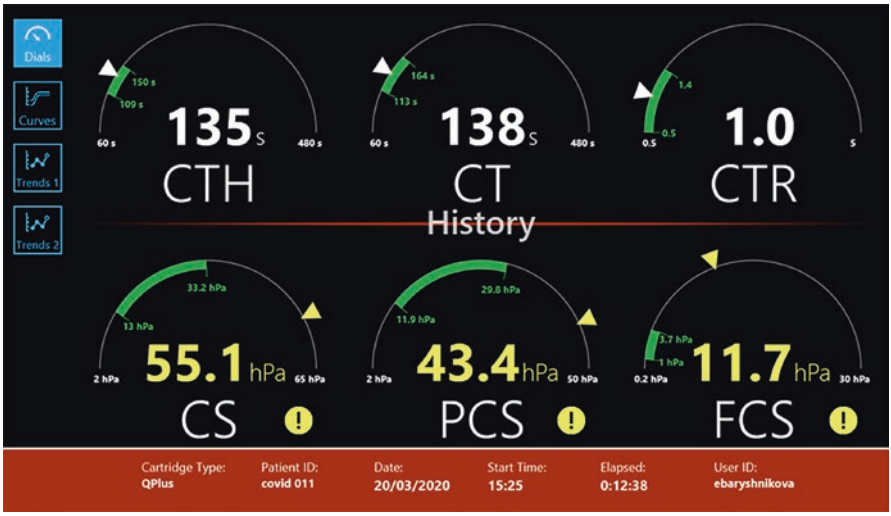

Fig. 3.3 Typical Quantra tracing from a representative critically ill COVID-19-positive patient with a procoagulant pattern. Very high level of fibrinogen contribution to clot stiffness (FCS) is of note. On the dial view of the results, the green space represents the normal range and the yellow arrow indicates the position of the patient's value. The exclamation point stands for a value out of normal range and worth of the operator's attention. Abbreviations: CT, coagulation time; CTH, coagulation time with heparinase; CTR, coagulation time ratio; CS, clot stiffness; PCS, platelet contribution to clot stiffness; FCS, fibrinogen contribution to clot stiffness

3.6 Viscoelastic Tests to Monitor Hypocoagulability and Bleeding in COVID-19 Patients

Hemorrhagic complications have been reported in a small but significant proportion of COVID-19 patients (8–21%), the most common being gastrointestinal bleeding [15, 39–41]. The extensive use of anticoagulation with some authors prompting more aggressive therapies in higher risk patients requires specific monitoring and established protocols for shifting therapies at varying conditions. Use of viscoelastic tests coupled to standard coagulation tests was suggested to be beneficial in monitoring coagulation by the recent ISTH guidance [42]. The American Society of Hematology (ASH) and American College of Surgeons (ACS) included viscoelastic tests (TEG and ROTEM) in their online COVID-19 resources for the management of coagulopathy and monitoring anticoagulation [43, 44].

Stillson and associates [45] investigated the use of TEG coupled to standard coagulation tests to predict bleeding as defined by the World Health Organization (WHO) bleeding scale score ≥2 [46] for COVID-19 intensive care unit patients who received intermediate or therapeutic anticoagulation. They were able to include in the analysis 10 patients who met the criteria of the WHO bleeding score of 2 or more (bleeding group) and 21 patients in the non-bleeding group. The following parameters were associated with bleeding: R ($P = 0.0001$), K ($P = 0.0002$), alpha angle ($P = 0.0001$) for the TEG, and aPTT ($P = 0.0006$) and fibrinogen ($P = 0.0019$)

for the standard coagulation tests. The findings of this investigation prompted the authors to modify their current anticoagulation protocol and adopt a TEG-guided protocol for anticoagulation management in COVID-19 critically ill patients that allowed them to significantly reduce bleeding events in their patient population.

3.7 Conclusions

All the available data agree that critically ill COVID-19 patients are affected by a complex hypercoagulable state where platelets and fibrinogen (expressed as clot strength/stiffness) seem to play a central role. In addition, the hypofibrinolytic condition contributes to the severity of the disease. Standard coagulation tests, though outlining the single alterations, lack the capacity to report the overall hemostatic competency of the patient. Point-of-care tests make up for this necessity.

At full value, inclusion of POC tests in international guidelines for monitoring and therapeutic decision-making in the setting of COVID-19 disease requires more rigorous studies and time but some indications have already been provided in interim recommendations and online resources of the major societies.

The main limitation of the above-presented studies is that most of them look at one moment in time (mainly ICU admission), present variable protocols for prophylactic and therapeutic anticoagulation, and differ in strategies for scanning for TE events.

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COVID-19 Associated Coagulopathy: The Thrombin Burst

4

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4.1 Introduction

Thrombin generation (TG) is a fundamental part of the coagulation process. Thrombin (FIIa) promotes the conversion of fibrinogen into fibrin leading, together with platelets and FXIIIa, to the formation of a stable clot. TG is a humoral/cellular process that finds its first step in the release of tissue factor (TF) by subendothelial vessel layers or by blood cells (blood-borne TF), mainly monocytes (initiation). TF forms a complex with FVIIa, and this complex activates FX leading to the FXa-Va complex, which promotes an initial and limited activation of FII to FIIa (propagation). Thrombin interacts with surface phospholipids located on the platelet surface (amplification) leading to a large degree of TG which is able to promote fibrinogen into fibrin conversion, platelet-fibrin(ogen) interaction, and finally onset of a stable clot. This is a dynamic process that includes the combination of procoagulant and anticoagulant factors. TF and all the soluble coagulation factors are procoagulants, and thrombin is certainly the most powerful: however, thrombin itself exerts a negative feedback on TG, by linking its endothelial receptor thrombomodulin, forming a complex that activates the protein C-S complex, which in turn inhibits FVIIIa and FVa. Other anticoagulant factors are the tissue factor pathway inhibitor (TFPI) inhibiting the complex TF-FVIIa and FXa, and the antithrombin (AT) which inhibits thrombin and its precursor FXa [1–4].

Figure 4.1 summarizes the main pathway(s) promoting and inhibiting TG. Overall, the net amount of TG depends on the balance between procoagulant and anticoagulant factors.

On a clinical basis, the assessment of TG may offer important insights into a number of pro-thrombotic and pro-hemorrhagic diseases, and the effects of

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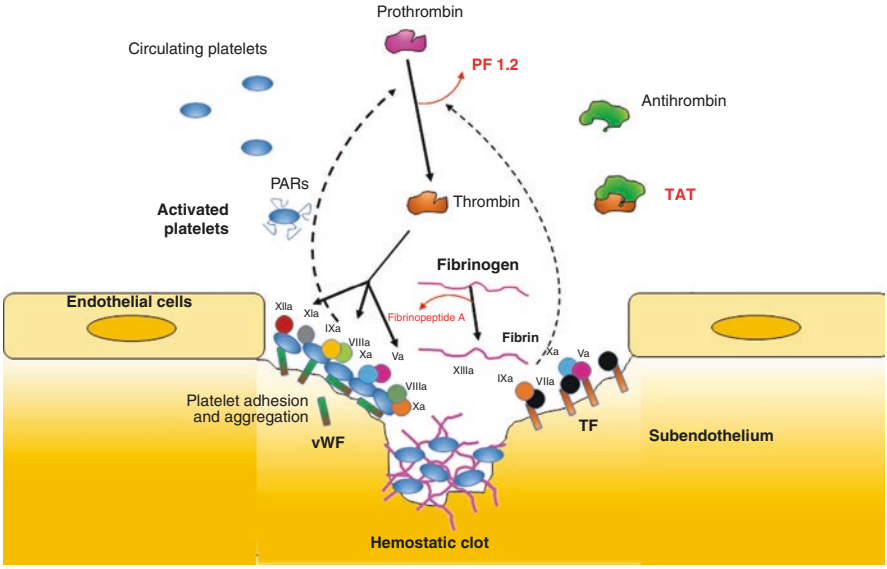

Fig. 4.1 Thrombin generation and clot formation. *PAR* protease activatable receptors, *PF* 1.2: prothrombin fragment 1.2, *TAT* thrombin-antithrombin complexes, *TF* tissue factor, *vWF* von Willebrand factor

procoagulant and anticoagulant drugs. TG is decreased in congenital (namely hemophilia) or acquired deficiencies of soluble coagulation factors, when this deficiency is not counterbalanced by an equivalent decrease in natural anticoagulants (like in liver cirrhosis, where TG is normal). Many drugs decrease TG, by directly or indirectly inhibiting thrombin or its precursor: heparin, bivalirudin, argatroban, warfarin, direct oral anticoagulants ….

Conversely, TG is increased in congenital deficiencies of natural anticoagulants (AT, proteins C-S), resistance to natural anticoagulants (factor V Leiden), and by the effect of specific procoagulant drugs or factor concentrates like prothrombin complex concentrate, cryoprecipitate, recombinant FVIIa, and others.

Therefore, a large number of conditions may benefit from a TG assessment. However, this measure is presently not routinely available in the clinical scenario. The standard coagulation tests prothrombin time (PT) and activated partial thromboplastin time (aPTT) do not reflect TG in an acceptable way [1]. The main reason is that they reflect congenital or acquired deficiencies in coagulation factors (prolonged aPTT in hemophilia, low-molecular-weight [LMWH] and unfractionated [UFH] heparin therapy or dabigatran, and prolonged PT in patients treated with warfarin, rivaroxaban, apixaban, edoxaban), but are not sensitive to procoagulant conditions related to deficiency of natural anticoagulants. In general, PT and aPTT are not shortened despite an increased TG, because plasma tends to rapidly clot after only 5% of the entire thrombin potential is formed [1–3]. Due to these reasons, a pro-hemorrhagic status may be detected by conventional laboratory tests, but not a pro-thrombotic state. The behavior of PT and aPTT in the setting of COVID-19 is addressed in Chap. 2.

Viscoelastic tests provide a measure that is theoretically related to thrombin generation. The reaction time (R) in thromboelastography and the clotting time (CT) in thromboelastometry represent the activity of soluble coagulation factors after stimulation with various activators (kaolin, ellagic acid, TF) in whole-blood tests. R-time and CT are prolonged in the same clinical conditions that prolong PT and aPTT. More controverse is the behavior of these "reaction times" in the setting of a hypercoagulable state. In patients with malignancies [5–7], and in some surgeries [8, 9], shortened reaction times are associated with a hypercoagulable state. In general, few studies investigated the correlation between reaction times at viscoelastic tests and the actual rate of thrombin generation, finding associations especially for CT at TF-activated thromboelastometry [10]. In general, the role of viscoelastic tests in assessing TG remains promising, but still elusive [1]. The behavior of viscoelastic tests in the setting of COVID-19 is addressed in Chap. 3.

In general, both routine laboratory tests and viscoelastic tests should be considered as surrogate of a direct TG measure. At present, only direct TG assays (TGA) or the measure of biomarkers of TG and of thrombin inactivation by AT (thrombin-antithrombin complex, TAT; prothrombin fragment 1.2, PF 1 + 2) should be considered reliable measures of TG.

4.2 TGA: Principles and How They Behave in COVID-19-Associated Coagulopathy (CoAC)

TGA is based on a direct detection of thrombin concentration, expressed in nmol/L, in plasma (or more recently in whole blood) after TG stimulation by TF. Thrombin is detected by a chromogenic substrate and, recently, by a fluorogenic substrate. In the first case, turbidity of the clotting plasma hampers the signal, and therefore the plasma must be defibrinated and should be platelet poor. In the second case, the test is applicable without these precautions. The output of TGA is represented in Fig. 4.2. Basically, there are some parameters derived by thrombin generation and

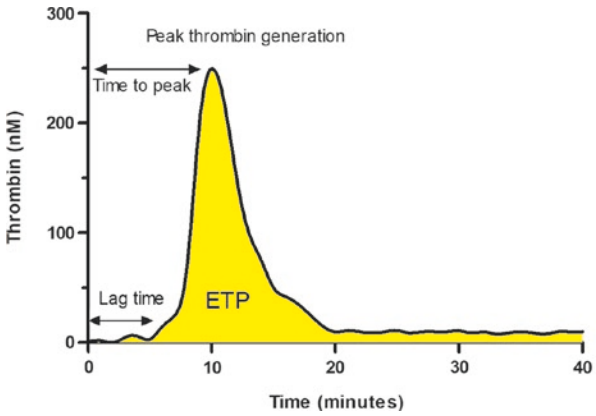

Fig. 4.2 A normal computerized automated thrombography output. *ETP* endogenous thrombin potential

inhibition: the lag time (from test beginning to the onset of TG); the peak height; the time to peak; and, probably the most important, the endogenous thrombin potential (ETP), that is, the area under the curve. The ETP represents the amount of thrombin generated and inhibited by the natural inhibitors present in plasma, and depends on the balance between procoagulant and anticoagulant factors. However, the lack of endothelial thrombomodulin and of platelets limits the transferability of this in vitro measure into ex vivo model. Modified TGA includes the supplementation with soluble thrombomodulin [11] and the use of whole blood instead of plasma [12].

Analyzing and comparing different studies that include a TG measure is a difficult task. This, because different TGA from different manufacturers provide different reference ranges; additionally, TGA may nowadays be performed on platelet-rich/poor plasma or whole blood, and with or without adding thrombomodulin. For this reason the (few) studies addressing TG in COVID-19 patients offer different and sometimes conflicting perspectives. White and associates [13] studied a series of 109 COVID-19 patients, 34 with noncritical patterns of disease and 75 with critical patterns. The patients were studied with calibrated automated thrombography (CAT) and received additional analyses of coagulation biomarkers. The authors found that the CAT-derived variables were basically within the normal range, both in tests done with or without thrombomodulin. The mean ETP was around 1240 nM·min (normal range 915–1716) at CAT without thrombomodulin and 760 nM·min (normal range 310–1550) with thrombomodulin. Of notice, no differences were found between patients in noncritical vs. critical state, unless a slight tendency towards hypocoagulation in critical patients. The rate of patients with a hypercoagulable state was low (9–12%) in both groups.

Nougier and associates [14] analyzed 78 COVID-19 patients, of whom 48 with severe patterns of the disease were treated in the intensive care unit (ICU) and 30 with minor degrees of the disease were treated in the internal medicine department. TG was assessed on platelet-poor plasma with a CAT system, with a reference range for peak thrombin of 350 ± 39 nM and for ETP of 1593 ± 206 nM·min (values obtained in 30 healthy volunteers). The patients were treated with conventional or high prophylactic dose of LMWH. Despite this, the patients showed elevated values of thrombin generation, with a mean peak value of 312 ± 127 nM and 391 ± 76 nM in patients in ICU or ward, respectively (P value for between-group difference = 0.004). The ETP was 1682 ± 610 nM·min and 1815 ± 357 nM·min in patients in ICU or ward, respectively. Based on these results, the authors conclude that thrombin generation in COVID-19 patients remains high despite LMWH treatment.

Similar results were obtained by Chistolini and associates [15] who investigated 27 patients admitted in the ICU due to COVID-19 pneumonia. A first series of 14 patients received a low-dose LMWH therapy (100 IU/kg/day) and the other 13 a high-dose regimen (100 IU/kg/b.i.d.). TG was assessed with a CAT system. The reference range was ≤106.2 nM for peak thrombin and of ≤984.12 for ETP. They

found that the mean value of peak thrombin (122.2 nM, range 5.31–268.48 nM) was higher than the upper limit of the reference range; this was mainly due to the high values of the low-dose group (148.4 nM), with the high-dose group showing a lower value (98.1 nM). The ETP provided even more striking differences, with a mean value of 953.51 nM·min (range 1–2357.21), but a significant ($P = 0.01$) lower value in the high-dose group (705.19 nM·min) than in the low-dose group (1222.52 nM·min).

Drawing conclusions and comparing different studies based on TGA and CAT is difficult. The main problem is the non-standardized measure of TG-related parameters with different CAT devices or other classical techniques, which leads to different ranges of normality. Additionally, practically all the patients included in the studies were receiving some sort of heparin prophylaxis or treatment. Therefore, even the presence of TG values within the normal range is not indicative of a normal thrombin generation, but only of its (partial) containment by heparin treatment.

Data from the above-cited studies are reported in Table 4.1. Overall, for their interpretation, at least two confounders should be considered: the severity of the disease (ICU or non-ICU) and the LMWH dose (low or high). In the study of White and associates [13] there were minimal treatment differences between ICU and non-ICU patients, with the majority of patients treated with prophylactic dose of LMWH. Not surprisingly, no differences were found between the groups in terms of ETP. Significantly longer lag times were found in the ICU group, probably as a reflection of a higher rate of patients treated with UFH. Based on these results, the

Table 4.1 Thrombin generation at calibrated automated thrombography in COVID-19 patients

Authors	Setting	General results	Critical vs. non-critical	Low vs. high anticoagulation
White et al. [13]	109 COVID-19 pts	Parameters within normal range despite anticoagulation	Peak TG = ETP = No patterns of hypercoagulation	Not addressed
Nougier et al. [14]	78 COVID-19 pts. 30 healthy controls	ETP/peak TG = ↑ controls despite anticoagulation	Peak TG ↑ in non-critical ETP =	ETP ↓ for higher anti-FXa values
Chistolini et al. [15]	27 COVID-19 ICU patients	Peak TG ↑ ETP = vs. normal range	Not addressed	Peak TG = ETP ↑ in low anticoagulation
Bouck et al. [16]	46 COVID-19 53 sepsis pts. 18 healthy controls	Peak TG ↑ in COVID-19 vs sepsis and controls ETP = COVID-19 vs. controls ETP ↑ COVID-19 vs. sepsis	No differences	Not addressed

ETP endogenous thrombin potential, *ICU* intensive care unit, *TG* thrombin generation

authors conclude that TG is independent from the severity of the disease. In the study of Nougier and associates [14], a subgroup of patients were assessed for anti-FXa levels, which demonstrated a substantial heparin activity (0.35 IU/mL), with a normal thrombin generation. Finally, Chisolini and associates [15] investigated only severe cases: in this setting, increasing the LMWH was an effective strategy to contain TG, which remained outside the upper limit of the normal range in patients receiving low-dose LMWH.

Within this context, an important contribution is the study of Bouck and associates [16].

The authors investigated 99 patients (46 with COVID-19 and 53 with bacterial sepsis). Twenty COVID-19 patients were in the ICU and 26 in the ward. A series of 18 healthy donors were included to settle the pattern of normality. Ninety percent of the COVID-19 and sepsis patients were receiving anticoagulants: COVID-19 patients were mostly treated with LMWH, while sepsis patients received UFH in 53% of the cases. Despite LMWH treatment, the ETP was not different between healthy donors and COVID-19 patients, but was higher in COVID-19 patients than in sepsis patients. Peak TG was higher in COVID-19 than in sepsis or healthy donors. The difference in TG values between sepsis and COVID-19 patients persisted in sub-analyses on patients under LMWH only or in the ICU. These results are consistent with the hypothesis that, despite heparin treatment, TG in COVID-19 patients remains unabated, differently from what happens in bacterial sepsis patients.

Given the inevitable limitations of these studies, it is reasonable to conclude that in COVID-19 patients (i) the disease severity is not "per se" a cause of higher thrombin generation; (ii) standard prophylactic LMWH treatment does not reduce TG; and, finally, (iii) a therapeutic dose of LMWH is probably more effective in limiting TG than a prophylactic dose.

4.3 TAT: Thrombin–Antithrombin Complexes. The Antithrombin (AT) Paradox

4.3.1 TAT Complexes

Once generated, thrombin is inactivated by AT, which acts as a "suicide substrate," being irreversibly bound to thrombin. AT inactivates FXa as well as thrombin. The velocity of these reactions is highly accelerated by both UFH and LMWH. However, LMWH only acts on FXa, whereas UFH accelerates both FXa and thrombin inactivation. The anticoagulation monitoring under heparin treatment is based on the anti-FXa level, measured with calibrated techniques for LMWH or UFH.

TAT is considered a biomarker of thrombin inactivation by AT, and therefore an indirect measure of thrombin generation. Under bacterial sepsis conditions, thrombin is highly generated, TAT increases, and AT is consumed. An association between high TAT levels, low AT levels, and bad outcomes has been shown in different studies [17–19]. This suggested that AT supplementation might be beneficial in septic

patients; however, randomized controlled trials failed to confirm any efficacy of this approach [20].

In the setting of CoAC and acute respiratory distress syndrome (ARDS) there are studies investigating the TAT behavior. The already cited work of Bouck and associates [16] found that circulating levels of TAT were not different in patients with COVID-19, sepsis, and healthy donors, even if COVID-19 patients showed a higher rate of outliers with high TAT levels.

However, various studies highlighted that COVID-19 patients show increased levels of TAT. Moosavi and associates [21] retrospectively studied a cohort of 81 patients, of whom 49 were in the ICU. Forty-seven percent of the patients were under LMWH treatment, 38% under UFH, and 11% under direct thrombin/FXa inhibitors. TAT levels (reference range < 5.5 μg/L) were abnormally elevated in 71% of the ICU patients and 53% of the non-ICU patients, with a median value of 8.9 μg/L and 5.9 μg/L, respectively ($P = 0.04$). No difference was found between patients with or without thrombotic events. Deceased patients had abnormally elevated TAT levels in 83% of the cases vs. 64% in survived patients.

Blasi and associates [22] studied 23 ICU and non-ICU COVID-19 patients, finding that COVID-19 patients had TAT levels of 7.30 (4.50–12.2) ng/mL, significantly ($P < 0.0001$) higher than healthy controls (1.55 ng/mL). Patients in the ICU had TAT levels not different from non-ICU patients. Xin and associates [23] in a large series of 147 COVID-19 patients found that COVID-19 patients had TAT values above the normal range in 96.6% of the cases vs. 11.1% in healthy controls ($P = 0.001$). Of notice, patients with a thrombotic complication had significantly higher TAT values than those without thrombosis, and patients under critical state had TAT values significantly ($P < 0.0001$) higher than patients with mild patterns of the disease and than patients with severe patterns of the disease ($P < 0.05$).

Based on these data, it is reasonable to conclude that thrombin generation and its inhibition by AT are increased in COVID-19 patients, and that the level of increase is a marker of the severity of the disease.

4.3.2 The AT Paradox

Under conditions of elevated thrombin generation and heparin or fondaparinux treatment, endogenous AT is consumed [24]. This is a common pattern both in chronic LMWH and in acute UFH treatment. Preoperative use of LMWH in cardiac surgery is associated with a poor heparin sensitivity, which is mainly related to decreased levels of AT [25, 26]. The magnitude of AT consumption is even larger with the combination of high-dose UFH and large thrombin burst. A typical example is cardiac surgery, where thrombin is greatly formed, and large doses of UFH are used. In this setting, an acute decrease in AT concentration (about 20–30% reduction) is observed [27].

From this perspective, CoAC should be the “perfect storm” for AT consumption, due to the concomitance of thrombin burst and heparin treatment. Despite this, the existing studies are far from being concordant on AT behavior in

Table 4.2 Antithrombin values in COVID-19 patients

Authors	Setting	General results	Critical vs. non critical	Low vs. high anticoagulation
White et al. [13]	109 COVID-19 pts	AT values within normal range	94.5 (21.2) vs. 94.6 (21.2) $P = 0.76$	Not addressed
Nougier et al. [14]	78 COVID-19 pts	AT values slightly decreased	87 (28) vs. 106 (14) $P = 0.016$	Patients in ICU more likely to receive UFH
Chistolini et al. [15]	27 COVID-19 ICU patients	AT values within normal range	Not addressed	87 vs. 90 $P = 0.64$
Dujardin et al. [28]	127 COVID-19 ICU patients	Normal values in non-VTE patients Decreased values In VTE-patients	Significant differences between VTE and non-VTE patients from day 5 through day 12 in the ICU	Not addressed
Calderon-Lopez et al. [29]	123 COVID-19 patients	AT activity decreased in 21% of the patients	In thrombotic patients only 8.3% had AT values <70	Not addressed
Ranucci et al. [30]	16 COVID-19 ICU patients	AT values slightly decreased at baseline (85, 69–91)	AT values normalized at 7 days (107, 81–130)	AT corrected with purified AT when <70
Correa et al. [31]	30 COVID-19 ICU patients	AT values slightly reduced	Significantly lower AT values in more critical patients from day 1 through day 14 in ICU	More critical patients more likely to receive UFH or high-dose LMWH
Zhang et al. [32]	19 COVID-19 ICU patients	AT values slightly reduced (72, 61–83)	Terminal stage 59 (59–85) Non-terminal 73 (67–89) (non-significant)	Not addressed
Gazzaruso et al. [33]	49 COVID-19 patientss	AT values slightly reduced 87 (23)	Non-survivors AT: 72 (23) Survivors AT: 95 (20) $P = 0.001$ Higher mortality in patients with low AT values	Not addressed

Antithrombin values are in %, expressed as mean (standard deviation) or median (interquartile range)

AT antithrombin, *ICU* intensive care unit, *LMWH* low-molecular weight heparin, *UFH* unfractionated heparin, *VTE* venous thromboembolism

COVID-19-associated coagulopathy. Some of the results are reported in Table 4.2. As for the previous biomarkers, there are subgroup analyses, based on the LMWH

dose (low-high), time-related course, and severity of the disease. A general confounder is that a variable percentage of patients were receiving UFH and not LMWH.

Some studies [13] report normal AT values both in critical and noncritical group, and regardless of the LMWH dose [15]. Patients without venous thromboembolism (VTE) have normal AT values, while patients with VTE have significantly lower values; however, AT has a poor predictivity for VTE (area under the curve 0.625) [28]. Calderon-Lopez and associates [29] investigated 80 COVID-19 patients (treated with low or high LMWH dose) finding a median AT value of 86 U/dL (reference range 70–120 U/dL), with only 23% of the patients showing values below the lower limit of normal range. Of notice, 12 patients had thromboembolic events: within this group, only one patient (8.3%) had abnormally low (61 U/dL) AT levels, whereas in the non-thrombotic group the rate of patients with low AT values was 25%.

Slightly reduced AT values (85%, 95% confidence interval 65–91) were found in a series of 16 ICU COVID-19 patients [30].

Conversely, other authors found that the severity of the disease was associated with lower AT values [31]. In a series of 19 patients, nonterminal COVID-19 patients had slightly reduced AT values (73%, interquartile range [IQR] 67–89.5), while terminal patients had nonsignificantly lower values (59%, IQR 59–85) [32]. Nougier and associates [14] found normal AT values in non-ICU COVID-19 patients (106% ± 14) and slightly reduced values in ICU patients (87% ± 28), with a significant ($P = 0.016$) inter-group difference, with all patients treated at variable doses of LMWH. Finally, in a series of 49 consecutive patients hospitalized for COVID-19, significantly ($P = 0.001$) lower AT values were found in non-survivors (72.2% ± 23.4) than in survivors (94.6% ± 19.5) [33]. A small meta-analysis confirmed that severe patterns of COVID-19 disease are associated with lower values of AT; however, this analysis is biased by an incorrect analysis of data from one [30] out of six studies [34].

Overall, it seems that patients with COVID-19 disease do not necessarily show abnormally reduced values of AT. There is a signal suggesting that more severe patterns may be associated with lower AT values; however, it is likely that both patients with the evidence of VTE or in critical state were treated more often with UFH, and that this may justify a larger AT consumption. Conflicting evidence exists with respect to the relationship between low AT values and thromboembolic events.

In any case, the global pattern is different from what is observed in bacterial sepsis, where AT consumption is more evident. It is difficult to find an interpretation for this apparent AT paradox. Actually, comparative studies have found a larger thrombin generation in COVID-19 than in bacterial sepsis [16]. A possible explanation is that in bacterial sepsis UFH is more often used than in COVID-19; this may justify a greater thrombin inactivation and consequent AT consumption. If this hypothesis is true, this may lead to the conclusion that LMWH, even in therapeutic doses, may be inadequate to antagonize the thrombin burst [15].

4.4 Prothrombin Fragment 1.2 (PF 1.2)

PF 1.2 comes from the in vivo cleavage of prothrombin by the prothrombinase complex on negatively charged phospholipids expressed on membranes of activated platelets [35] (Fig. 4.1). When TG is accelerated, increasing concentrations of PF 1.2 are detectable in plasma. Recently, Capecchi and associates [35] could demonstrate that PF 1.2 is significantly ($P < 0.001$) decreased in patients under vitamin K inhibitors (51 pmol/L, IQR 36–79 pmol/L) vs. normal controls (159 pmol/L, IQR 124–202 pmol/L); additionally, PF 1.2 levels, in a range between low and normal values, have a good correlation with TGA parameters peak thrombin generation, lag time, and ETP.

In the setting of increased TG, the association between TGA parameters and PF 1.2 is not demonstrated. In 27 patients with Puumala hantavirus infection [36], PF 1.2 was increased (704, 284–1875, vs. 263, 118–556 pmol/L; $P < 0.001$) during the acute phase, but the ETP was not associated with PF 1.2 ($r - 0.164$, $P = 0.415$). In noncomplicated pregnancy, PF 1.2 progressively increases while TGA parameters, after an initial increase, remained stable, and no association was found between TGA parameters and PF 1.2 [37].

Despite the fact that PF 1.2 does not match TGA parameters when TG is enhanced, there is an overwhelming evidence that in prothrombotic conditions and whenever thrombosis is established, PF 1.2 values are increased.

PF 1.2 is one of the markers included in the Markers of Coagulation and Hemostatic Activation (MOCHA) profile, together with TAT, D-dimer, and fibrin monomer. The MOCHA profile is associated with a number of thrombotic conditions, including embolic stroke malignancy, venous thromboembolism, and hypercoagulable disorders [38]. Individually, PF 1.2 is associated with a combined outcome of malignancy, venous thromboembolism, hypercoagulable disorders, and atrial fibrillation [39].

PF 1.2 is increased in bacterial sepsis with or without associated disseminated intravascular coagulopathy [40–42].

There are few studies with PF 1.2 assessment in COVID-19 patients. They are summarized in Table 4.3. Comparison between the studies is not direct, since different assays with different reference ranges and unit of measure are reported. Basically, when the unit of measure is pmol/L, values below 300–370 pmol/L are to be considered within the normal range, and for pg/mL values below 100–150 pg/mL are within the normal range [43].

White and associates [13] found largely elevated PF 1.2 values both in critical (1530 pg/mL, IQR 910–2530) and in noncritical (1550 pg/mL, IQR 1330–2230) COVID-19 patients, without significant inter-group differences. Blasi and associates [22] did not find increased values of PF 1.2 (206 pmol/L, IQR 158–269) despite an increase in TAT; no differences were detected in ICU vs. non-ICU patients. Moosavi and associates [21] found that 39% of COVID-19 patients had PF 1.2 values above the reference range, with significantly ($P = 0.03$) higher values in ICU vs. non-ICU patients. Of the 9 patients who developed a thrombotic event, 3 (33%) had abnormally elevated PF 1.2 values. Al-Samkari and associates [44] found elevated

Table 4.3 Prothrombin fragment 1.2 in COVID-19 patients

Authors	Setting	General results	Critical vs. non critical	Thrombosis vs non thrombosis
White et al. [13]	109 COVID-19 pts	Generally elevated values	1.53 pg/mL vs. 1.55 pg/mL $P = 0.964$	Not addressed
Blasi et al. [22]	23 COVID-19 pts. 20 healthy controls	Normal values despite anticoagulation	218 pmol/L vs. 186 pmol/L $P = 0.964$	Not addressed
Moosavi et al. [21]	81 COVID-19 ICU patients	Generally elevated values	Significantly ($P = 0.03$) higher values in ICU patients No differences in survivors or non-survivors	No differences in thrombotic vs. non-thrombotic patients
Al-Samkari et al. [44]	115 COVID-19 ICU patients	Slightly increased values; in patients not on therapeutic anticoagulation, increased values	Not addressed	Trend ($P = 0.082$) towards higher values in patients with VTE; higher values ($P = 0.006$) in patients with thrombosis
Ranucci et al. [45]	20 COVID-19 ICU patients	Generally elevated values	Survivors had a significant ($P = 0.025$) decrease at follow-up Survivors: 237 pg/mL vs 557 pg/mL at follow-up ($P = 0.247$)	Not addressed

Values are median. *ICU* intensive care unit, *VTE* venous thromboembolism

values of PF 1.2 (397 pmol/L, IQR 260–611) in 115 COVID-19 patients. Patients with VTE had nonsignificantly ($P = 0.082$) higher values of PF 1.2 (611 pmol/L, IQR 331–1333) than patients without VTE (402 pmol/L, IQR 263–679). Patients with any kind of thrombotic event had significantly ($P = 0.006$) higher PF 1.2 values (611 pmol/L, IQR 333–1148) vs. patients without thrombotic events (374 pmol/L, IQR 230-542). From a predictive perspective, the area under the curve of PF 1.2 values was 0.654 for VTE and 0.687 for any thrombotic event.

Finally, our group performed a wide analysis of coagulation immunoassays in 20 ICU COVID-19 patients [45]. Various markers of thrombin generation and fibrinolysis were measured in 20 ICU patients with severe patterns of COVID-19 ARDS at two points in time: at the admission in the ICU and at follow-up after 5–7 days. PF

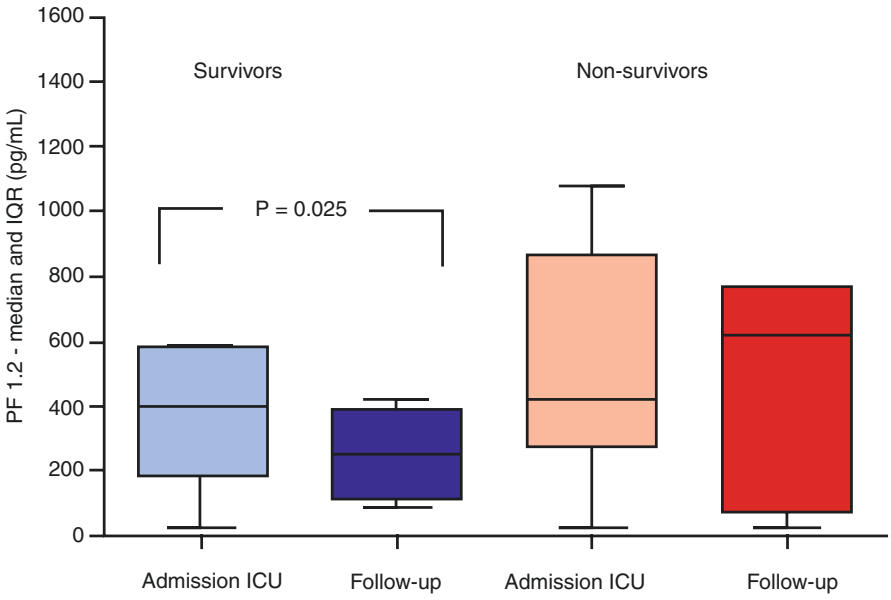

Fig. 4.3 Prothrombin fragment 1.2 (PF 1.2) levels at the admission in the intensive care unit (ICU) and at follow-up in COVID-19 survivors and non-survivors. *IQR* interquartile range

1.2 at baseline was higher than the upper limit of the reference range (442 pg/mL, IQR 302–649), without significant differences in survivors (396 pg/mL) vs. non-survivors (442 pg/mL). However, in surviving patients there was a significant ($P = 0.025$) decrease of PF 1.2 to a value of 237 pg/mL, whereas in non-survivors the value increased to 557 pg/mL (Fig. 4.3).

4.5 Conclusions

Overall, the existing body of literature highlights that COVID-19 patients suffer from different degrees on increased thrombin generation that in some cases configures an important "thrombin burst." At CAT analysis, this is more often seen as an increased peak of thrombin generation; when biomarkers are measured, elevated values of TAT and PF 1.2 are common. However, not all the studies are concordant to this respect. This reflects the different role of anticoagulant therapies, of the severity of the disease, of the presence of thrombotic complications, and finally of the time course of the disease. In general, low levels (prophylactic) of anticoagulation seem to only partially contain the thrombin burst that is better controlled by high-dose LMWH or UFH [14, 15]. Severe patterns of the disease are associated with higher levels of thrombin generation and/or AT consumption in the majority of studies [14, 24, 31–33, 45], even if others offer different results [13, 22]. Thrombotic complications are associated with higher levels of thrombin generation and/or AT

consumption in many studies [22, 28–44], but not in others [21, 24]. Finally, in survivors, a progressive decrease of thrombin generation is observed [45].

Given the limitations of the existing studies, it is reasonable to conclude that thrombin generation should be considered the main target of anticoagulation in COVID-19 patients, in order to prevent the progression of the disease and the onset of thrombotic complications.

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COVID-19 Associated Coagulopathy: D-Dimer and Fibrinolysis

5

Nathan D. Nielsen, Dawn Swan, and Jecko Thachil

5.1 Introduction

The first case of severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) was described at the end of 2019 and within 3 months, the World Health Organization had declared the disease, coronavirus disease 2019 (COVID-19), a pandemic. Observations of increased rates of thrombosis were made from an early stage during the pandemic. Initial anecdotal evidence of high levels of venous thromboembolism (VTE) have been confirmed in case series with incidence rates of 8–54% reported [1–4]. The majority of VTE events have been pulmonary thrombi with far fewer deep vein thromboses. For example, of 184 intensive care unit (ICU) patients with severe COVID-19 infection receiving low-molecular-weight heparin (LMWH) prophylaxis, 87% of thrombotic events were pulmonary thrombosis [5]. Autopsy studies have identified microthrombi within pulmonary vessels as well as renal and other affected organs in patients with multisystem organ failure [6]. The presence of fibrin and platelets with inflammatory cellular infiltrates has also been seen, as well as evidence of direct viral damage to pneumocytes infected with the virus via binding to angiotensin-converting enzyme 2 (ACE2) [7]. It has therefore been suggested that the thromboses seen in COVID-19 may be mediated primarily by local effects rather than embolization from peripheral veins [8].

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5.2 Fibrinolysis

Fibrinolysis is the process of breakdown of fibrin exerted by plasmin (Fig. 5.1). Plasmin derives from plasminogen, produced by the liver. Plasminogen cannot break down fibrin, but is however incorporated in the clot. The activation of plasminogen to plasmin is triggered by tissue plasminogen activator (t-PA) and by the serine protease urokinase-type plasminogen activator (uPA). t-PA is released by the damaged endothelium. Physiological inhibitors of t-PA and uPA are the plasminogen activator inhibitors (PAI) 1 and 2. Alpha 2-antiplasmin and alpha 2-macroglobulin inactivate plasmin. Plasmin activity is also reduced by thrombin-activatable fibrinolysis inhibitor, which modifies fibrin making it more resistant to plasmin. The breakdown of fibrin produces the fibrin degradation products. D-dimer is a term defining multiple peptide fragments derived from plasmin degradation of fibrin polymer.

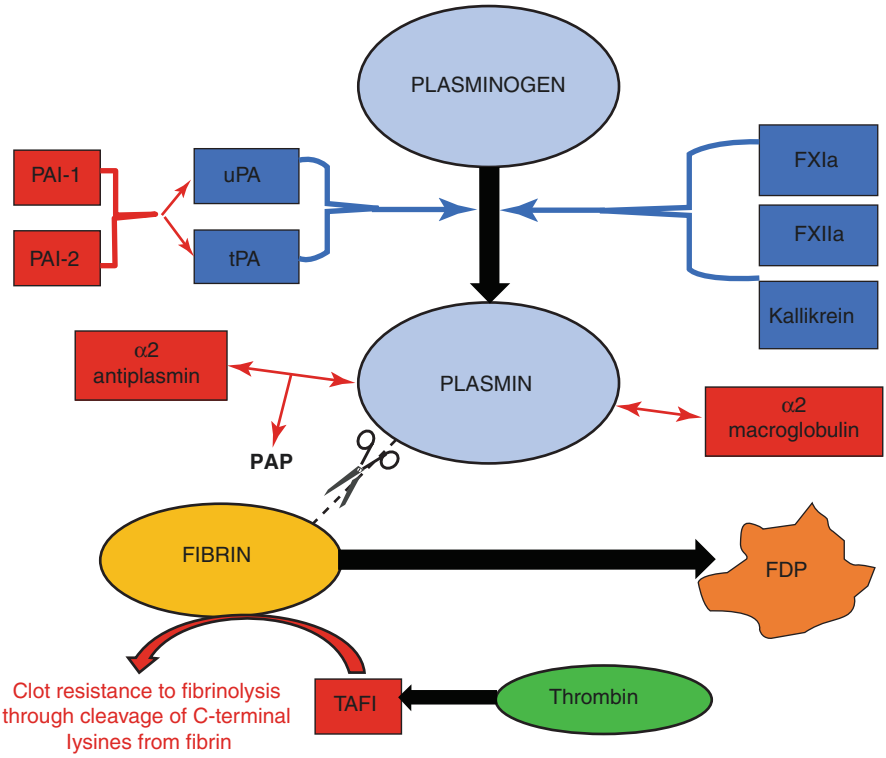

Fig. 5.1 Fibrinolytic pathways. Red boxes and lines are antifibrinolytic; blue boxes and lines are pro-fibrinolytic. *FDP* fibrin degradation products (including D-dimer), *PAI* plasminogen activator inhibitor, *PAP* plasmin-antiplasmin complex, *TAFI* thrombin-activatable fibrinolysis inhibitor, *t-PA* tissue plasminogen activator, *uPA* urokinase-type plasminogen activator

5.3 D-Dimer in COVID-19 and Mortality

In the first published academic accounts out of Wuhan describing the emergence of SARS-CoV-2, one of the consistently reported laboratory findings was hemostatic perturbations, specifically mild thrombocytopenia and elevated levels of D-dimer [9]. As the pandemic blossomed, further studies from China reported striking correlations between elevated D-dimer levels and mortality—for example, Zhang et al. reported that a D-dimer cutoff value of 2.0 μg/mL predicted mortality with a sensitivity of 92.3% [10]; Guan and colleagues found that COVID-19 non-survivors had a median D-dimer of 2.12 μg/mL whereas survivors had median levels of 0.61 μg/mL [11]; and Zhou et al. claimed that a D-dimer level >1 μg/mL at the time of admission carried a greater than 18-fold risk of in-hospital death with COVID-19 [12].

Subsequent studies conducted across the globe have generally corroborated these early observations [13, 14]. In a comparative study of COVID and non-COVID acute respiratory distress syndrome (ARDS) cases, Helms et al. noted that >95% of all patients studied had a D-dimer well above the normal range—a mean value of 2.27 mg/L, where the normal range was <0.5 mg/L [4]. Another French multicenter study confirmed some of the earlier Chinese findings: an admission D-dimer level >1129 ng/mL was a robust predictor of mortality with an area under the curve (AUC) of 64.9% and was associated with three times higher odds of mortality. Notably, this did not differ for the subgroup of patients diagnosed with VTE during their hospitalization [15]. A large cohort study in the United States reported that not only did 93.6% of COVID-19 patients admitted to the ICU have a D-dimer above the upper limit of normal (ULN), but those with D-dimer levels ≥8 times the ULN had 3.11-fold higher odds of death compared to those with <2 times the ULN. Strikingly, this association held despite extensive multivariate adjustments for baseline and severity of illness variables and even for the initiation of therapeutic anticoagulation efforts [16]. Valerio et al. opined that while admission D-dimer levels possessed some prognostic capacity, *daily* inpatient D-dimer levels were much more robust predictors of survival. In particular, the highest level of D-dimer ("the peak") and the higher velocities of increase (the "delta") were much more accurate prognosticators of mortality than baseline values [17]. Overall, multiple authors worldwide have independently concluded that elevated D-dimer levels in COVID-19 are indicative of a pathological prothrombotic (or alternatively a fibrinogenic-fibrinolytic) state which in turn predicts an unfavorable course of the disease.

5.4 The Problems with D-Dimer and Mortality Correlations

A number of serious caveats to these conclusions for correlation between D-dimer and mortality must be acknowledged. Several critics have voiced the concern that the earliest studies, particularly the studies from China, were too flawed as to be in any way conclusive. These critics noted that pooling data from studies using retrospective end points, widely heterogenous patient populations undergoing variable treatment strategies would result in data too noisy to be relied upon [18]. Other

critics were concerned about the preponderance of single-center, retrospective studies with small-to-modest sample sizes, and a widespread failure to perform adequate statistical adjustments in the reported D-dimer-to-mortality correlations to account for confounding severity of illness variables [16], and yet others critiqued the absence of clarity as to when in the course of illness D-dimer levels were obtained, and raised concerns regarding the reliability of baseline measurements for prognosticating a highly dynamic disease [17]. On a more technical level, wide variations in laboratory methods employed introduced limitations into cross-study comparisons, as differing assays use different limits of detection, ULN (or "cutoff" values), and even units of measurement. Lippi et al. cautioned that as there are up to 28 possible reporting units for D-dimer assays and that the major manufacturers of these assays globally employ up to five different units, true harmonization of results is an unlikely proposition due to technical discordances alone [9]. As such, while the correlations between D-dimer levels and clinical outcomes in COVID-19-related illness have been too widely and too consistently reported to be dismissed, it is likely prudent to take a *caveat emptor* approach to the interpretation of individual reports.

5.5 D-Dimer and Venous Thromboembolism in COVID-19

Being a thrombotic marker (or at least widely accepted to be so), D-dimer levels were used to correlate with the likelihood of venous thromboembolism. A cutoff level of 1.5 μg/mL for the D-dimer could predict the development of VTE with a sensitivity of 85.0% and specificity almost reaching 90% (negative predictive value 95%) [3]. A radiography-based analysis went with a D-dimer threshold of 2660 μg/L to accurately detect all patients with pulmonary embolus with a chest CT [19]. A US group even categorized patients into three groups based on their D-dimers—low-probability (<1000 ng/mL), intermediate-probability (1000–7500 ng/mL), and high-probability groups (>7500 ng/mL), whereby they identified posttest probabilities of VTE of 3%, 18%, and 43%, respectively [20].

Clinical, radiological, and even autopsy series [21, 22] have independently reported that episodes of VTE can be found in up to one-third of patients with severe COVID-19. However, these same studies, and others, have detailed the presence of *both* micro- and macrovascular thrombi within the pulmonary vasculature in a majority of patients who died as a result of COVID-19 [22–24], with localized pulmonary microemboli observed to be even more common than macro-thrombi [9]. In the setting of potentially disseminated thrombosis or even significant clot burden at both the micro and macro level, does the D-dimer level still carry robust prognostic value for VTE? On the one hand, Pellegrini et al. found that the incidence of VTE was much higher in patients with respiratory failure from COVID-19 compared to those with respiratory failure from other causes (36.8% vs. 0%, $p = 0.023$), and that in a multivariate regression model the incidence of VTE was independently associated with a diagnosis of COVID-19 *and* a rising D-dimer concentration (an OR of 1.15 per 1 ng/mL increase) [25]. Additionally, in one of the few systematic screening studies for VTE in the context of COVID-19, Demelo-Rodriguez and colleagues

found that among non-ICU COVID-19 patients with D-dimer levels >1000 ng/mL, screening compression ultrasound revealed that 14.7% had asymptomatic DVT. They further noted that patients with DVT had higher D-dimer levels than those without (4527 vs. 2050 ng/mL, $p < 0.001$) and that a D-dimer level of >1750 ng/mL was strongly associated with the development of asymptomatic DVT (OR 9.1) [26]. On the other hand, a French study performed a propensity-matched analysis of COVID-19 ARDS cases with historical "classical" ARDS cases and found that while the COVID-19 cohort had a significantly higher rate of VTE, especially of pulmonary emboli (11.7% vs. 2.1%, $p < 0.008$), mean D-dimer levels were much higher in the "classical" ARDS cohort (4.30 mg/L in the "classical" ARDS group vs. 2.27 mg/L, $p < 0.001$) [4]. Even Demelo-Rodriguez et al. cautioned that while the D-dimer cutoff level they observed possessed a robust negative predictive value, it lacked the specificity required for reliable clinical use [26].

Thus, it is reasonable to conclude that while D-dimer levels are almost universally high in severe cases of COVID-19, increased D-dimer values in patients with COVID-19 cannot be solely attributed to VTE, certainly not at typical cutoff points, nor from levels obtained at the time of ICU admission. More recent studies have suggested that peak levels or the change ("delta") in levels over the course of an ICU admission more accurately predicts VTE than admission D-dimer levels [9, 27]. The trend in D-dimer values over the course of admission was also of greater utility than any single measurement—for example, Naymagon et al. showed that patients with *stable* D-dimer levels had a greater than 80% lower risk of experiencing a VTE event than those with rising levels (OR 0.18) [14].

In summary, D-dimer may serve a role in the diagnosis of VTE in COVID-19 patients, though with several important caveats; among them peak levels or changes in levels are much more robust predictors than single values obtained at the time of admission; D-dimer levels have a far greater negative predictive value than positive predictive value; and uncertainty persists regarding the optimal cutoff threshold. Given these constraints, a fair conclusion is that D-dimers are not a reliable diagnostic nor screening tool for VTE in COVID-19.

5.6 D-Dimer and ARDS

D-dimer levels have been reported in the setting of ARDS essentially ever since the recognition of ARDS as a pathophysiologic entity. In fact, as far back as 1980, investigators stated, "higher concentrations of circulating fibrin/fibrinogen degradation products are associated with ARDS itself, either as a marker of more extensive microvascular injury or as a possible mediator of injury" [28]. Multiple observational studies have reported the same association between acute pulmonary insults and elevated D-dimer levels, not just in ALI/ARDS. Snijders et al. reported significant elevation in D-dimer levels in community-acquired pneumonia (CAP) and noted higher levels in severe CAP cases and in CAP fatalities [29], and Querol-Ribelles et al. reported abnormally high levels of systemic D-dimer in >80% of CAP cases and noted that even higher levels were seen in CAP cases that led to

ARDS or to demise [30]. More recently, Yu et al. also reported abnormally high mean D-dimer levels in a cohort of CAP patients of varying severity [31]. Nor are D-dimer abnormalities limited to bacterial processes, for Wang and colleagues reported extremely elevated levels in H1N1 influenza cases who went on to develop respiratory failure as opposed to those that did not (mean 6.74 mcg/mL vs. 1.13 mcg/mL, $p = 0.004$), and further noted a negative linear correlation between D-dimer levels and oxygenation indices. Higher levels of D-dimer (at the time of admission) were seen in non-survivors as compared to survivors even in the respiratory failure cohort [32].

5.7 The Concept of a Localized Fibrinolytic System in the Lungs

The marked elevations in plasma fibrin degradation products in the setting of significant pulmonary pathology led multiple independent authors to posit the theory that pulmonary microthrombi were present and likely played a significant role in the injury process. Other authors opined that in the setting of lung injury, abnormalities in both the coagulation cascade and fibrinolytic pathways would predispose to fibrin deposition in the *air spaces*, not just the pulmonary vasculature, and that fibrin degradation products thus entered the systemic circulation because of fibrinolytic activity taking place in the alveolar spaces. This would lead to the expectation that markers of dysregulated (i.e.: depressed) fibrinolytic activity would be even more prominent in the alveoli than in the plasma [33]. This theory has subsequently been proven to be true. For example, Fuchs-Buder et al., after stating that intra-alveolar fibrin deposition was in fact a typical finding in acute lung injury (ALI), went on to study D-dimer levels in the alveolar spaces as measured by bronchoalveolar lavage fluid (BAL) analysis. They found that BAL levels of D-dimer were markedly elevated in every stage of ARDS for patients who developed the syndrome as compared to those who were at risk but did not develop it. In fact, there was a 400- to 500-fold increase in BAL D-dimer levels in patients with frank ARDS as compared to normal control subjects; in patients only "at risk" for ARDS, levels were only elevated 50- to 60-fold. Strikingly, in their study, they found no correlation between plasma and BAL D-dimer levels, leading them to conclude that degradation of fibrin in the alveolar space in ARDS was "determined by local factors and probably independent of the systemic turnover of fibrin" [34]. Prabhakaran et al. took the examination of these processes one step further and studied not only the D-dimer levels in BAL fluid of ALI patients, but also plasminogen activator inhibitor-1 (PAI-1) levels. While alveolar D-dimer levels were surprisingly similar in both hydrostatic edema and in ALI, both were almost 17-fold higher than plasma concentrations—further bolstering the theory of compartmentalized fibrin turnover in the alveolar space. However, in ALI cases, PAI-1 levels were eightfold higher in edema fluid than in plasma whereas in hydrostatic edema the ratio was close to 1:1—this was taken as evidence of significant *intrapulmonary* production of PAI-1 rather than of accelerated systemic production in the setting of acute pulmonary insult. Furthermore,

elevated levels of both plasma and BAL PAI-1 activity correlated with poorer clinical outcomes, suggesting a relationship between the degree of fibrinolytic dysregulation and tissue injury [35].

5.8 Balancing Hyperfibrinolysis with Antifibrinolysis

Other authors have further expanded the mechanistic understanding of these processes—Hasday et al. showed that in BAL fluid from patients with ARDS, urokinase concentrations (the predominant plasminogen activator in the lung, and the cell surface protein most responsible for the regulation of fibrinolysis at the tissue level) were markedly decreased, whereas PAI-1 and α2-antiplasmin activities were elevated [36]. Idell et al. found that while plasminogen levels were elevated in the BAL fluid of ARDS cases, the majority of the plasminogen activator in these samples was complexed with inhibitory molecules [37]. Bertozzi et al. generally concurred, stating that the reduced fibrinolytic activity in ARDS was not the lack of urokinase per se, which could be elevated in some cases, but its profound inhibition by an array of antifibrinolytic factors [38]; similarly, Grau et al. reported that while pulmonary endothelial cells normally secreted urokinase plasminogen activator (uPA), cells from ARDS patients expressed more PAI-1 than controls and also had a lower fibrinolytic capacity as measured by tPA:PAI-1 ratios [39], and Gunther et al. reported that while all severe pneumonia cases had reduced fibrinolytic capacity and elevated uPA activity, only the most severe groups also had an increase in PAI-1 activity [40].

However, if it is to be believed that the pulmonary coagulopathy seen in ARDS and other types of pulmonary inflammation/infection is a localized hemostatic disturbance, and fibrin generation is restricted solely to the site of infection [41], then how to explain the elevated plasma levels of D-dimer reported in numerous clinical studies? Additionally, if intrapulmonary fibrinolysis is effectively quenched by dramatic elevations in pulmonary PAI-1 activity, how then to explain the impressive coincident elevations in BAL D-dimer levels? The resolution to both of these apparent paradoxes may be obtained by considering the sheer scale of hemostatic dysregulation in these conditions. The degree of fibrin generation in the injured lung parenchyma (the alveoli, and probably to a lesser degree the microvasculature) is so *massive* that even dysregulated, suppressed fibrinolytic processes still generate impressive quantities of degradation products; consequently, the level of D-dimers produced locally in the injured organ are high enough such that even a small proportion entering into the systemic circulation registers as abnormally high plasma levels.

In conclusion, the lung, unlike other organs and vascular beds, in its basal state possesses a profibrinolytic environment which is often lost in the setting of acute lung injury. Multiple forms of pulmonary injury, particularly ARDS, result in a marked dysregulation of both fibrinogenesis and fibrinolysis—procoagulant activity becomes predominant while fibrinolytic activity is (relatively) reduced. Systemic D-dimer levels and plasma procoagulant/fibrinolytic enzymatic activity are poor reflections of the disruptions to the local fibrinolytic environment in the lung and give a very limited view as to the extent of alveolar and microvascular injury taking

place. Simply put, plasma measurements do not always reflect what is occurring in the alveoli or even in the pulmonary microvasculature. Unfortunately, our capacity to understand *tissue-level* hemostatic and fibrinolytic activity remains limited, even forty-plus years on.

Thus, based on the evidence from years of research into fibrinolysis in the setting of acute pulmonary injury, it is reasonable to state the following:

1. Plasma D-dimer levels are consistently elevated in clinical studies of ARDS, CAP, and viral pneumonia.
2. Intra-alveolar (i.e.: BAL) D-dimer levels are often discordant with, or at the very least much more highly elevated than, plasma D-dimer levels in ARDS.
3. Intra-alveolar D-dimer levels are due to fibrin deposition in the air spaces from *localized* tissue factor-mediated thrombin generation and not necessarily the pulmonary vasculature, and correlate with the degree of pulmonary injury.
4. Pulmonary fibrinolytic processes are depressed in the setting of acute injury, where even elevated levels of uPA are inhibited by even higher relative levels of PAI-1 activity (secreted by pulmonary epithelial cells, fibroblasts, endothelial cells [41], and macrophages [42]) and other fibrinolysis inhibitors, while systemic levels of these same inhibitors are proportionally low.

5.9 So Then Why Is the D-Dimer High in COVID-19?

The question is thus begged: If the observed correlation between D-dimer elevations and poor clinical outcomes in COVID-19 is to be believed, what is the purported mechanism(s) driving these elevations? And a related query: Are the D-dimer elevations noted in severe COVID-19 merely illustrative of the overall severity of illness or do they reflect a dysregulated thrombotic (and fibrinolytic) pathophysiological process with direct implications for survival? Perhaps the clearest answer to the latter question was provided by Short et al., who reported an *independent*, proportional risk for mortality with increasing D-dimer levels despite extensive adjustments for both baseline and in-ICU severity of illness variables [16]. As such, D-dimer levels are less likely to be mere signposts for impending danger than they are indicators of active, life-threatening pathophysiological processes. The precise nature of these processes, however, remains poorly understood, and may well vary from patient to patient. D-dimer elevations may reflect underlying thrombus formation (micro- or macrothrombi, venous or arterial, localized or disseminated, occult or overt), accelerated fibrinolysis, immunoinflammatory processes, pulmonary endothelial/epithelial injury [17, 19], or other, as-yet-undescribed, pathological processes. A final, as-yet-unanswered, question is whether these pathophysiological processes are unique to COVID-19 or whether we are collectively suffering from immediacy bias, for elevated D-dimer levels have been reported in a host of other respiratory infections [43, 44] and in critical illness in general [45].

5.10 Conclusion

In summary, COVID-19 has put the fibrinolytic system into the spotlight. D-dimers have become a very important, but at the same time, controversial test. It has the potential to be a marker for acute lung injury and not exclusively venous thromboembolism. However, the conundrum of localized hyperfibrinolysis as evidenced by high D-dimers occurring simultaneously with systemic hypofibrinolysis as evidenced by increase in more sophisticated markers for this part of the hemostatic system is intriguing and requires much deeper investigation.

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COVID-19 Associated Coagulopathy: Platelet Count and Function

6

Ekaterina Baryshnikova

6.1 Introduction

Platelets play a complex and multifactorial function both in physiological and pathological conditions.

Platelets are nonnucleated cells derived from the cytoplasm of megakaryocytes, highly specialized cells residing in the bone marrow. Platelets are the smallest corpuscular components of the bloodstream and have a 2–4 μm diameter. Platelets exhibit no or minimal ability for *de novo* synthesis of proteins, but store a great variety of molecules required for the completion of its functions within internal granules that degranulate and release their content upon specific activation signals. The physiological platelet count is usually considered to be $150–450 \times 10^3$ for μL of peripheral blood but the definition of the reference range varies to a certain grade among different institutions. The physiological lifetime of circulating platelets is approximately 7–10 days with a daily renewal rate of about 20% of the total platelet count. Degradation of the senescent or damaged platelets occurs in the reticuloendothelial system of the liver and spleen. About one-third of platelets are stored in the spleen and undergo constant exchange with the circulating population [1].

Platelets are involved in both primary hemostasis and immune response.

The small size and the discoid shape of the quiescent platelets make them circulate pushed to the vessel wall by the much larger and more abundant red cells, positioning them in the right place to rapidly detect and respond to vascular damage. The inner surface of the blood vessels is lined with a thin layer of endothelial cells that, in normal conditions, inhibit spontaneous platelet activation by producing

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nitric oxide, endothelial ADPase, and prostaglandin PGI_2. Endothelial cells produce von Willebrand factor (VWF), a cell adhesion molecule helping endothelial cells adhere to collagen in the basement membrane. Under physiological conditions, collagen and VWF are not exposed to the bloodstream.

Platelet activation takes place when the continuous endothelial layer is broken and the injury exposes VWF and collagen fibers from the subendothelium to the bloodstream, to which fast-moving platelets can attach through a dedicated cell surface receptor, the glycoprotein Ib (GP Ib). This interaction is insufficient to firmly adhere the platelets to the matrix but rather allows them to slow down, roll along the vessel wall, and finally stop by the site of the vessel damage. This interaction lasts long enough for other collagen receptors to engage, generating intracellular signals that activate platelets. The exposed VWF starts recruiting coagulation factors to the lesion site, initiating the coagulation cascade [1].

Activated platelets release the contents of their granules into the blood plasma. The molecules in the granules include adenosine diphosphate (ADP), serotonin, platelet-activating factor, VWF, platelet factor 4 (PF4), and thromboxane A_2 (TxA_2), which, in turn, activate additional platelets. The activation cascade inside platelets finally leads to the modification of the integrin membrane glycoprotein IIb/IIIa (GP IIb/IIIa), increasing its affinity to bind fibrinogen. The activated platelets change shape from spherical to stellate, and stick tightly to the collagen fibrils, forming a monolayer on the lesion. Additional platelets are recruited to the lesion and activated, sticking to each other and accumulating on the top of the initial monolayer, forming a primary platelet clot at the site of the initial activation [1].

Moreover, platelets play an important role in the response to infections, both bacterial and viral, and modulate various immune cells. Previous observations showed that platelets are able to engage direct cell-to-cell interactions with pathogens and hosts (including leukocytes and macrophages) and to release soluble mediators of the immune system [2]. Notably, platelet normal count and function offer a protective effect in viral infection. Following activation and engagement in coagulation and immune response to virus, platelets are irreversibly cleared out from the circulation leading to thrombocytopenia that is often observed during sepsis. Moreover, excessive activating stimulation of platelets during its exposure to pathogens and the following hyperinflammatory environment potentially lead to a dysregulation of its function with further strengthening of the prothrombotic tendency [3]. Circulating pro-inflammatory biomarkers induced by the infection have profound agonistic effects on the platelets. In these conditions, platelets are able to bind the intact endothelium endowing it with pro-inflammatory phenotype. Activated platelets attract circulating leukocytes further enhancing inflammation. In this way, platelets adhere to phagocytes enhancing pathogen killing and clearance [4]. Activated platelets release the content of their granules, including soluble and surface-bound molecules. Some of these mediators can exit the vasculature and penetrate the underlying tissues where they activate additional leukocytes to their modulatory and effector functions [5].

6.2 Platelet Interaction with SARS-CoV-2

The novel coronavirus SARS-CoV-2 infects host human cells via its spike protein binding to angiotensin-converting enzyme 2 (ACE2) located on the host cell membrane. Meanwhile, transmembrane protease serine 2 (TMPRSS2) proteolytically cleaves and activates the spike protein to allow membrane fusion of SARS-CoV-2 virus with the infected cell [6]. The ACE2 surface receptor is expressed in hematopoietic and lymphoid tissues, as well as lung and gastrointestinal epithelium [7]. Human platelets strongly express ACE2 at both the RNA and protein levels, as well as TMPRSS2 [8]. Zhang and colleagues directly observed SARS-CoV-2 particles on the platelet membrane using scanning electron microscopy, and, using transmission electron microscopy and fluorescent confocal microscopy, they demonstrated that SARS-CoV-2 particles were present inside the platelets [8], confirming the internalization of the virus and the potential role of the platelets in its clearance from the circulation.

The binding of SARS-CoV-2 to platelets potentiated platelet aggregation in response to its main agonists—collagen, ADP, and thrombin. The expression of GP IIbIIIa and P-selectin increased, following agonist activation [8]. P-selectin is a cell adhesion molecule located on the surface of activated platelet, and thus it is often used as a surrogate marker for platelet activation. Analogous results were observed incubating platelets with spike protein. Notably, spike protein induced GP IIbIIIa activation and P-selectin expression in the absence of an agonist. Notably, expression of P-selectin from the very early phase of COVID-19 hospitalization (sampling made within 24 h from the admission) is independently associated with the composite outcome of thrombosis or death [9]. In the same study, other biomarkers were found to associate with death and thrombosis—thromboxane B2 (TxB_2, the metabolite of thromboxane A_2 and surrogate marker of platelet activation via COX-1) and soluble CD40 ligand (sCD40L, located on activated platelets, whose interaction with endothelial cells leads to the production of reactive oxygen species, chemokines, cytokines, and expression of adhesion molecules on the endothelium) [9].

Moreover, SARS-CoV-2 virus directly stimulates platelets to release coagulation (mainly factors V and XIII) and inflammatory and other factors (including PF4, tumor necrosis factor TNF-α, interleukins IL-8 and IL-1β) stored in their granules. Remarkably, the severe and critically ill COVID-19 patients had the highest plasma levels of PF4 [10]. SARS-CoV-2 also promotes the formation of leukocyte-platelet aggregates, another marker of platelet activation [8].

Platelets from COVID-19 patients are more activated, aggregate faster, and have increased expression of monocyte tissue factor, further reinforcing thrombin generation [11, 12]. Functional assays showed that platelets from patients infected with COVID-19 are hyperresponsive and sensitized to release inflammatory cytokines and to adhere more efficiently [13]. Taken together, the overall platelet-activating events, including aggregation, infiltration, and inflammatory response, contribute to lung injury and microvascular thrombosis in SARS-CoV-2-associated pneumonia [12]. Interestingly, ACE2 polymorphisms were linked to hypertension and diabetes mellitus, specifically in Asian population [14].

6.3 Platelet Function and COVID-19

There are various methods and technologies available to test the efficacy of platelet aggregation in vitro. Point-of-care techniques include multiple electrode aggregometry (Multiplate®), modified thromboelastography TEG® Platelet Mapping, and aggregometry with ROTEM® Platelet device. Laboratory methods include light transmittance aggregometry and dosing of selected biomarkers associated with platelet activation (soluble CD ligand and P-selectin, for instance).

Briefly, the multiple electrode aggregometer Multiplate® is based on measuring increasing electrical impedance between two metallic wires covered by platelets aggregating upon specific activation. Whole blood, where free thrombin is inhibited with hirudin, is used and platelet aggregation is specifically triggered by an activator. Arachidonic acid (ASPI test) explores the platelet aggregation upon stimulation of the COX-1 pathway; adenosine diphosphate (ADP) test specifically measures the availability of P2Y12 receptor, strongly engaged during platelet activation; and thrombin-receptor-activating peptide (TRAP) test is targeted against the thrombin PAR receptors; thrombin being the most potent platelet activator, in the absence of drugs targeting the thrombin or the final aggregation receptor GP IIbIIIa, TRAP test is an indicator of the maximum platelet aggregating potential. An example of ADP and TRAP tests is described in Fig. 6.1.

The TEG® Platelet Mapping measures platelet aggregation taking advantage of the viscoelastic properties of the coagulating blood, joining indications from different channels. Briefly, in the first channel, a basal test activated with kaolin or kaolin with heparinase (depending on the clinical scenario) is run on heparinized whole blood, standing for 100% platelet activation. In the second channel, a 100% fibrin clot (and thus, with no platelet contribution) forms, following the addition of reptilase, an enzyme triggering a direct fibrinogen-to-fibrin conversion. At this point, the addition of an activator of specific platelet receptors (ADP or arachidonic acid, AA) to the blood with reptilase will give the exact proportion of platelet activation.

ROTEM® Platelet is an additional module to the ROTEM® delta device. It brings two channels measuring platelet aggregation via electrical impedance, analogously to Multiplate® aggregometer. The available tests include ARATEM (using arachidonic acid as agonist), ADPTEM (using ADP and targeting P2Y12 receptors), and TRAPTEM (using TRAP and targeting thrombin PAR receptors) test. An example of ADPTEM and TRAPTEM tests is described in Fig. 6.2.

Light transmittance aggregometry is performed on platelet-rich plasma samples. When platelets are stirred inside the measuring cuvette and placed between a light source and a photocell, the amount of light passing through the sample is prevented because of the turbidity of the solution. After the addition of an agonist (AA, ADP, or TRAP, for instance), platelet aggregation takes place and the solution becomes gradually clearer, letting more light to pass. The increasing light transmittance is recorded in the function of time, as percent of aggregation. Light transmittance aggregometry is considered the gold standard method for the assessment of platelet aggregation but its application is limited to specialized laboratories and not widely available.

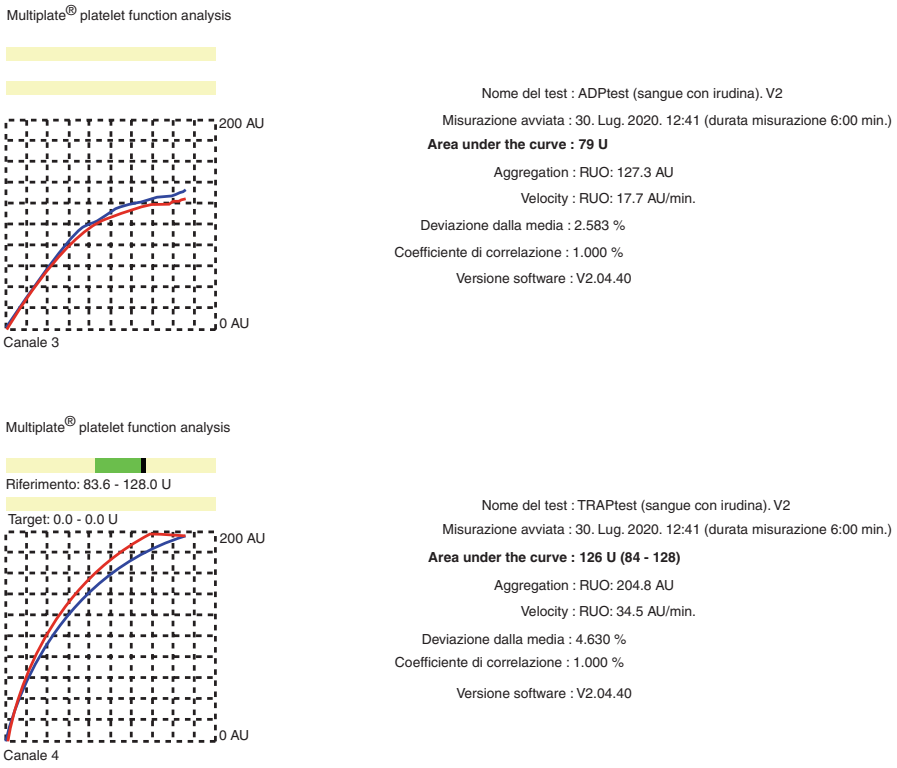

Fig. 6.1 Example of Multiplate® assessment of platelet function in a critically ill COVID-19 patient. All the values are within the normal range. *ADP* adenosine diphosphate, *TRAP* thrombin receptor-activating peptide

6.3.1 Studies on COVID-19 and Platelet Function Assessed by Point-of-Care Devices

In contrast to that found with surrogate biomarkers for platelet activation, available studies assessing the overall platelet function do not support these data.

Heinz and collaborators assessed platelet function with multiple electrode aggregometry [15]. They compared platelet aggregation in response to stimulation with agonists to adenosine diphosphate (ADP) receptor P2Y12, to arachidonic acid, the substrate of cyclooxygenase pathway, and to thrombin receptor-activating peptide (TRAP) 6 between 27 patients admitted to intensive care unit (ICU) with acute respiratory distress syndrome (ARDS) diagnosis and healthy controls. The results showed significantly lower area under the curve (AUC) values only for ADP test (68 ± 37 U *vs.* 91 ± 29 U, $p = 0.043$). Nevertheless, this difference became insignificant after adjusting for the confounding effects of sex. The differences in platelet aggregation values were previously reported and may partially account for this finding [16].

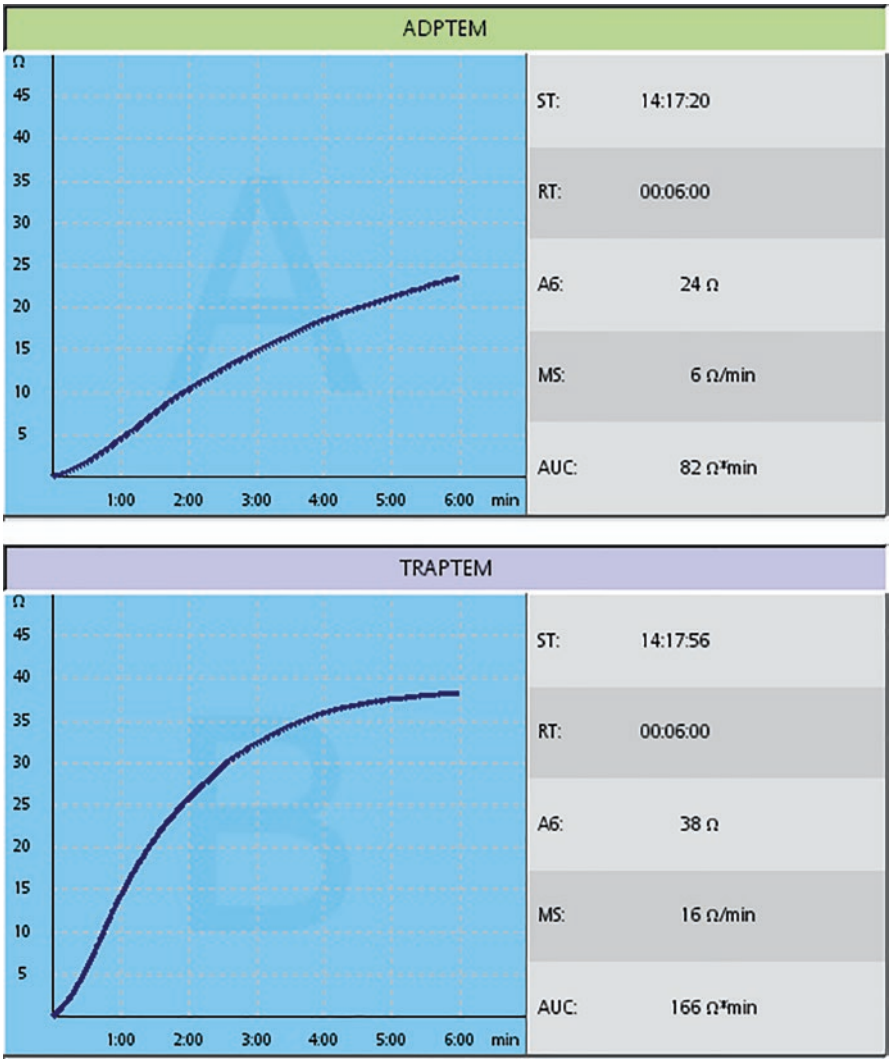

Fig. 6.2 Example of ROTEM® Platelet assessment of platelet function in a critically ill COVID-19 patient. No reference range is reported by the manufacturer in the software. *ADP* adenosine diphosphate, *TRAP* thrombin receptor-activating peptide, *AUC* area under the curve

Correa and colleagues addressed the platelet function issue by the ROTEM® Platelet device in 30 COVID-positive patients treated in ICU and followed for up to 14 days during hospitalization [17]. They found that the values of ARATEM (platelet activation with arachidonic acid) and ADPTEM (platelet activation with ADP) remained within the normal range during the study period, although they tended to increase over time ($p = 0.014$ and $p = 0.004$ for time effect in ARATEM and ADPTEM, respectively). No association with outcome was investigated.

Light transmittance aggregometry performed by Campo and coworkers found no difference in platelet aggregation in response to arachidonic acid, low- and high-dose ADP, and TRAP between COVID-19-positive patients and healthy controls. The aggregation values were stable from baseline through the 14-day observation period. Some differences were observable when comparing critically ill COVID-19 patients requiring ICU versus non-ICU patients but the difference became nonsignificant after adjustment for confounding factors. Nevertheless, when stratifying patients based on the outcome parameters, myocardial injury, or mortality, patients who experienced myocardial injury or fatal outcome showed higher values of platelet aggregation [18].

The group of Hranjec et al. assessed 72 patients diagnosed with COVID-19 with Platelet Mapping on TEG 5000 Thrombelastograph (Haemonetics, USA) [19]. They built a TEG-guided institutional protocol for COVID-19 anticoagulation monitoring, where besides interventions on anticoagulation, they considered to add, hold, increase, or decrease the antiplatelet agent aspirin (eventually supplemented with clopidogrel or ticagrelor, in case of an inadequate antiaggregation) based on the value of the maximum amplitude of the AA (arachidonic acid) and/or ADP test taken every 48–72 h. Compared to the 28 patients who were not on the protocol and whose anticoagulation was managed independent of TEG variables, the TEG-guided patients had a decreased frequency of eventual ICU admission (67.9% *vs*. 29.2%, $p = 0.0004$), shorter ICU and hospital length of stay (11.0 ± 3.3 *vs.* 3.7 ± 1.1 days, $p = 0.0456$, and 23.2 ± 3.1 *vs.* 14.2 ± 1.3 days, $p = 0.0122$, respectively), and decreased incidence of acute kidney injury and need for hemodialysis (68% *vs*. 29.4%, $p = 0.0007$, and 22.2% *vs*. 2.8%, $p = 0.0017$, respectively). The mortality rate was significantly different between the two groups: 5.6% in the TEG-guided group vs. 60.7% in the non-TEG group, $p < 0.0001$. Despite the limitations of the study, treating patients presenting high values of platelet aggregation apparently improved the outcome parameters, including the likelihood of survival.

The role of antiplatelet therapies in critically ill COVID-19 patients is uncertain and should be explored. The employment of antiaggregation is anecdotally reported in the studies published so far with no thorough statistical exploration of its clinical benefits.

6.4 Platelet Count and Other Platelet-Related Parameters in COVID-19 Patients

Thrombocytopenia at the hospital admission manifests in up to 40% of the patients [20, 21]. The incidence of thrombocytopenia varies depending on the reports and is different in different stages of the COVID-19 disease. Moreover, the definition of thrombocytopenia itself is not homogeneous among the different studies.

In particular, stratifying patients for disease severity, in the population described by Liao and coworkers, the frequency of thrombocytopenia (defined as platelet count lower than 100,000/μL) was 6% in the moderate disease (fever and

respiratory symptoms with radiological findings of pneumonia), 14% in the severe disease (respiratory distress with ≥30 breaths per minute; oxygen saturation of 93% or less at rest; ratio of partial arterial pressure of oxygen to fractional concentration of oxygen in inspired air of 40 kPa or less; or more than 50% lesion progression over 24–28 h in pulmonary imaging), and 49% in the critical disease (any of the following: respiratory failure requiring mechanical ventilation; shock, other organ failure that requires ICU monitoring and treatment) groups. The median platelet counts in the groups were 198 × 10^3/μL (IQR 145.5–249.5 × 10^3/μL), 227 × 10^3/μL (IQR 142–328 × 10^3/μL), and 105 × 10^3/μL (IQR 55.75–200.75 × 10^3/μL), respectively. Overall, the moderate *vs.* critical and the severe *vs.* critical group differences all had a significance of $p < 0.001$ [22].

In a large series of 1099 patients from an early report, Guan et al. found that the incidence of thrombocytopenia (platelet count <150 × 10^3/μL) was 31.6% in the non-severe vs. 57.7% in the severe group of patients ($p < 0.001$). The median platelet counts were 172 × 10^3/μL (IQR 139–212 × 10^3/μL) and 137.5 × 10^3/μL (IQR 99–179.5 × 10^3/μL), respectively [23].

In a cohort of 261 COVID-19 patients Chen et al. reported significantly different platelet counts in patients stratified for disease severity [24]. Critically ill patients showed the lowest count (117 ± 38.31 × 10^3/μL), significantly different from severe patients (188 ± 71.56 × 10^3/μL) and moderate and mild patients (169 ± 62.85 × 10^3/μL), $p < 0.001$. Comer and colleagues confirmed significantly lower platelet count in severe COVID-19 patients at the time of ICU admission as compared to the non-severe group ($p = 0.014$) [10].

Other reports found a less pronounced difference between groups. For instance, Ding et al. stratified patients as non-severe (including the mild and moderately ill patients) and severe (including the critically severe patients) and reported a median of 180 × 10^3/μL (IQR 149–227 × 10^3/μL) in the first vs. 160 × 10^3/μL (IQR 134–216 × 10^3/μL) in the latter group. The incidence of thrombocytopenia in this population was 10.5% *vs.* 26.7% ($p = 0.108$), respectively [25].

The association of platelet count on admission with outcome, including mortality, is not unanimously established.

Guan explored the association of thrombocytopenia (platelet count <150 × 10^3/μL) with a composite outcome of admission to intensive care unit, use of mechanical ventilation, or death and found that low platelet count was present in 46.6% of patients with outcome *vs.* 35.5% of patients without ($p = 0.091$) [23]. In a small series, a higher incidence of ICU treatment requirement was found in patients with thrombocytopenia (<100 × 10^3/μL)—8% vs. 4%, $p = 0.45$ [26]. However, in larger cohorts the association between baseline platelet count and admission to ICU was not confirmed [27, 28]. Analogously, platelet count did not differ between patients with and without ARDS [29].

In a population of 338 patients, thrombocytopenia (defined as platelet count lower than 125 × 10^3/μL) was associated with mortality of almost three times as high as that for those without thrombocytopenia ($p < 0.05$) [30]. Other reports confirmed the statistical difference in the proportion of patients with thrombocytopenia between survivors and non-survivors [22, 31, 32]. In the population

analyzed by Yang and coworkers, the proportion of patients with thrombocytopenia among non-survivors was 72.7% vs. 10.7% in the surviving group ($p < 0.001$) [32]. He and colleagues observed a higher survival rate in patients with high platelet count (not defined in the text, HR 0.28, 95% CI 0.11–0.69; $p = 0.0057$) [33]. Liao found a significant difference between survivors and non-survivors both in platelet count, 225.5 (IQR 130.5–327.0) × 10^3/μL *vs.* 75 (IQR 39–130) × 10^3/μL ($p < 0.0001$), and in incidence of thrombocytopenia (defined as platelet count <100 × 10^3/μL), 15.34% *vs.* 63.64% ($p < 0.0001$), respectively. In this series, after multivariate analysis, thrombocytopenia was associated to the death outcome with an OR of 8.33 (95% CI 2.56–27.15, $p = 0.00045$) [22]. In contrast, Zhao and colleagues in a cohort of 532 COVID-19 patients reported that the difference in platelet count on admission between the surviving and the non-surviving group showed no statistical significance, both in overall population and in male and female patients analyzed separately [34]. This finding was further confirmed by other reports [35].

The nadir platelet count has been found to be associated with disease severity and survival. Chen et al. outlined that in critically ill patients the nadir platelet count was greatly below the normal range (84 ± 57.80 × 10^3/μL) and significantly lower than that in the severe (171 ± 69.96 × 10^3/μL) and the mild/moderate (164 ± 55.53 × 10^3/μL) groups ($p < 0.001$) [24]. In a large cohort of 1476 COVID-19 patients, Yang et al. reported that the nadir platelet count in survivors, 203 × 10^3/μL [IQR 155–257 × 10^3/μL], was significantly higher than that in non-survivors, 79 × 10^3/μL [IQR 43–129 × 10^3/μL], $p < 0.001$ [32]. After the stratification of patients based on the nadir platelet count, the authors found that the in-hospital mortality was 92.1% in patients with count lower than 50 × 10^3/μL, 61.2% in patients with 50–100 × 10^3/μL platelets, 17.5% for counts 100–150 × 10^3/μL, and 4.7% for patients who never went below 150 × 10^3/μL. Taking the 150 × 10^3/μL platelet count as reference, the abovementioned groups had a relative risk of 13.68 (95% CI 9.89–18.92, $p < 0.001$), 9.99 (95% CI 7.16–13.94, $p < 0.001$), and 3.42 (95% CI 2.36–4.96, $p < 0.001$), respectively. The in-hospital mortality trend remained significant even after adjusting for age and gender [32].

As many authors highlighted, however, the platelet count parameter (and other parameters of blood count, as well) had a variable trend over time from the admission through the hospitalization. Many authors reported that different groups of patients, stratified on the basis either of the survival or of the disease severity, showed different trajectories of variation eventually associated with the outcome.

In the cohort of Zhao and colleagues, there was no difference in platelet count between survivors and non-survivors on the admission day (no stratification for disease reported); however on days 5–6 the difference was significant for males, 218.5 × 10^3/μL *vs.* 142.3 × 10^3/μL ($p = 0.003$), and on days 14–15 the difference became significant for females too, 239.9 × 10^3/μL *vs.* 114.3 × 10^3/μL ($p < 0.001$) for males and 250.7 × 10^3/μL *vs.* 151.1 × 10^3/μL ($p = 0.001$) for females. When assessing the relationship between the early (0–7 days) changes in platelet count and death, the authors confirmed that the platelet count in the non-surviving group was significantly lower than that in the surviving one. The difference between the

two groups had an increasing trend already in that early phase of hospitalization which increased by an average of $5.3 \times 10^3/\mu L$ per day [34].

In the population of Liu and colleagues, the difference between survivors and non-survivors was significant at all points of the monitoring, on days 1, 3, 7, and 14 after admission ($p < 0.05$). Notably, all the points stood higher than the lowest limit of normal range ($125 \times 10^3/\mu L$). The dynamics in platelet count in the first 7 days was negatively correlated with prognosis. An increment of $50 \times 10^3/\mu L$ in platelets was associated to a 40% decrease in death (HR 0.60, 95% CI 0.43–0.84) [31]. Patients with a platelet count of less than $200 \times 10^3/\mu L$ at admission and a decrease within the first week had the highest mortality rate, whereas those with platelets higher than $200 \times 10^3/\mu L$ and showing an increase in platelet count after 1 week had the lowest mortality rate [31].

Liao assessed the dynamics in platelet count changes from admission for up to 25 days. In this small series (12 survivors *vs.* 8 non-survivors) platelet count clearly decreased in non-survivors compared to survivors throughout the follow-up. In non-survivors, platelet count decreased far beyond the $100 \times 10^3/\mu L$ threshold [22].

Ding and colleagues followed patients for up to 15 days after admission and found a growing trend in platelet count in non-severe patients—379 (IQR 310–426) $\times 10^3/\mu L$ on day 15 *vs.* 166 (IQR 132–232) $\times 10^3/\mu L$ on day 1, $p = 0.008$. In contrast, severe patients (including the critically ill) showed no such dynamics, 180 (IQR 91–279) $\times 10^3/\mu L$ on day 15 *vs.* 160 (IQR 111–206) $\times 10^3/\mu L$ at baseline, $p = 0.893$ [25].

Most of the above-reported findings and associations were confirmed by meta-analyses [20, 21]. In a first early analysis pooling together patient data from nine studies, data showed a significant association between lower platelet count and more severe disease status, even if a high heterogeneity was outlined. For the mortality outcome, a stronger drop of platelet count in non-survivors compared to survivors was verified [20]. In a later meta-analysis with four additional studies for a total of 5252 participants, the association of the decreased platelet count with more severe disease was further substantiated, whereas no such evidence was found for the mortality [21].

6.5 Conclusions

Considered the available evidence, platelets represent the crossroad between hemostasis, inflammation, and immune response, even if there are still gaps in knowledge requiring additional research to draw a complete picture of the phenomenon. Unbalance in platelet contribution to the cross talk between these systems could lead to deleterious effects, as observed in the COVID-19 disease progression. Continuous and sustained stimulation of platelets for activation potentially leads to platelet exhaustion as function and consumption as count. Platelets brought to aggregation following strong agonistic stimuli increase the overall thrombotic risk of the patient. On the other hand, the resulting platelet depletion and thrombocytopenia are associated with worse outcome and greater

likelihood of bleeding diathesis, as well as a decreased efficacy of the infection resolution. In the light of what is described for the platelet engagement in the response to the viral infection by the SARS-CoV-2 as function, it is logic to consider platelet engagement as count being highly variable as variable is the individual susceptibility to the virus.

Platelet count must be considered as a dynamic parameter, rather than a single point in time (for instance, at admission to the hospital or to the ICU). Observation of the trend in platelet count as a continuous variable, rather than dichotomizing this parameter in thrombocytopenia vs. non-thrombocytopenia, is more informative as the trending is significative even when it lies within the normal range of the platelet count. Assessment of the platelet count as part of the routine blood count examination is simple, economic, rapid, and widely available in clinical structures. Daily observation of the platelet count and dynamic trend observation, especially in the early phase of the hospitalization, could give useful information for the treating specialist on the disease progression, recovery, or increase of the severity grade.

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The Overall Scenario of COVID-19-Associated Coagulopathy

7

Marco Ranucci and Dario Niro

7.1 Introduction

This chapter tries to fulfill the difficult task of summarizing the information delivered in the previous chapters into a single scenario. COVID 19-associated coagulopathy (CoAC) is the result of multiple factors and mechanisms mutually interacting. Additionally, there is certainly a time-related course of the disease, where inflammation, coagulation activation, thrombosis, fibrinolysis, and coagulation factors/platelet consumption interact. These factors can be distinguished into three phases: systemic and local inflammation (the "cytokine storm"); coagulation system activation (the "prothrombotic phase"); and overt thrombotic state with or without disseminated intravascular coagulation (DIC).

For the purpose of clarity, these phases will be separately analyzed. This is only partially adherent to the real scenario; even if in the majority of cases these phases are temporally subsequent, this is not always true, as they may interact even in a chaotic, not predefined pattern. However, the pharmacological approach to these phases is certainly different, and possible interventions will be separately addressed.

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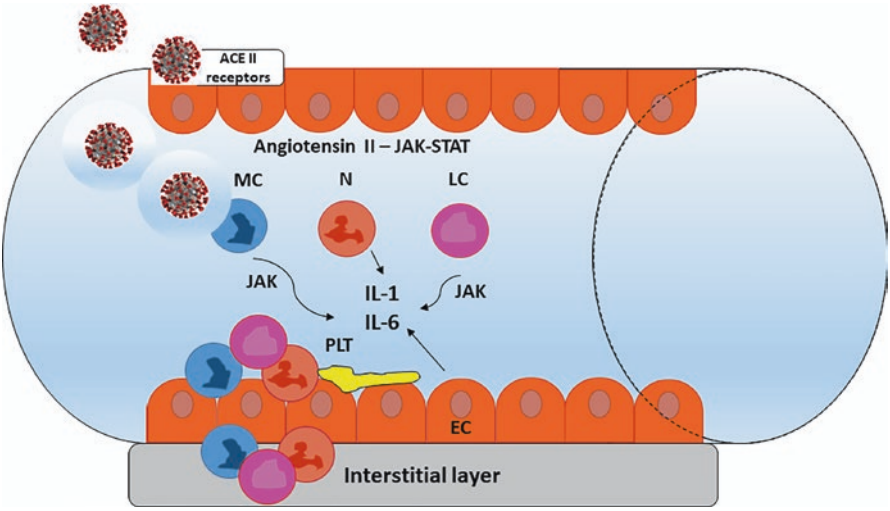

Fig. 7.1 The inflammatory reaction in COVID-19. ACE: angiotensin-converting enzymes; *EC* endothelial cells, *IL* interleukin; *JAK-STAT* Janus kinase-signal transducer and activator of transcription, *LC*: lymphocytes, *M* monocytes, *N* neutrophils, *PLT* platelets

7.2 Inflammation and the Cytokine Storm

The cytokine storm (Fig. 7.1) and its associated cellular mechanisms are the result of a complex chain of events. The first step is the SARS-CoV-2 endocytosis that is mediated by the angiotensin-converting enzyme II (ACE II) receptors. ACE II binds to the SARS-CoV-2 capsid with internalization within the host cell. ACE II receptors are expressed on cell surface of the heart, kidney, and endothelial cells, and in 83% of alveolar epithelial cells, which makes the lung the favorite target of SARS-CoV-2 infection [1].

The interaction of the SARS-CoV-2 spike protein with ACE II leads to shedding of the ACE II from the cell surface [2], producing increased levels of angiotensin II and hyaluronan, which are determinants of the acute respiratory distress syndrome (ARDS) [3, 4].

Angiotensin II mediates its action through the Janus kinase-signal transducer and activator of transcription (JAK-STAT). This induces an immune cell response with production of pro-inflammatory cytokines: IL-1, IL-2, IL-6, IL-7, IL-10, and TNF-α [4].

Among the different cytokines, IL-6 is considered the main player within the cytokine storm in COVID-19 [5].

IL-6 is a polypeptide consisting of four α helices. It is produced by a number of cells, including B-lymphocytes, T-lymphocytes, macrophages, monocytes, fibroblasts, and endothelial cells [6]. In infectious inflammation, the main cells producing IL-6 are monocytes and macrophages [5].

With different mechanisms, IL-6 interacts with its receptor IL-6R, which may be found at the level of the cell membrane or in soluble form. The biological activity of IL-6 is multifactorial, involves different cellular types, and is associated with a number of acute and chronic diseases. IL-6 induces proliferation of β-lymphocytes, promoting the production of IgA, IgM, and IgG [7]. At the level of T-lymphocytes, IL-6 is a pro-inflammatory regulator, promoting even self-inflammatory diseases [8]. At the hepatic level, IL-6 is a strong trigger of synthesis of acute-phase proteins (fibrinogen among the others) [9, 10]. Chronic cardiovascular and cerebral diseases are accompanied by an IL-6-mediated systemic inflammatory state [11]; however, the most important effects of IL-6 overproduction can be observed at the level of the lung, with an increased alveolo-capillary permeability as a marker of the lung insult [12].

In COVID-19 ARDS, the role of IL-6 overproduction has been widely stressed by an incredible number of reports and meta-analyses. Our group could identify the link between inflammation and coagulation in COVID-19 ARDS patients since March 2020, demonstrating the association between IL-6 values and fibrinogen levels in a series of 16 ICU patients [13]. The early reports from China immediately pointed out elevated values of IL-6 in COVID-19 patients: Chen and associates, in a population of mainly non-ICU patients, showed a mean IL-6 value of 7.9 pg/mL (normal range 0–7 pg/mL), with 52% of the patients with values above the upper limit of normal range [14]. Wu and associates, in a mixed patient population, confirmed this finding, with 49% of the patients showing IL-6 values above the upper limit of normal range; additionally, IL-6 was significantly higher in patients with overt ARDS ($P = 0.03$) and in non-survivors ($P < 0.001$) [15].

IL-6 is not only generally increased in COVID-19, but is even associated with the severity of the disease. A number of studies showed that in the severe vs. mild cases there was a significantly higher IL-6 level [15–19]. Two meta-analyses confirmed that patients with poor clinical outcomes had significantly higher IL-6 values [20, 21].

7.3 Therapeutic Interventions to Tackle Inflammation and Cytokine Storm

7.3.1 Hydroxychloroquine

At the beginning of the pandemic, hydroxychloroquine (HCQ) was widely used in the treatment of COVID-19. The rationale was based on its properties in inhibiting virus cell-binding receptors and virus replication. Besides this, HCQ has anti-inflammatory properties exerted through the inhibition of different cytokines' (IL-1, IL-6, TNF-alpha) release [22].

HCQ has additional antithrombotic effects, especially in the setting of lupus erythematosus, rheumatoid arthritis, and antiphospholipid syndrome [23, 24].

Three trials investigated the effects of HCQ in determining "viral clearance" [25–27]; when pooled in a meta-analysis, no effects of HCQ were found [28]. Given the lack of specific studies, its routine use for limiting thromboembolic complications of COVID-19 cannot be recommended [29]. The last release of the World Health Organization (WHO) COVID-19 clinical management living guidance (January 25th, 2021) reports a strong recommendation against the use of HCQ in COVID-19, regardless of the disease severity [30].

7.3.2 Statins

Statins preserve the endothelial function, have anti-inflammatory effects, and reduce thrombogenicity [31]. This last effect is mediated by a reduced expression of tissue factor and thromboxane A, and an upregulation of thrombomodulin [32]. Therefore, there is a rationale for their use in COVID-19. At present, there are ongoing randomized trials on statin use in COVID-19, but no study has been published yet.

7.3.3 Steroids

At the beginning of the COVID-19 pandemic, the use of steroids was highly controversial. Voices against the use of steroids were loud, stressing the risk for potential harm related to increased susceptibility to viral replication and supra-imposed bacterial infections [33]. The Interim Guidance of the World Health Organization (WHO) released on May 27th, 2020, advised against the use of steroids in COVID-19 unless for other reasons [33].

However, randomized controlled trials have subsequently challenged this attitude. The RECOVERY trial randomized hospitalized patients for receiving dexamethasone (6 mg once daily, oral or intravenous) for ten days [34]. Mortality was significantly lower in the dexamethasone group (29.3% vs. 41.4%; rate ratio: 0.64; 95% confidence interval [CI]: 0.51–0.81) among patients under mechanical ventilation and in those receiving oxygen in other forms (23.3% vs. 26.2%; rate ratio: 0.82; 95% CI: 0.72–0.94), but not in patients without respiratory support.

A French study [35] randomizing ICU patients to receive low-dose hydrocortisone or placebo was stopped at 50% of enrollment. The primary outcome (treatment failure, defined as death or persistent dependency on mechanical ventilation or high-flow oxygen therapy) occurred in 32 of 76 patients (42.1%) in the hydrocortisone group compared with 37 of 73 (50.7%) in the placebo group (difference of proportions, −8.6% [95% CI, −24.9% to 7.7%]; $P = 0.29$). An almost concomitant Brazilian study [36] randomized 299 ICU patients with COVID-19 ARDS to receive 20 mg of dexamethasone intravenously daily for 5 days, 10 mg of dexamethasone daily for 5 days or until ICU discharge, plus standard care, or standard care alone. The primary outcome was ventilator-free days. Patients randomized to the dexamethasone group had a mean 6.6 ventilator-free days (95% CI, 5.0–8.2) during the

first 28 days vs. 4.0 ventilator-free days (95% CI, 2.9–5.4) in the standard care group (difference, 2.26; 95% CI, 0.2–4.38; $P = 0.04$).

In patients with mild-moderate patterns of COVID-19 ARDS, dexamethasone did not improve the outcome in a recent randomized clinical trial [37].

Overall, steroid treatment appears justified at a low dose, for a limited period of time, and in patients with severe respiratory patterns. The last release of the WHO Guidance reports a strong recommendation for systemic corticosteroids in patients with severe and critical COVID-19, and a conditional recommendation against systemic corticosteroids in patients with non-severe COVID-19 [30].

7.3.4 Tocilizumab

Given the fact that elevated levels of IL-6 are a marker of COVID-19 inflammatory reaction, associated with the severity of the disease, therapies targeted to antagonize IL-6 release and action have been proposed since the beginning of the pandemic. Tocilizumab is an IL-6 receptor antagonist approved for the treatment of rheumatoid arthritis. At present, there are a few randomized controlled trials on tocilizumab in COVID-19. Veiga and associates [38], in a series of 129 patients receiving supplemental oxygen or mechanical ventilation, could not find a superiority of tocilizumab vs. standard of care. Conversely, the study was prematurely halted due to a significant increase of mortality in tocilizumab group. Almost simultaneously, in a patient population ($N = 389$) not receiving mechanical ventilation, a randomized controlled trial could demonstrate that tocilizumab administration significantly decreased the rate of progression to the composite outcome of mechanical ventilation or death, but did not improve survival [39]. Opposite results were found in a population of patients with moderate patterns of disease (not under mechanical ventilation): patients in the tocilizumab group did not show a reduced rate of progression to mechanical ventilation or death [40]. A nonsignificant trend toward a reduced rate of progression to noninvasive ventilation, mechanical ventilation, or death was found in a recent study [41]. Finally, an Italian study randomized non-ICU patients to receive standard of care with or without tocilizumab. The primary outcome was defined as entry into the intensive care unit with invasive mechanical ventilation, death from all causes, or clinical aggravation documented by the finding of a Pao_2/Fio_2 ratio less than 150 mmHg, whichever came first. No benefit of tocilizumab treatment was found [42]. The existing meta-analyses suggesting an efficacy of tocilizumab in decreasing mortality pooled together prospective and retrospective studies [43, 44]. Therefore, the role of tocilizumab in decreasing the rate of progression to severe patterns or mortality still appears elusive.

7.3.5 Other Cytokine Inhibitors

Inhibition of other cytokines has been addressed by some studies. IL-1 is an important player within the cytokine storm, and the IL-1 receptor antagonist anakinra was

used empirically in small series [45–49]. In a cohort study, patients receiving anakinra had a significantly reduced mortality risk (hazard ratio 0.450, 95% CI 0.204–0.990, $P = 0{\cdot}047$) than those who did not receive interleukin inhibitors [50]. A meta-analysis of non-randomized studies (four studies) suggests that anakinra may reduce the risk of progression to mechanical ventilation and death [51].

7.3.6 JAK Inhibitors

JAK inhibitors are biological agents inhibiting the type I/II cytokine receptors [4]. Tofacitinib is a JAK inhibitor approved for the treatment of rheumatoid arthritis, acting through a blockade of IL-2, IL-7, and IL-6. Baricitinib, ruxolitinib, fedratinib, momelotinib, gandotinib, and oclacitinib belong to the same group of JAK inhibitors. There are very few clinical studies on JAK inhibitors in COVID-19. Kalil and associates [52] performed a randomized controlled trial on baricitinib plus remdesivir vs. remdesivir alone in 1033 hospitalized patients with COVID-19. Patients receiving baricitinib had a median time to recovery of 7 days (95% CI 6–8), as compared with 8 days (95% CI, 7–9) in control (rate ratio for recovery, 1.16; 95% CI, 1.01–1.32; $P = 0.03$), and a 30% higher odds of improvement in clinical status at day 15 (odds ratio, 1.3; 95% CI, 1.0–1.6). Patients receiving high-flow oxygen or noninvasive ventilation at enrollment had a time to recovery of 10 days with combination treatment and 18 days with control (rate ratio for recovery, 1.51; 95% CI, 1.10–2.08). The 28-day mortality was 5.1% in the combination group and 7.8% in the control group (hazard ratio for death, 0.65; 95% CI, 0.39–1.09).

Cao and associates [53], in a small study on 43 patients, found a marginal and nonsignificant improvement in patients receiving ruxolitinib vs. placebo.

7.3.7 Others

The complement cascade is a possible target for controlling inflammation in COVID-19. Eculizumab and ravulizumab are anti-C5 monoclonal antibodies clinically applied in some complement-mediated diseases. Their use has been proposed in COVID-19 [54], but no clinical studies have been published so far.

7.4 The Prothrombotic State: Therapeutic Interventions

The link between inflammation and activation of the hemostatic system is represented by thrombin generation that is widely addressed in Chap. 4. Briefly, cytokines induce the release of tissue factor from circulating white cells, endothelial cells, and other cell types. This initially small amount of thrombin acts on the protease activatable receptors (PAR) placed on the platelet surface, triggering the formation of large amounts of thrombin which, in turn, converts fibrinogen into fibrin (Fig. 7.2).

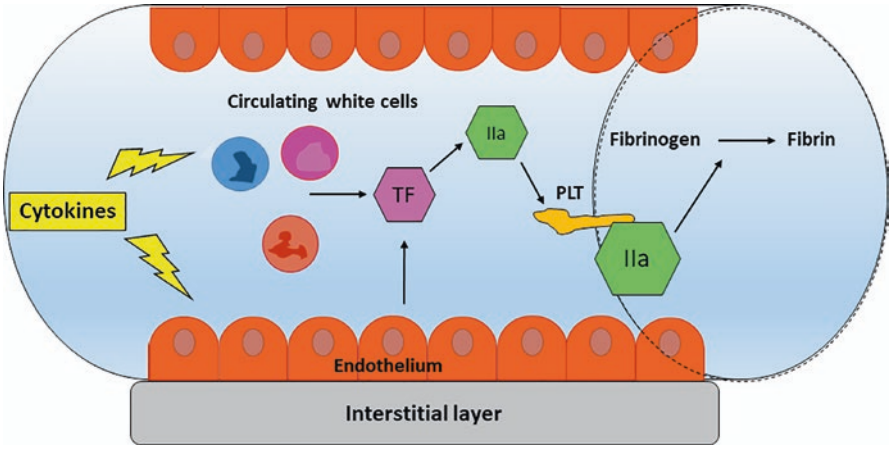

Fig. 7.2 The prothrombotic reaction in COVID-19. *PLT* platelets, *TF* tissue factor

Given this chain of events, prevention and control of thrombin generation are the main target of therapeutic interventions aimed to avoid the progression toward an overt thrombotic condition.

7.4.1 Heparin

Heparin inactivates thrombin by accelerating the action of the endogenous anticoagulant antithrombin (AT). The antagonizing reaction occurs at the level of factor IIa (thrombin) and of its precursor factor Xa. However, unfractionated heparin (UFH) acts both on thrombin and FXa, while low-molecular-weight heparin (LMWH) acts mainly on FXa, with an anti-FXa/anti-FIIa ratio between 2.0 and 4.0, and fondaparinux acts on FXa only.

Given its (indirect) action as a thrombin inhibitor, heparin is the logical approach to tackle the thrombin burst, and its role since the early phases of COVID-19 is nowadays widely recognized. Heparin use is widely addressed in Chap. 11. The existing debate is focused on the dose (prophylactic vs. therapeutic) of heparin to be used in the different phases of the disease.

The multiplatform RCT (mpRCT) is a collaboration between three trial platforms: ATTACC: antithrombotic therapy to ameliorate complications of COVID-19—58 sites in Canada, the USA, Brazil, and Mexico; REMAP-CAP: randomized embedded multifactorial, adaptive platform trial—290 sites in Canada, the USA, the UK, Ireland, the EU, Saudi Arabia, Australia, New Zealand, Nepal, India, and Pakistan; and ACTIV-4a: accelerating COVID-19 therapeutic interventions and vaccines—60 activated sites in the USA and Spain.

On January 28th, 2021, the mpRCT released preliminary, interim analysis, not peer-reviewed results of the randomized controlled trial comparing therapeutic LMWH dose or UFH vs. prophylactic LMWH dose [55]. The patient population

was analyzed according to a predefined separation between patients in severe state/critically ill patients (receiving organ support/ICU-level care) and moderate-state patients (hospitalized but not initially requiring ICU therapies/level of care). The primary outcome was organ support-free days. After the interim analysis (2895 patients enrolled), the three platforms agreed in stopping enrollment of the patients because the superiority of therapeutic heparin dose was achieved in the moderate-state patients (regardless of the D-dimer value). The odds ratio for superiority of therapeutic heparin dose was 1.57 (95% CI 1.14–2.19) in patients with low D-dimers and 1.53 (1.09–1.17) in those with high D-dimers. Conversely, in severe-state patients, the enrollment was stopped for futility. This important study introduces the concept of an aggressive use of heparin in the early phases of the disease, to prevent the progression to more severe states; once this happens, the heparin dose appears not relevant.

A recently published randomized controlled trial confirmed that, in ICU COVID-19 patients, intermediate-dose vs. prophylactic dose heparin treatment did not change the composite outcome of thrombosis, need for extracorporeal membrane oxygenation (ECMO), or death rate [56].

7.4.2 Warfarin and Direct Oral Anticoagulants (DOAC)

From a theoretical perspective, oral drugs reducing thrombin generation through a reduced synthesis of vitamin K-dependent coagulation factors (warfarin) or antagonizing factor Xa or IIa (DOAC) could be an interesting perspective for tackling the procoagulant phase of COVID-19. However, the extensive use of LMWH, especially in hospitalized patients, results in similar effects, and no studies addressed the possibility to prevent thromboembolic complications by a de novo therapy with oral anticoagulants. However, patients who were already receiving thromboembolic prophylaxis with warfarin or DOAC (mainly carriers of mechanical valve prostheses in the first case, and those with atrial fibrillation in the second) represent an interesting segment of population to better understand the role of thrombin generation and its antagonization in COVID-19.

Very recently, Denas and associates [57] reported the results of a wide epidemiological study carried in the Veneto region (Italy). The authors reviewed all patients aged 65 years or older, with a laboratory-confirmed COVID-19 diagnosis. They compared, after propensity score matching, those who received chronic anticoagulation for atrial fibrillation with those who did not. The main outcome was all-cause mortality. After propensity matching, two groups of 599 patients were compared. Those anticoagulated had a significant ($P = 0.036$) lower mortality rate (26.5%) vs. those non-anticoagulated (32.2%).

A totally different result was found in a Swedish population study. The authors found that ongoing DOAC use was not associated with reduced risk of severe COVID-19 (mortality or ICU admission) [58].

The real clinical question remains whether we should switch from warfarin/DOAC to LMWH in hospitalized patients with COVID-19. There are different reasons to support this option: basically, the higher predictivity of the efficacy of a parenteral treatment vs. oral administration. Additionally, there are factors in COVID-19 disease and treatment which could modify the response to DOAC therapy [59]. DOAC interacts with P-glycoprotein and/or cytochrome P450 (CYP)-based metabolic pathways, and this may modify their pharmacokinetic profile due to drug-drug interaction. Dexamethasone, antiviral, antibiotics, tocilizumab, and immunosuppressive drugs are some of the drugs used in COVID-19 which may interact with DOAC, resulting in a higher or lower clinical effect, and therefore potentially exposing the patient to a thrombotic or hemorrhagic risk [60].

7.4.3 Alternative Anticoagulants

Direct thrombin inhibition can be achieved with alternative parenteral anticoagulants, namely bivalirudin and argatroban. These drugs are usually applied in the management of heparin-induced thrombocytopenia and during ECMO. Their use in the setting of ECMO in COVID-19 patients will be addressed in Chap. 12. Outside these indications, their use in COVID-19 patients is mainly anecdotic. A randomized controlled trial comparing bivalirudin to LMWH/UFH in COVID-19 patients under mechanical ventilation has been registered at clinicaltrials.gov (NCT04445935) [61].

Hypotheses on a potential role of argatroban have been raised [62], but no clinical studies exist.

7.5 Overt Thrombotic Complications: Antiplatelet Agents Prophylaxis and Thrombolytic Treatment

The transition from a prothrombotic state, characterized by thrombin and fibrin generation, and overt thrombus formation, requires the contribution of platelets. Chapter 6 extensively addresses the platelet behavior in COVID-19, and Chap. 9 the clinical manifestations of thrombus formation. Prophylaxis of thrombus formation therefore may have a role in the overall scenario of COVID-19 treatment.

7.5.1 Aspirin

Aspirin has been extensively studied in the setting of ARDS, showing beneficial effects in terms of mortality [63, 64]. Recently, a cohort study on 991 patients hospitalized for COVID-19 demonstrated a reduction of mortality (odds ratio 0.746,

95% CI 0.560–0.994, $P = 0.046$) in patients treated with aspirin [65]. However, no randomized controlled trials on aspirin use in COVID-19 have been published so far.

7.5.2 Other Antiplatelet Drugs

$P2Y_{12}$ receptor antagonists are powerful antiplatelet agents widely used in the setting of double-antiplatelet therapy for the prevention of thrombotic complications in cardiovascular disease. Their use in COVID-19 patients has been hypothesized when thrombocytosis is present [13]. In a small study, patients treated with clopidogrel and tirofiban showed a better oxygenation profile than controls [66]. A randomized controlled trial evaluating the effects of double-antiplatelet therapy in COVID-19 patients at risk for cardiovascular disease is underway (NCT04333407).

Dipyridamole has been tested by one small study on COVID-19 patients [67]. Those receiving dipyridamole showed significantly decreased concentrations of D-dimers, increased lymphocyte and platelet recovery in the circulation, and an improved clinical outcome [67].

Other antiplatelet agents like vorapaxar have been hypothesized for blunting cytokine storm and platelet activation in COVID-19, but no studies are available so far.

The use of powerful antiplatelet agents in COVID-19 should be confronted with their potential drawbacks, which include the interaction with other drugs like lopinavir/ritonavir and remdesivir, the possible onset of thrombocytopenia, and the bleeding risk.

7.5.3 Thrombolysis

Once a clinically relevant thrombosis is evident, there is the challenge of treatment. The main patterns are pulmonary embolism (PE) and stroke.

The approach to PE is based on diagnosis and treatment. The National Pulmonary Embolism Response Team Consortium has published specific recommendations for PE in COVID-19 [68]. According to this statement, the presence of PE complicating COVID-19 should be considered when a patient exhibits hemodynamic instability or poor gas exchange that is not fully explained or is out of proportion to the stage, duration, and rate of progression of COVID-19 infection. In this case, a diagnostic algorithm should be followed.

Biomarkers, and namely D-dimer, are not recommended as diagnostic tools, since they are often elevated in COVID-19; CT angiography is the favorite choice. However, when CT angiography is not feasible, transthoracic echocardiography is helpful in finding indirect signs of PE like right ventricular dilation and intracardiac thrombi.

Once PE is diagnosed, a risk stratification is needed. This is based on the PE severity index, and on imaging for right ventricular dysfunction and/or biomarkers (troponin, brain natriuretic peptide, or NT-pro-brain natriuretic peptide).

In case of low or intermediate risk, systemic anticoagulation is needed and considered sufficient. In the severe cases, and namely in hemodynamically unstable patients, other treatments should be considered, including invasive procedures in cath lab or operating room and extracorporeal membrane oxygenation. Systemic thrombolysis is always recommended in severe cases, with dose and agent selected according to institutional protocol and by consensus of the treating team.

Subclinical cases characterized by microvascular thrombi do not justify the use of therapeutic anticoagulation or thrombolysis.

The therapeutic approach to stroke has been addressed by a position paper from an International Panel of Experts [69]. The general approach is consistent with the current guidelines on stroke management in non-COVID 19 patients [70], including mechanical thrombectomy when stroke is caused by the internal carotid artery or proximal middle cerebral artery occlusion, when treatment is feasible within 6 h of symptom onset. Same applies to intravenous thrombolysis with rtPA, with some additional concern related to a possible concomitant state of pro-hemorrhagic pattern.

7.6 Vaccines and Thrombotic Complications

Recently, the establishment and diffusion of vaccination campaign against COVID-19 have introduced a novel thromboembolic entity indirectly related to COVID-19. Vaccine-induced thrombocytopenia and thrombosis (VITT) is a clinical entity that has been reported after COVID-19 vaccination with vaccines containing replication-incompetent adenoviral vectors that encode the spike glycoprotein on SARS-CoV-2. It is believed that DNA that leaks from the adenovirus-infected cells binds to platelet factor 4 (PF4) and triggers the production of autoantibodies [71]. This induces a pattern of platelet hyperaggregability similar to what is seen in heparin-induced thrombocytopenia (HIT).

The International Society on Thrombosis and Hemostasis has delivered an Interim Guidance for the diagnosis and treatment of VITT [72]. The clinical manifestations include, but are not limited to, cerebral venous sinus thrombosis, mesenteric infarction, and pulmonary embolism, occurring 4–28 days after COVID-19 vaccination. The suspicion of VITT is based on symptoms and medical history. The diagnosis is based on (i) acute thrombosis and platelet count <150,000/μL and (ii) positive immunoassay for PF4 antibodies (HIT ELISA is the most reliable).

The treatment includes (i) intravenous immunoglobulin with steroids to be considered if platelet count <50,000/μL; (ii) avoidance of platelet transfusions, UFH, LMWH, and vitamin K antagonists; (iii) anticoagulation with fondaparinux or argatroban or a DOAC, if platelet count >50,000 cells/μL and no serious bleeding; and (iv) early plasma exchange or fibrinogen substitution to >1.0 g/L which can be considered if platelet count remains <30,000 cells/μL despite immunoglobulin and steroid treatment or fibrinogen level < 1.0 g/L.

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Endothelial Function and Microcirculation

8

Umberto Di Dedda

8.1 The Vascular Endothelium

The vascular endothelium, which forms the inner lining of all blood vessels of the vascular system, is an expansive cell layer, and represents the cellular interface between flowing blood and underlying tissues. Its weight is approximately 1 kg in an average-sized human [1]. The endothelium is formed by a single layer of endothelial cells (ECs) connected to each other by intermixed adherent and tight junctions. The shape of ECs varies along the vascular tree, but in general they appear as cobblestone-shaped, slightly elongated cells, with their dimension approximately 30–50 micrometer (μm) in length, 10–30 μm wide, and a thickness of 0.1–10 μm [2]. ECs are orientated along the axis of the vessel wall and thus in parallel with the blood flow, in order to minimize the flowing blood-induced shear stress. The basolateral surface of ECs is mounted on a glycoprotein basement membrane, filled with fibroelastic extracellular matrix, pericytes (primarily in capillaries and postcapillary venules), and smooth muscle cells (primarily in arteries and arterioles). The luminal side of endothelium is in contact with blood constituents and circulating cells through the interposition of the glycocalyx, a gel-like protective structure. The glycocalyx is a multicomponent layer consisting of proteoglycans (of which 50–90% is heparan sulfate and hyaluronan) and glycoproteins, anchored to ECs by glycosaminoglycans, and adherent plasma proteins [3]. The endothelium is not a mere inert barrier but rather is metabolically active, participating in many homeostatic processes, including the control of vasomotor tone, the regulation of permeability and trafficking of cells and nutrients, the maintenance of blood fluidity and control of hemostatic balance, the regulation of immune and inflammatory responses, and the control of new blood vessel formation [4].

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8.2 Endothelial Functions

8.2.1 Barrier and Transport Functions

These consist in the regulation of cellular and nutrient trafficking, and extravasation of solutes, fluid, macromolecules, and hormones, as well as that of platelets and blood cells. Located between the bloodstream and the endothelium, the endothelial glycocalyx is an important determinant of vascular permeability ("endothelial gatekeeper") [5, 6]. It is able to regulate vascular permeability by sieving molecules from plasma to the interstitium, repelling negatively charged molecules (e.g., albumin) as well as white and red blood cells and platelets [7, 8]. The endothelium is a "smart" structural barrier. The permeability depends on two main mechanisms. The paracellular pathway is regulated by endothelial cell–cell junctions ("tight junctions"), which act as a selective gate to the egress of water, cells, and molecules from the circulation [9]. The transcellular transport (transcytosis via caveolae) is the primary means of transport of many macromolecules (e.g., albumin), and is involved in transcellular ions' signaling [10]. Amino acid transport mechanisms are multiple but probably the most relevant is the system y+ cationic amino acid transporter, since it allows the transport of L-arginine, the substrate for nitric oxide (NO). Permeability and transport capabilities of the endothelium change along the vascular tree, reaching the maximal at micro diameters: capillaries are actually vessels meant for the exchange of nutrients and fluids. Furthermore, a great variability in EC permeability is found in different capillary regions, depending on organ-specific functions. ECs in the central nervous system, for example, form a "continuous" intima layer, conferring the blood-brain barrier an extremely low permeability (protective function). Conversely, EC permeability in renal glomeruli and endocrine glands are "fenestrated" and exhibit a high intra-extracellular exchange [2]. The integrity of endothelial transport function is essential: tissue edema or jaundice, as examples, is the result of tight junction disruption or dysfunction. Moreover, endothelial tight junctions are target sites of some viruses or pathogenetic bacteria. As a consequence, permeability function may be affected leading to endotheliitis in different organ systems (edema, diarrhea, acute respiratory distress syndrome) [11].

8.2.2 Vascular Tone Control

ECs are able to synthetize and release a broad array of factors with vasoactive properties in response to humoral and mechanical stimuli. Under physiological conditions, endothelium-derived relaxing and contracting factor production is balanced, with a net effect slightly in favor of vasodilation [12].

Vasoregulation by ECs is influenced by mechanical stimuli (i.e., hemodynamic forces, injury), and by both systemic and local biochemical inputs. Among others, the latter include local temperature variations and tissue oxygen tension changes, which represent powerful stimuli for ECs to modulate local vessel diameter and thus capillary blood flow [13]. Endothelium-derived factors inducing vasodilation

include NO, prostacyclin (PGI2), and endothelium-derived hyperpolarizing factor (EDHF) [13]. NO is a powerful endogenous vasodilator. The physiologic NO production by ECs is mediated by constitutive endothelial NO synthase (eNOS), which catalyzes the conversion of L-arginine to NO by means of the fundamental role of amino acid transport and caveolae transcytosis [14]. The direct effect of NO on vascular smooth muscle cells is cGMP-mediated vasodilatation.

Normally, a constitutive production of NO is guaranteed and maintains the vasculature in a basal state of relaxation and quiescence. The principal physiological cue that assures the basal, constitutive NO generation by resting ECs is blood flow-induced shear stress, which upregulates eNOS, a process termed "flow-mediated vasodilation" [13, 15–17] (Fig. 8.1).

The endothelial glycocalyx acts as a mechanotransducer by sensing blood flow-induced shear stress and, through conformational changes, transmits these forces to ECs and vascular smooth muscle cells which promote oxide-mediated vasorelaxation [8]. Besides the constitutive quote, an inducible amount of NO is generated by humoral stimuli. Indeed, both eNOS and cytokine-inducible NO production are activated via changes in intracellular calcium concentration in response to changes in shear forces. NO synthesis increases when shear stress increases [18] in order to preserve an adequate vasorelaxation in case of perturbations of blood flow. Laminar (normal) shear maintains endothelium in a NO-dominated quiescent state [19]. NO release occurs also in response to chemical stimuli from a variety of circulating vasoactive mediators including acetylcholine, angiotensin II (Ang II), adenosine diphosphate (ADP), thrombin, histamine, bradykinin, serotonin, or vascular endothelial growth factor (VEGF) [20, 21]. These mediators induce a vasodilation response by enhancing NO production when the endothelium is intact, or

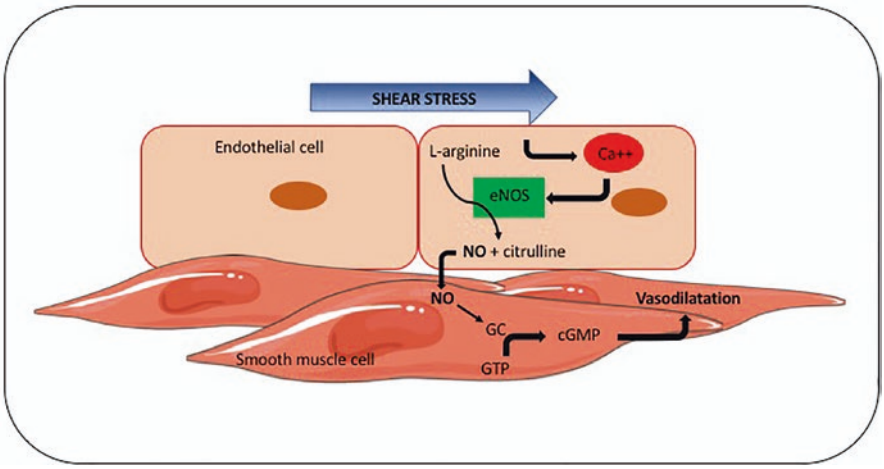

Fig. 8.1 Shear stress induces calcium-dependent activation of constitutive nitric oxide synthetase (eNOS) resulting in smooth muscle relaxation. *NO* nitric oxide, *GTP* guanosine triphosphate, *cGMP* cyclic guanosine monophosphate, *GC* guanylate cyclase

vasoconstriction when the integrity of vascular wall is compromised [22, 23]. Constitutive NO maintains the quiescent state of the endothelium also by a number of other effects. Indeed, NO targets key regulator molecules involved in the immune and inflammatory responses reducing their biological activity [24], such as nuclear factor kappa B (NF-kB), and limits phosphorylation in mitochondria [25] which leads to silencing of cellular processes. NF-kB is a redox-sensitive transcription factor which controls the expression of a number of cytokines, growth factors, and adhesion molecules involved in the inflammatory cascade. It is normally maintained in a nonactivated state by an inhibitor subunit (IkB). Constitutive NO production by ECs inhibits adhesion molecule expression through stabilization of IkB, thus attenuating pro-inflammatory responses [26]. Constitutive NO is also sufficient to inhibit platelet adhesion and aggregation by raising intracellular cyclic arginine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) [27, 28], an effect that may be synergistic with that of PGI2 [29]. PGI2, an eicosanoid constitutively generated by the vascular endothelium [30], is a potent vasodilator and an inhibitor of platelet aggregation by increasing cAMP levels [31]. Endothelium-dependent relaxation cannot be fully attributed to the release of NO or PGI2 [32]. Indeed, the endothelium also mediates vasodilatation via an NO-independent pathway which involves the activity of the EDHF [33]. EDHF becomes the predominant endothelium-derived vasodilator when NO bioavailability is absent or reduced [34], and its role in vasodilatation seems to be particularly important in the microcirculation [35]. Bradykinin stimulates release of NO, PGI2, and EDHF which contributes to inhibition of platelet aggregation [36]. Bradykinin also stimulates production of tissue plasminogen activator (t-PA), thus playing an important role in fibrinolysis [37]. The endothelium modulates vasomotion also by vasoconstriction. Endothelium-derived vasoconstrictor factors include endothelin-1 (ET-1), Ang II, thromboxane A2, platelet-activating factor (PAF), and reactive oxygen species (ROS) [38, 39]. Ang II not only acts as a vasoconstrictor but is also a prooxidant and stimulates production of ET-1 [40].

8.2.3 Blood Fluidity Maintenance and Antithrombotic Properties

The resting endothelium maintains blood fluidity by promoting the activity of numerous anticoagulant pathways.

The glycocalyx is a binding site for crucial anticoagulant mediators such as antithrombin (AT), thrombomodulin (TM), and tissue factor pathway inhibitor (TFPI). AT inhibits coagulation by lysing factor II (thrombin), factor IXa, and factor Xa, and its activity is increased by anticoagulant heparin [41]. Healthy ECs express surface heparan sulfate proteoglycans which exert a heparin-like activity by binding circulating AT, thus creating a substrate trap for active thrombin. Tissue factor (TF) is a procoagulant transmembrane glycoprotein synthesized by the endothelium and leukocytes, which, by creating complexes with factor VIIa, activates factors IX and X, ultimately leading to clot formation. The endothelium regulates TF by producing

TFPI, which prevents the formation of TF-factor VIIa complex by binding to factor Xa, and consequently the initiation of intrinsic coagulation cascade. TM is an integral membrane protein expressed on the surface of endothelial cells and serves as a cofactor for thrombin. TM is an important natural anticoagulant. Indeed TM-thrombin complex formation suppresses the procoagulant functions of thrombin [42, 43]. Protein S, synthetized by ECs, forms a complex with activated protein C on EC surface; this complex cleaves and inactivates several components of the clotting cascade, as factor VIIIa or Va. The endothelium further regulates anticoagulation by activating protein C via TM and endothelial protein C receptor, which inhibits factor V, factor VIII, and plasminogen activator inhibitor-1 (PAI-1) [44].

Furthermore, ECs synthesize and release tPA in constitutive and/or induced manners, potentiating plasmin-mediated fibrinolysis [45]. Indeed, PAI-1, a glycoprotein synthesized by the endothelium and the liver, regulates fibrinolysis by inhibiting tPA in health, but is incrementally released during inflammation [46]. Finally, resting ECs synthetize von Willebrand factor (VWF), a platelet adhesion protein. VWF functions in primary hemostasis by forming an adhesive bridge between platelets and vascular subendothelial structures, as well as between adjacent platelets at sites of endothelial injury [47]. In healthy status, ECs sequester VWF in intracellular storage granules (Weibel-Palade bodies, WPB), making it inaccessible to circulating platelets. VWF dimers and granular VWF multimers in WPB are rapidly mobilized in response to activating molecules such as thrombin. The VWF binds and stabilizes factor VIII and is a cofactor for platelet binding to exposed extracellular matrix in injured vessel walls. Both infection and inflammatory stimulation by tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-8, and high shear stress conditions can lead to elevation of plasmatic VWF [48]. Under high shear stress conditions, VWF promotes platelet aggregation [49]. Finally, as already described, endothelium-derived NO and PGI2 act synergistically for the maintenance of a silenced platelet activity [50].

8.2.4 Host Defense Function

Resting ECs do not interact with circulating leukocytes [51, 52]. This occurs because quiescent ECs sequester leukocyte-interactive molecules, like P-selectin and chemokines, within intracellular vesicles [53]. Interactions between leukocytes and endothelium are mediated by several families of adhesion molecules, including selectins (E-selectin, P-selectin, L-selectin) located on both leukocytes and ECs, integrins on the leukocyte surface, and immunoglobulin superfamily molecules expressed on ECs, including intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1); selectin-integrin interaction results in leukocyte firm adhesion and transendothelial migration [54, 55]. The surface expression of these molecules is tightly controlled in normal conditions, and amplified after EC activation, as during an inflammatory response [56].

The anatomical and functional integrity of ECs is fundamental in the maintenance of all these functions. As a central orchestrator of vascular permeability,

Table 8.1 Functions and features of the endothelial glycocalyx

• Major controller of interactions between the vessel wall and blood cells
• Its exact composition varies greatly according to the local microenvironment
• Plays a key role in shear stress-dependent NO production ("mechanotransducer")
• Key regulator of regulation of vascular permeability, trafficking of cells and macromolecules ("endothelial gatekeeper")
• Modulator of inflammatory cell adhesion and platelets to the endothelium
• Retains natural anticoagulants (e.g., AT, TM, TFPI) and antioxidants (e.g., superoxide dismutase)
• Glycocalyx may be damaged by exposure to shear and oxidative stress (e.g., sepsis, ischemia/reperfusion injury), thereby losing its protective functions

AT antithrombin, *NO* nitric oxide, *TM* thrombomodulin, *TFPI* tissue factor pathway inhibitor

vascular tone, and leukocyte and platelet adhesion, an intact glycocalyx is essential to the healthy functions of ECs in the maintenance of vascular homeostasis [57] (Table 8.1).

The glycocalyx is by no means a static structure. It sheds in response to numerous physiological and pathological stimuli [58]. The exact composition and thickness of the glycocalyx (0.2–2 μm) vary, depending on the vessel type, flow shear stress, and vascular bed [59]. A preserved glycocalyx thickness represents a sign of healthy endothelial function. Actually, its dimension fluctuates physiologically. Glycocalyx dimensions depend upon the balance between biosynthesis and enzymatic or shear-dependent shedding of its components [60]. In the presence of pathological stimuli, the best characterized being ischemia and hypoxia, sepsis and inflammation, or malignancies, components of the glycocalyx are shed from the endothelial surface into the plasma, thereby representing sensitive biomarkers of glycocalyx degradation [61]. As a result, glycocalyx is degraded, becoming thinner and more sparse, and loses its normal functions.

Glycocalyx degradation leads to enhanced vascular permeability, fluid shift and tissue edema, augmented leukocyte adhesion, platelet aggregation, and dysregulated vasodilation [62, 63]. Conversely, reconstitution of glycocalyx restored protective abilities of the vessel wall [64, 65].

Vascular homeostasis refers to the maintenance and preservation of vascular functions over time, and thus also includes the adaptation to persistent environmental signals [66]. ECs receive and respond to signals and stimuli in a variety of pathways which are dramatically broad, depending on the spatial origin of the stimulus, the local extracellular environment, and its changes over time [66].

8.3 Endothelial Activation

In normal conditions, the endothelium is at rest and it exhibits anticoagulant, antiadhesive, and vasodilatory properties. "Resting" is not describing a passive state, rather it refers to a potential condition. Activation is a switch from a quiescent "protective" phenotype towards one involving mechanisms related to host defense

Table 8.2 Endothelial activation and dysfunction manifestations

• Increased vascular permeability
• Change from a vasodilator to a vasoconstrictor phenotype
• Increased adhesiveness of the endothelial cells to inflammatory cells (leukocytes) and platelets
• Switch from an anticoagulant to a procoagulant phenotype
• Change from a vasodilator to a vasoconstrictor phenotype
• Change from a growth-inhibiting to a growth-promoting phenotype through elaboration of cytokines

response [22]. Differently from the quiescent phenotype, the activated EC phenotype consists of a combination of pro-adhesive, procoagulant, and vasoconstricting properties (Table 8.2). Triggers of endothelial activation include pro-inflammatory cytokines such as TNF-α, IL-1, bacterial lipopolysaccharide (LPS), viruses, PAF, shear and oxidant stress, hyperglycemia, and hypoxia/reperfusion [67]. An extensive description of EC activation is reviewed elsewhere [68, 69]. Briefly, endothelial activation involves de novo or enhanced expression of specific leukocyte adhesion molecules, such as P- and E-selectin ICAM-1 and VCAM-1, which promote leukocyte adhesion and tissue migration [70].

Furthermore, cytokine-induced EC activation is accompanied by loss of vascular integrity, leading to vascular leakage of fluid and plasma proteins. These alterations are particularly prominent on postcapillary venules of the microcirculation [71]. Inflammatory mediators interact with ECs to induce a loss of the physiologic thromboresistant phenotype. The prothrombotic effects of EC activation include loss of the surface anticoagulant molecules TM and heparan sulfate (glycocalyx shedding); reduced fibrinolytic potential due to enhanced PAI-1 release; and loss of the platelet antiaggregatory effects of NO and prostacyclin. Furthermore, TNF and IL-1 enhance the production of PAF and induce the synthesis of TF, the principal initiator of coagulation [72]. The distinct effects of EC activation share a common intracellular control mechanism through the activation of transcription factor, NF-kB [73]. The NF-κB system is recognized as the common denominator of endothelial activation [74]. Once activated, NF-κB upregulates the expression of genes which characterize endothelial activation, including surface adhesion proteins, cytokines, growth factors, and components of the coagulation system [68]. The quiescent state of the endothelium is mediated by the NO generated by eNOS. As mentioned before, NO targets the NF-κB system, inhibiting its pro-inflammatory signaling pathways. Reduced bioavailability or unbalanced metabolism of endothelium-derived relaxing factors, specifically NO, occurs in the presence of risk factors or during disease involving the vascular endothelium [75]. Conditions of hypoxia, inflammation, or ischemia-reperfusion, and the presence of cardiovascular risk factors, increase the expression and/or activity of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidases in the vascular wall, thereby enhancing the production of ROS. Furthermore, in these conditions, eNOS can switch to generate ROS (eNOS uncoupling). eNOS uncoupling has also been seen in patients with endothelial dysfunction resulting from hypercholesterolemia, diabetes mellitus, or essential

hypertension or in chronic smokers [14]. Endogenous ROS (e.g., superoxide, hydrogen peroxide) are formed as a natural by-product of the normal aerobic metabolism of oxygen and have important roles in cell signaling, but they are deleterious at high concentrations [56]. When oxidative stress is uncontrolled, redox signaling becomes predominant, leading to sustained endothelial activation and dysfunction, thus contributing to vascular disease [76].

8.4 Endothelial Dysfunction

The transition from endothelial activation to endothelial dysfunction is subtle and implies a fundamental feature: the excessive, dysregulated, and sustained response of the endothelium to a pathological input, which ultimately poses a net liability to the host [1]. Disturbed endothelial function is a hallmark in many pathophysiological conditions, including aging, hypertension, male gender, diabetes, obesity, and smoking [77]. The hallmark of endothelial dysfunction is impaired endothelium-dependent vasodilation mediated by NO. A defect in NO production or activity has been proposed as a major mechanism of endothelial dysfunction [78].

8.5 Sepsis: A Model of Endotheliopathy

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection [79]. Many types of microbes can cause sepsis, including bacteria, viruses, and fungi. Sepsis primarily affects the vascular endothelium, leading to detrimental changes in endothelial function. The aggression on the endothelium during sepsis occurs through a direct infection of ECs by pathogens, and an indirect assault on ECs exerted by components of bacterial wall, such as LPS, and host-derived factors, such as cytokines, chemokines, proteases, ROS, hypoxia-induced factors, and shear stress changes, which ultimately activate ECs. The cytokines released to combat the infection trigger sustained and uncontrolled endothelial activation until the loss of normal endothelial functions. The septic endothelium exhibits structural changes, such as nuclear vacuolization, swelling, denudation, and fragmentation and detachment from the underlying layer [80]. Anatomical changes are accompanied by functional changes, including loss of barrier function and hyper-permeability, altered vasoregulation, increased leukocyte adhesion, and shifts in hemostatic balance [46, 80–82].

8.5.1 Hyper-Permeability and Loss of Barrier Function

A first central feature of the endothelium during sepsis is its loss of barrier function and the increased permeability. During sepsis, the glycocalyx becomes thinner and degraded, allowing uncontrolled passage of plasma proteins and fluid across the vascular wall. As a consequence, tissue edema occurs [62]. A number of cytokines and factors are responsible for this process. Pro-inflammatory cytokines released during sepsis, such as TNFa and IL-6, and thrombin are known to increase EC

permeability both in vitro and in vivo [83]. Elevated levels of TNFα contribute to glycocalyx shedding via increased metalloproteinase activity and syndecan loss [84]; VEGF, which is primarily a stimulator of angiogenesis, also increases vascular permeability and its levels are elevated during sepsis [85]. Ang II release from WBP during inflammation leads to enhanced barrier disruption via heparanase-mediated degradation of heparan sulfate [86]. Ang II levels are increased during sepsis and have been associated with bad outcome [87, 88]. In septic shock, LPS exerts a direct anatomical damage on the endothelium. A single injection of LPS in animals denudates endothelium [89], ECs become detached, and subendothelial edema occurs.

Nieuwdorp and associates [90] reported a significant decrease in glycocalyx thickness (0.60 ± 0.1 μm to 0.30 ± 0.1 μm, $p < 0.01$) in healthy human subjects who received low dose of intravenous endotoxin, together with a concurrent increase of plasmatic hyaluronan concentration (62 ± 18–85 ± 24 ng/mL, $P < 0.05$).

8.5.2 Dysregulated Vasodilation

The balance between vasodilators, such as NO and prostacyclin, and vasoconstrictor levels, such as endothelin, PAF, and thromboxane A2, is altered in sepsis. Because NO metabolism also plays a key role in the regulatory function of the ECs, reduced activity of eNOS exacerbates organ injury. NO levels are increased during sepsis. NO overproduction is generated by inducible NOS upregulation in response of a variety of cytokines and microbial mediators. Conversely, constitutive production of NO is impaired in sepsis. Pro-inflammatory cytokines and ROS-induced NF-kB activation can turn eNOS into a generator of superoxide anion (eNOS uncoupling), thereby reducing NO bioactivity [91]. Shedding of the glycocalyx may also hamper the ability to sense and transduce blood flow-induced shear stress, resulting in the altered endothelial release of NO and ET. Increased plasma concentrations of NO and ET metabolites have been reported in endotoxic shock [92].

8.5.3 Pro-adhesive Phenotype

See “Endothelial Activation.”

8.5.4 Procoagulant Phenotype

Sustained EC activation during sepsis rapidly confers a procoagulant profile to the surrounding environment. Inflammation-related coagulation is characterized by a number of derangements from normal endothelial functions.

– Upregulation of procoagulant factors: Pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α can activate ECs leading to synthesis and release of procoagulant particles such as the WPB [93]. These organelles contain ultra-large VWF

and P-selectin, which can together form a network with other coagulant factors, platelets, and inflammatory cells. At the same time, cytokines increase the expression of TF from activated ECs and on circulating monocytes, leading to intravascular activation of coagulation. Moreover, the cytoskeletons of ECs are rearranged to expose the procoagulant collagen. Furthermore, in response to microbial stimuli, activated neutrophils release neutrophil extracellular traps (NETs), which can provide a scaffold and stimulus for intravascular coagulation by activating the intrinsic coagulation pathway [94].

– Downregulation of anticoagulant factors: The natural anticoagulant pathways are inhibited when cytokines are present through a variety of mechanisms such as loss of heparan sulfates via glycocalyx degradation, and decline of anticoagulant proteins such as TM [95], TFPI, and AT [96]. Furthermore, there is decreased expression of endothelial protein C receptors [97], or inhibited synthesis of a disintegrin and metalloproteinase with thrombospondin motifs 13 (ADAMTS-13). ADAMTS-13 deficiency may lead to insufficient cleavage of VWF resulting in enhanced platelet-vascular wall interaction [98].
– Blunted fibrinolysis: The fibrinolytic system is inhibited in septic condition through the enhanced expression of PAI-1, which can deactivate tPA and result in less plasmin, thus preventing fibrin thrombus removal [99].

The net effect of these changes is to enhance thrombin generation and to allow thrombin to cleave fibrinogen to fibrin. At the same time, the blunted fibrinolysis may exacerbate microvascular thrombosis.

8.5.5 The Septic Glycocalyx

Glycocalyx breakdown represents the earliest step and the most significant site of injury during severe states of inflammation, as in sepsis [100]. A degraded glycocalyx is unable to perform its normal protective functions on the endothelium. Indeed, glycocalyx shedding causes abnormal transcellular communication, dysregulated NO metabolism, increased ROS generation, exposure of adhesion molecules, and activation of TF. All these mechanisms are behind the development of progressive EC dysfunction, manifesting as hyper-permeability, tissue edema, dysregulated vasodilation, inflammatory cell attraction, platelet aggregation, and microvascular thrombosis [101] (Fig. 8.2).

The severity of glycocalyx breakdown is associated with adverse clinical outcome [102, 103].

During sepsis, the glycocalyx is degraded via several enzymes such as metalloproteinases, heparanase, and hyaluronidase causing the release of glycocalyx components (such as syndecan-1, heparan sulfate, hyaluronan, chondroitin sulfates) into the plasma. Heparanase directly cleaves the heparan sulfate chains attached to core proteoglycans [104]. Metalloproteinases, such as ADAM17, are known to cleave proteoglycans (e.g., syndecan-1) directly from the endothelial cell membrane, and are activated in inflammatory states by ROS and pro-inflammatory cytokines such as TNF-α and IL-1β [105, 106]. In contrast to ADAM17, ADAMTS-13 activity is

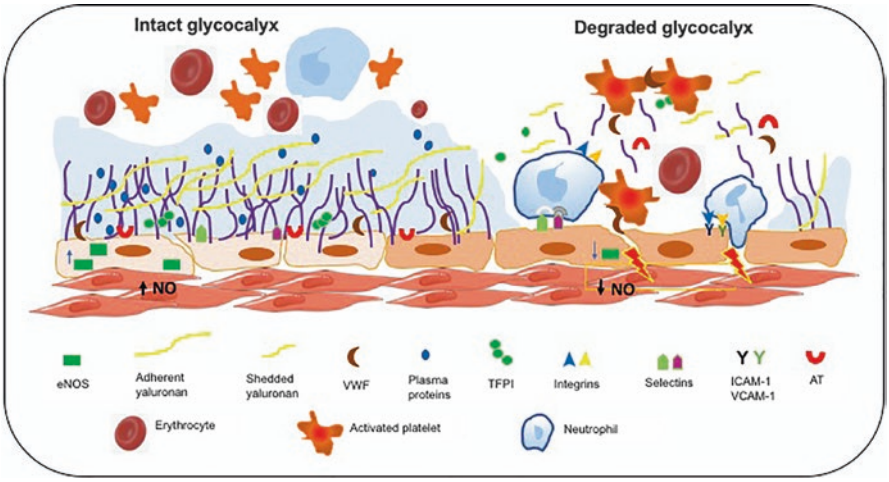

Fig. 8.2 In healthy conditions, the endothelial glycocalyx acts as an exclusion zone for cellular blood components. When degraded, platelets and leukocytes are allowed to interact with adhesion molecules expressed on the endothelial surface as modulators of coagulation and inflammation are displaced from the endothelial surface. Shedding of the glycocalyx leads to intercellular gap formation resulting in hyper-permeability and tissue edema, dysregulated vasoregulation, inflammatory cell attraction, platelet aggregation, and microvascular thrombosis. *AT* antithrombin, *eNOS* constitutive nitric oxide synthetase, *vWF* von Willebrand factor, *TFPI* tissue factor pathway inhibitor, *ICAM* intercellular adhesion molecules, *VCAM* vascular cellular adhesion molecules, *NO* nitric oxide

suppressed in sepsis [107], thus compromising cleavage of VWF multimers leading to enhanced platelet aggregation.

In turn, fragmented glycocalyx components induce the synthesis of cytokines, intensifying inflammation. The degradation of glycocalyx is also thought to contribute to microcirculatory dysfunction in sepsis and septic shock [108–110].

8.6 The Microcirculation

The microcirculation is defined as the smallest unit of the cardiovascular circulation [111]. The microcirculation consists of a branching network of arterioles, capillaries, and venules with lumen diameter less than approximately 100 μm. The cell types comprising the microcirculation are the endothelial cells lining the inside of the microvessels, smooth muscle cells (mostly in arterioles), erythrocytes, leukocytes, and plasma components in blood. The structure and function of the microcirculation are highly heterogeneous throughout organ systems [111–113].

8.6.1 Microcirculation Function: General Principles

The smallest blood vessels of the microcirculation (<20 μm in diameter) are the principal site of gas and nutrient exchange between blood and underlying tissues

[112]. A functional microcirculation is essential for adequate tissue perfusion and thus organ function. Tissue perfusion is determined by (i) the vascular density (the diffusive component of oxygen transport), (ii) the capillary flow providing oxygen-carrying red blood cells (the convective component), and (iii) the heterogeneity of microvascular flow [114].

Convective and Diffusive Oxygen Transport

Convective oxygen transport is determined by microvascular oxygen content and flow.

Capillary blood flow is determined by the driving pressure, arteriolar tone, rheologic properties (i.e., red blood cell deformability and viscosity), and capillary patency. More specifically, the autoregulatory mechanisms, intrinsic to the microcirculation, controlling microcirculatory perfusion are classed as myogenic (sensing strain and stress), metabolic (regulation based on oxygen, carbon dioxide, lactate), and neurohumoral [112].

Depending on local oxygen requirements, indeed, tissue blood flow regulation is achieved by shutting down or limiting flow in capillaries or, conversely, increasing flow by vasodilation. Two main mechanisms may contribute to autoregulation: first, perivascular sympathetic nerves influence arteriolar tone in a cross talk manner with ECs [115]; second, red blood cells may act as intravascular sensor of oxygen and enhance local endothelial derived NO release when exposed to a low-oxygen environment [116]. Capillary vasodilation in response to hypoxia and preservation of capillary flow despite changes in systemic blood pressure are examples of autoregulation.

Diffusive oxygen transport is determined by the intercapillary distance, namely the distance between oxygen in the red blood cells and mitochondria within tissue cells. A reduction in tissue capillary density increases the diffusion distance for oxygen. A critical distance exists. At that point, anaerobic metabolism will occur. Capillary density increases during training [117], and in response to chronic hypoxia [118].

Finally, compared with baseline, the heterogeneity of the microcirculation increases by close to 10% during hypoxia or hemorrhage [119].

8.7 Microvascular Dysfunction in Sepsis and Septic Shock

As stated by Ince [112] in a milestone review, the microcirculation is the motor of sepsis, as microcirculatory abnormalities play a key pathophysiological role in such a condition.

In a pioneering study, De Backer and associates [120] first demonstrated that, in humans, the sublingual microcirculation is altered in septic patients. After this publication, multiple experimental studies confirmed that sepsis induces marked alterations in the microcirculation, and that such alterations are associated with a worsened outcome [120–122]. Sepsis-induced microvascular dysfunction is characterized by a decreased capillary density (quantitative alterations), and

heterogeneity of perfusion, namely the presence of capillaries with altered blood flow (sluggish, intermittent, or stopped) in close vicinity of normal to overperfused capillaries (qualitative alterations) [123]. These two microcirculatory alterations lead to both diffusive and convective oxygen transport disturbances, respectively. Microvascular alterations can lead to cellular injury [124], while their reversal is associated with improvement in lactate [125] and NADH levels [126], suggesting that microvascular alterations directly impair tissue oxygenation.

Heterogeneous capillary blood flow: During sepsis, the microvascular blood flow becomes heterogeneous, promoting the presence of well-oxygenated tissue areas in contiguity with hypoxic pouches, even when total blood flow and oxygen delivery to the organ are preserved. Indeed, despite a preserved or even increased systemic oxygen delivery during sepsis, the septic microcirculation promotes the shunting of blood and hence oxygen, from arterial to venous compartment, leaving the microcirculation hypoxic. The impaired oxygen delivery in the dysfunctional microcirculation results in oxygen extraction deficit. When the local microcirculatory partial pressure of oxygen drops below the venous oxygen pressure a "pO2 gap" occurs, representing an indicator of the severity of functional shunting [127]. Studies report that heterogeneity in microvascular blood flow is associated with heterogeneity in tissue oxygenation and with altered local oxygen extraction capabilities [128, 129].

Decreased perfused capillary density: The immediate consequence of this alteration is the increase of intercapillary space and, thus, the increase of oxygen diffusion distance, leading to compromised oxygen supply for mitochondrial efficiency. If the diffusion distance for oxygen exceeds a critical threshold in tissues, then anaerobic metabolism occurs. Bateman and associates [130] demonstrated that in rat cardiomyocytes, LPS administration induces hypoxia with increased oxygen diffusion distances in the microcirculation.

Heterogeneity of microvascular perfusion is a crucial aspect. In physiological conditions or in response to systemic low flow, the microcirculation tends to adapt by recruiting closed capillaries, thus minimizing perfusion heterogeneity. This is possible when autoregulatory mechanisms, intrinsic to the microcirculation, are intact. When heterogeneity is associated with microcirculatory dysfunction, as happens during sepsis, such adaptive mechanisms are lost, and tissue perfusion and oxygenation are compromised [131]. Importantly, tissues tolerate a homogeneous decrease in blood flow better than a heterogeneous one [132].

8.7.1 Mechanisms Underlying Microcirculatory Alterations During Sepsis

Endothelial dysfunction, and the broad spectrum of its manifestations, is a key mechanism underlying sepsis-induced microvascular alterations.

Endothelial functions are strictly dependent on the integrity of the glycocalyx. Sepsis causes a ubiquitous degradation of the glycocalyx, leading to NO metabolism alteration, blunted reactivity to shear stress changes, and impaired flow-mediated vasoregulation. Sensitivity to vasodilating and vasoconstrictive substances and/or stimuli (shear stress) is impaired, contributing to microvascular shunting and alteration in the distribution of local perfusion. Sepsis-induced endothelial activation with concomitant glycocalyx breakdown promotes both a pro-inflammatory state with upregulation of adhesion molecules, enhancing leukocyte-endothelial interactions, and a shift to procoagulant state via the loss of heparan sulfate, with microthrombosis leading to capillary obstruction, further impairing local oxygen delivery.

Sepsis induces morphological and functional changes in red blood cells, leukocytes, and platelets. These abnormalities in blood rheology contribute to the decrease of functional capillary density [133]. Of note, the persistence of these alterations—and thereby the subsequent microcirculatory dysfunction—is associated with adverse clinical outcome even though macrohemodynamic variables have been corrected [111].

8.7.2 Hemodynamic Coherence Between Macro- and Microcirculation

A hallmark aspect of the septic microcirculation is its functional disconnection from the systemic hemodynamic status. During states of shock, resuscitation goals are aimed to normalize systemic variables of perfusion (cardiac output, blood pressure, volemia) and oxygenation (arterial oxygen content), and it is expected that a parallel improvement in microcirculatory perfusion will result in restoration of tissue oxygenation. In landmark paper [134], Ince introduced the concept of loss of hemodynamic coherence between macro- and microcirculation in states of shock. In conditions of severe inflammation and infection, which often accompany states of shock, vascular regulation and microcirculatory compensatory mechanisms needed to regulate tissue perfusion and adequate oxygen delivery are lost, and hemodynamic coherence is not guaranteed. In these cases, despite successful macrocirculatory parameter restoration, resuscitation becomes ineffective in restoring the microcirculation and in correcting tissue hypoperfusion. The pathogenic mechanism underlying the loss of hemodynamic coherence is the development of microcirculatory dysfunction secondary to endothelial dysfunction, changes in blood viscosity and shear stress, changes in erythrocytes deformability, glycocalyx degradation, pathologic inflammation, and coagulation activation. Some studies have described the condition of loss of hemodynamic coherence between macro- and microcirculation in states of shock [135–138], Ince identified four types of microcirculatory alterations underlying the loss of hemodynamic coherence [134]. Loss of hemodynamic coherence is frequent in septic patients in whom a lack of microcirculatory recruitment is observed despite successful macrocirculatory resuscitation [134].

8.8 Monitoring the Microcirculation

Different techniques may be used to assess the microcirculation at the bedside, either indirectly using indices of tissue oxygenation (i.e., near-infrared spectroscopy, NIRS) or directly measuring microvascular perfusion (videomicroscopic techniques). The reader is referred to an exhaustive description of the past and current techniques for microcirculation monitoring [139–141]. Direct evaluation of sublingual microcirculation by handheld vital microscopes (HVM) is today the technique of reference [139–141].

Figure 8.3 depicts two patterns of sublingual microcirculation.

Differently from NIRS, these bedside devices monitor and measure the actual blood flow, perfusion, and heterogeneity of flow in the microvessels. The first-generation HVM such as orthogonal polarization spectral imaging pioneered the research in the field. The new-generation sidestream dark-field (SDF) and incidence dark-field (IDF) imaging techniques improved optical resolution, and are currently used. HVM works using a scattered light which, penetrating the mucosa, is absorbed by hemoglobin in the erythrocytes flowing in superficial vessels, giving back a well-defined image of blood flowing through the microvessels.

Massey and Shapiro [142] published an exhaustive and comprehensive review of the technical aspect for the acquisition of high-quality videos.

The different techniques and microcirculatory variables introduced to assess the microcirculation are described in depth in the Second Consensus on the assessment of sublingual microcirculation in critically ill patients [143], published in 2018 by a task force of the European Society of Intensive Care Medicine.

8.8.1 Microcirculatory Variables

A proper evaluation of microcirculatory status should include an assessment of vascular density, capillary perfusion, and heterogeneity of perfusion. For such purposes

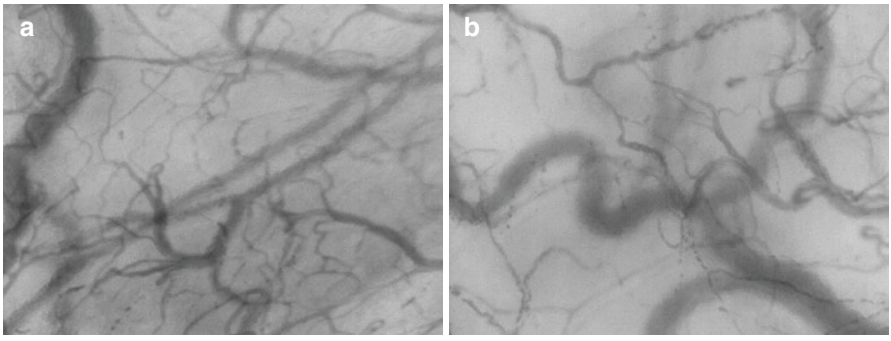

Fig. 8.3 (**a**) The sublingual microcirculation in a healthy patient and (**b**) in a septic patient. The septic microcirculation is characterized by decreased capillary density and heterogeneity of perfusion, namely the presence of capillaries with altered blood flow (sluggish, intermittent, or stopped) in close vicinity to normally perfused capillaries

a number of microcirculatory parameters have been employed in clinical practice [144].

Briefly, the De Backer score approximates vessel density using a line-crossing method.

Perfused vessel density (PVD) and proportion of perfused vessels (PPV) are related to the density of functionally flowing capillaries. PVD is equal to the functional capillary density (FCD) and is probably the most important variable to determine because it is the factor with the greatest influence on perfusion.

The microvascular flow index (MFI) is a semiquantitative measure of capillary perfusion quality. Perfused vessels are those with a constant or sluggish flow, and non-perfused vessels are those with intermittent or absent flow.

Finally, the heterogeneity index (HI) evaluates the heterogeneity or variability of predominant blood flow (typical of distributive abnormalities) between sequences at a single time point at different sites of detection. HI is the difference between the highest MFI minus the lowest MFI divided by its mean value of all sublingual sites at a single time point.

These main functional microcirculatory variables are related to their oxygen-carrying capacity, diffusive capacity, and heterogeneity of perfusion.

- Oxygen-carrying capacity is defined by a measure of convective transport, namely the flow of red blood cells through the capillaries. It is quantified by PVD and STD. Space-time diagrams were developed to describe quantitative red blood cell velocity (RBCv) profiles.
- The diffusive capacity refers to diffusive transport of oxygen and is related to the oxygen diffusion distance between the red blood cells and the tissue cells. It is quantified by PVD, which includes TVD and PPV.

Any derangement in one or more of the microcirculatory variables and endothelial function may cause microcirculatory dysfunction and lead to inadequacy of tissue perfusion [145].

8.8.2 Microcirculatory Alterations During Sepsis and Septic Shock

Septic patients with significant alterations in sublingual microcirculation had a worst prognosis compared with those who did not show microcirculatory alterations [146]. In early septic patients, De Backer and colleagues [120] reported a reduction in PPV as close as 50% compared to controls (48% vs. 90% in volunteers, $P < 0.001$), and that PPV reduction was more severe in non-survivors. Another hallmark study by De Backer and associates [135] demonstrated that, among microcirculatory variables, PPV was the strongest predictor of outcome (receiver operating characteristic curve area 0.818, $P < 0.001$). Of importance, microcirculatory alterations in terms of PPV were less severe in the later than in the earlier phase of sepsis (74% vs. 63%,

$P = 0.004$) and, together with lactate levels, independent predictors of outcome in the early period of sepsis.

In the ProCESS trial, Massey and associates [147] demonstrated that, in patients with septic shock, microcirculatory parameters of vessel density were more significantly associated with mortality than parameters of flow alone (i.e., MFI and PPV), whereas no association was found between microcirculatory perfusion parameters at 72 h and mortality.

Other studies reported that microcirculatory alterations during septic shock improved over time only in survivors [122], even in the pediatric population [148].

8.9 Endothelial Dysfunction and Microcirculatory Alterations in COVID-19

The adverse effects of SARS-CoV-2 infection were initially considered to mainly affect the respiratory tract by causing pneumonia and acute respiratory distress syndrome (ARDS). Nevertheless, a strong evidence has highlighted that COVID-19 is associated with a significant risk of thrombotic complications, ranging from micro- to macrovascular thrombosis, both in venous and arterial districts, and in multiple organs. In particular, patients with severe COVID-19 frequently develop pulmonary embolism, deep vein thrombosis, stroke, and thrombosis in extracorporeal circuits [149]. Such complications are markers of severe form of the disease and portend an adverse prognosis.Accumulating evidence indicates that SARS-CoV-2 infection adversely affects the endothelium of the microcirculation by altering the integrity of vessel barrier, inducing endothelial inflammation and promoting pro-coagulative state [149, 150]. The characteristic hyperinflammatory and procoagulant state of COVID-19 implies a critical role of the vascular endothelium for two main reasons: first, the endothelium is the target organ of SARS-CoV-2, and virus entry and proliferation in ECs directly induce damage and apoptosis [151]; second, the endothelium is the main effector contributing to inflammatory process and thrombosis. SARS-CoV-2 may cause endothelial dysfunction directly through EC infection, or indirectly through the sustained and exaggerated activation of ECs secondary to viral infection [152].

8.9.1 The Assault on the Endothelial Cell by SARS-CoV-2

The SARS-CoV-2 accesses host cells via the binding of its spike glycoprotein to angiotensin-converting enzyme 2 (ACE2) [153, 154]. Cell invasion also depends on the presence of membrane protein called transmembrane protease serine 2 (TMPRSS-2), able to cleave the viral spike. Another pathway involves cathepsin L and cathepsin S, responsible for the endosomal pathway, a protease-independent virus cleavage [152, 155].

ACE2 is a type I transmembrane protein ubiquitously expressed in endothelial cells of several organs, with the highest levels in the cardiovascular system and lungs [156].

ACE and ACE2 are intimately linked to vascular physiology as a part of the renin-angiotensin-aldosterone system (RAS), which controls blood pressure by modulating vascular tone. ACE and ACE2 have different biochemical functions. ACE2 converts angiotensin I into angiotensin 1–9 which, in turn, is converted into angiotensin 1–7, a vasodilator, by ACE. ACE also converts angiotensin I into angiotensin II, which is a potent vasoconstrictor. Angiotensin II has also pro-inflammatory, prothrombotic, and other metabolic effects on the vasculature [157, 158]. Finally, ACE2 is an inhibitor of angiotensin II by processing angiotensin II into angiotensin 1–7.

In brief, whereas ACE/Ang I-II axis induces vasoconstriction, ACE2/Ang 1–7 axis counterbalances such effects, promoting vasodilation and reducing hypertension, and attenuating the pro-inflammatory and cardiovascular complications of angiotensin II [157].

The RAS is a complex cascade of vasoactive peptides controlling vascular tone (blood pressure), tissue perfusion, cardiac function, and fluid balance [159, 160]. SARS-CoV-2 infection causes the disruption of ACE2 function, shifting the balance of the RAS towards the pressor arm, triggering vasoconstriction, inflammation, and a procoagulant status (immunothrombosis) (Fig. 8.4).

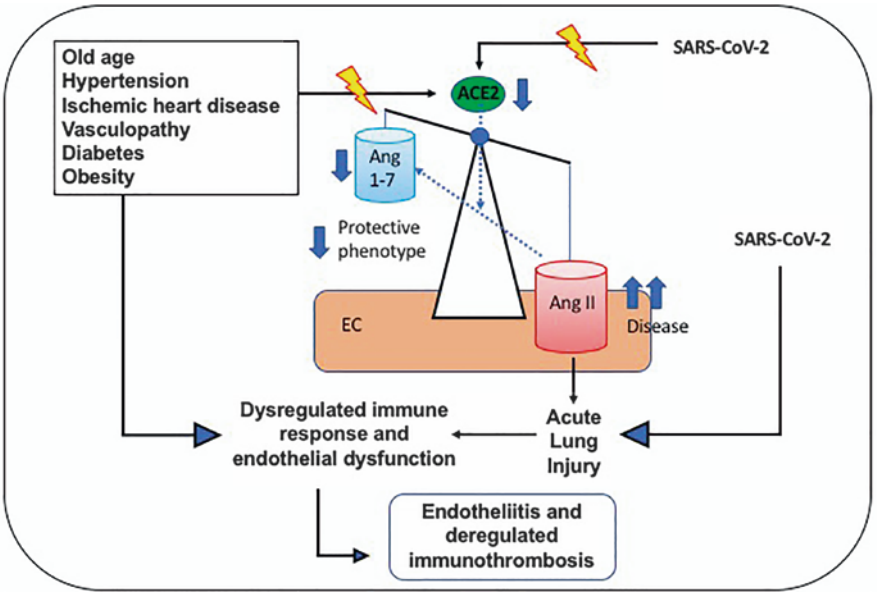

Fig. 8.4 Through the disruption of ACE2 function, SARS-CoV-2 unbalances the RAS towards the pressor arm, promoting vasoconstriction, inflammation, and a procoagulant status via the ACE/Ang I-II axis. The coexistence of SARS-CoV-2 infection and preexisting endothelial dysfunction (diabetes, obesity, heart disease, or aging) may further aggravate the impairment of ACE2 protective action and the detrimental effects of Ang II

ACE2 angiotensin-converting enzyme 2, *RAS* renin-angiotensin system, *Ang II* angiotensin II, *Ang 1–7* angiotensin 1–7

Accordingly, ACE2-deficient mice exhibit cardiovascular complications, including hypertension [161] and endothelial dysfunction [162]. Furthermore, the ACE2 protein has been shown to play an important role in protecting against some disorders such as cardiovascular complications, chronic obstructive pulmonary disease, and diabetes, among other COVID-19 comorbidities [163]. Notably, the difference in ACE2 expression levels also depends on factors such as lifestyle and age. There are evidences that ACE2 activity differs between males and females, with males having higher levels in the lungs [164]. ACE2 gene is under-expressed in the nasal mucosa of children younger than 10 years of age [165].

Several potential therapeutic approaches to address ACE2-mediated SARS-CoV-2 infection have been proposed, including *spike protein-based vaccine, blocking ACE2 receptor, inhibition of TMPRSS2 activity, and delivering excessive soluble form of ACE2 [166].*

SARS-CoV-2 directly infects engineered human blood vessel organoids [167]. There is also evidence of SARS-CoV-2 infection of vascular ECs in patients with severe COVID-19 [168]. As ACE2 is also widely present in extrapulmonary sites [169] the virus can enter and infect other organ tissues.

Varga and associates [151] described EC involvement in different organs, including the heart, lung, liver, and kidney, reporting evidence of diffuse endothelial inflammation with mononuclear cell infiltrates and intracellular viral inclusion, cell necrosis, as well as evidence of endotheliitis of submucosal vessels in different organs.

8.9.2 Pathophysiology of COVID-19

Following entry of the virus in the upper and lower respiratory tract, viral infection likely occurs first in alveolar type II epithelial cells, vascular endothelial cells, and immune cells in the lung. The replication and release of the virus from the infected alveolar cells result in a high form of inflammatory cell death (i.e., pyroptosis) [170]. SARS-CoV-2 infection via ACE2 binding critically unbalances the fragile equilibrium of the RAS towards a disease-promoting direction, namely inducing vasoconstriction, inflammation, and fibrotic remodeling [171]. The lung injury caused by the virus involves also the endothelium of the perialveolar capillaries inducing (or turning the preexisting EC dysfunction into) endotheliitis. Endotheliitis promotes alveolar edema formation due to vascular leakage resulting from the increased gaps between the inflamed EC. Furthermore, the disruption of vascular integrity and EC apoptosis lead to exposure of the thrombogenic basement membrane and the activation of the clotting cascade. Clogging by inflammatory cells and microthrombus formation in the alveolar capillaries worsens the ventilation-perfusion mismatch causing the impaired oxygen uptake by the lung. ECs release cytokines that further augment platelet production. Meanwhile, an overwhelming immune response and the associated massive cytokine release lead to further lung injury with the development of ARDS [172].

In normal physiology, the microvascular endothelium of the alveolar capillaries and precapillary arterioles may trigger hypoxic pulmonary vasoconstriction in response to alveolar hypoxia, a mechanism that provides protection against ventilation/perfusion mismatch in the lung by redistribution of blood flow [173]. In severe COVID-19, the deregulated immune response and coagulopathy lead to the high incidence of micro- and macrothrombosis observed in the lungs [174, 175], and other arterial and venous vessels [176]. The dysregulated pulmonary perfusion secondary to microcirculatory flow abnormalities in COVID-19 may explain the remarkable dissociation between the severe hypoxemia and the preserved lung mechanics in patients with L-type ARDS phenotype [177]. Indeed, when the hypoxic vasoconstriction response is impaired, increased intrapulmonary shunt increases, leading to marked arterial hypoxemia, as in severe COVID-19 patients.

The transfer or formation of microthrombi in the systemic circulation increases the risk of formation of deep vein thrombosis, which may further cause pulmonary embolism and stroke. The excessive cytokine release into the systemic circulation may also lead to vasculitis. In COVID-19, edema, inflammation, and microthrombi work together to cause ARDS. The suggested underlying pathophysiological processes are endotheliitis with subsequent endothelial dysfunction and immunothrombosis.

8.9.3 COVID-19-Induced Endothelial Dysfunction

SARS-CoV-2 may cause endothelial damage either through direct EC infection or indirectly by the milieu of pro-inflammatory cytokines elicited by the hyperactivation of the immune system, finally leading to widespread endothelial dysfunction.

Old age, hypertension, cardiovascular disease, diabetes, and obesity are the most prevalent comorbidities in COVID-19 patients [178, 179]. The mortality rate of COVID-19 patients without any documented comorbidities was 0.9% compared to 10.5% for patients with cardiovascular disease and 7.3% for patients with diabetes [179]. The high prevalence of such comorbidities in patients with severe COVID-19 suggests shared pathophysiological mechanisms. All the mentioned conditions, indeed, are characterized by a common feature: years of endothelial dysfunction [53], which may increase the susceptibility of the cells for infection by SARS-CoV-2 in such patient populations. Old age is characterized by a progressive dysregulation of the innate immune system (i.e., immunosenescence) leading to a persistent basal state of inflammation and delayed or impaired response to infections [180]. Furthermore, aging is accompanied by structural and functional modification of the vasculature, leading to progressive endothelial cell dysfunction. The ability of the aged ECs to maintain vascular homeostasis by production of NO or by sensing environmental inputs is significantly compromised [181].

Therefore, preexisting endothelial dysfunction is often present in patients who develop severe COVID-19 (Fig. 8.5). Such conditions, combined with the direct assault of SARS-CoV-2 on the vascular endothelium, may account for a high mortality in COVID-19 in this patient population.

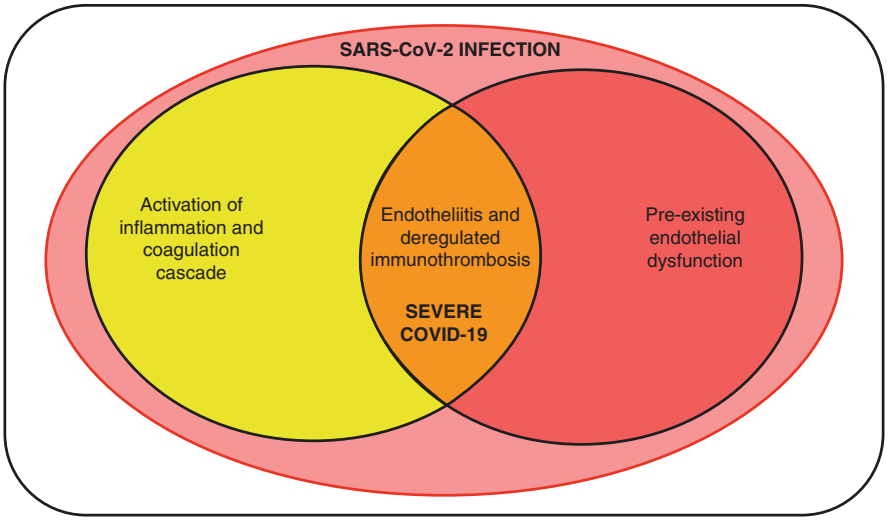

Fig. 8.5 The degree of preexisting endothelial dysfunction may represent a crucial condition for developing moderate-severe SARS-CoV-2 infection and its consequences

The pulmonary complications of SARS-CoV-2 infection result from a vascular barrier breach leading to interstitial edema, endothelial inflammation with deregulated inflammatory cell infiltration, and activation of coagulation pathways (immunothrombotic dysregulation).

8.9.3.1 Mechanisms of Endothelial Dysfunction in COVID-19

The underlying mechanisms for endothelial activation and dysfunction in COVID-19 may be summarized as follows:

1. SARS-CoV-2 directly infects ECs which therefore become dysfunctional and lyse and die [151]. Autopsy pathology describes the presence of the virus and disruption of pulmonary EC membranes [168].
2. The marked reduction of ACE2 expression on EC surface causes an imbalance in the RAS system, leading to an increase in Ang II plasma levels. Increased Ang II suppresses NO production which in turn triggers thrombogenicity due to leukocyte and platelet adhesion to the endothelium, and vasoconstriction [182]. Furthermore, Ang II also acts as a pro-inflammatory molecule via the activation of the ADAM17 [105]. ADAM17 is the first shedding protease to be identified, and is understood to play a role in the release of a diverse variety of membrane-anchored cytokines, cell adhesion molecules, receptors, and enzymes. ADAM17 cleaves IL-6R and TNF from EC membrane releasing them in their soluble active form [183]. In addition, Ang II promotes thrombosis, through a thrombin-dependent pathway [184]. Infection-induced reduction of ACE2 indirectly activates the kallikrein-bradykinin system, leading to leukocyte adhesion and complement activation [185]. Finally, the excessive Ang II increases the expres-

sion of PAI-1 in EC [186], which inhibits tPA and uPA, two proteins that mediate fibrinolysis. The increased PAI-1-to-tPA/uPA ratio is observed in COVID-19 and results in hypofibrinolysis, which likely leads to vascular microthrombosis and unresolved fibrin deposits in the alveoli [187, 188].

3. Oxidative stress plays an important role in promoting endothelial dysfunction through reduced NO bioavailability. Serum level of NO is decreased in patients with COVID-19, implying oxidative stress [189]. ROS-induced NF-kB signaling promotes expression of adhesion molecules, release of pro-inflammatory cytokines by the ECs, and increase of permeability with subsequent vascular leakage and formation of interstitial edema [190].
4. First-line pro-inflammatory cytokines IL-6R and TNFα, together with the virus itself, lead to ECs and macrophage activation causing a massive production of cytokines, triggering the acute and sustained inflammatory response, known as "cytokine storm" [191]. This cytokine-induced EC activation (type II activation) may result in the loss of the normal anti-inflammatory and antithrombotic functions of endothelial cells, leading to coagulation dysregulation, complement and platelet activation, leukocyte recruitment, and inflammation in the microvasculature. No single definition of cytokine storm is widely accepted. There is a disagreement about the distinction between cytokine storm and a physiologic inflammatory response. Recently, Fajgenbaum and June [192] proposed three criteria for its definition: elevated circulating cytokine levels, acute systemic inflammatory symptoms, and secondary organ dysfunction beyond that which could be attributed to a normal pathogen, if a pathogen is present.

   In COVID-19, serum levels of a number of cytokines are elevated above the normal range including, among others, IL-1β, soluble IL-2R and IL-2, IL-6, IL-8, Il-10, TNF, interferon-gamma, macrophage inflammatory protein 1α and 1-β, and VEGF [193–195].

   Higher IL-6 levels have been proposed as a signature predicting survival in COVID-19 [196] and severity of the disease [197, 198]. However, median IL-6 levels in severe COVID-19 are much lower than plasma levels typically reported in patients with ARDS [199].
5. Complement activation has been confirmed in the pathogenesis of COVID-19. Excessive complement activation may cause endothelial dysfunction and microthrombus formation [200].
6. C-reactive protein (CRP) plays a significant role in vascular inflammation. It can promote EC damage and apoptosis [201] and can suppress eNOS transcription, thus promoting EC dysfunction [202]. Finally, CRP has been shown to upregulate adhesion molecules and transcription of inflammatory genes [203]. In COVID-19, an increase in CRP is associated with poor prognosis [204].
7. Local lung hypoxia is likely to activate hypoxia-inducible factor (HIF)-1α which induces endothelial dysfunction and thrombosis [205].
8. The massive cytokine production and ROS generation associated with COVID-19 lead to glycocalyx degradation [206].

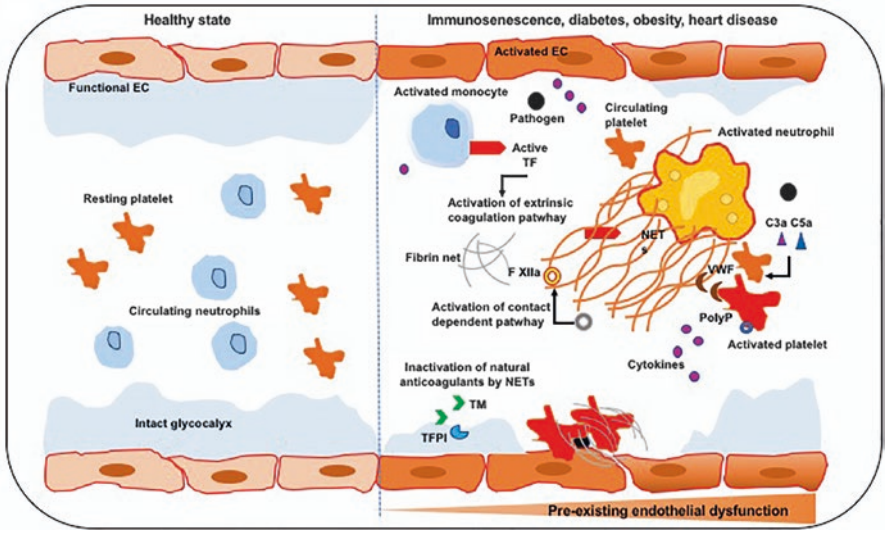

Fig. 8.6 In immunothrombosis, activated neutrophils and monocytes express and release TF at sites of pathogen localization, leading to activation of the extrinsic coagulation pathway (coagulation induced by inflammation).[208] The concomitant release of pro-inflammatory cytokines further recruits and activates neutrophils. Neutrophils contribute to immunothrombosis through the release of NETs. NETs are organized extrusions of the chromatin of mature neutrophils and exert many functions, including antibacterial and prothrombotic activity. NETs, indeed, directly activate factor XII, and thereby the contact-dependent coagulation pathway. NETs also bind VWF, thus promoting platelet recruitment and can directly activate platelets.[209] NETs are able to degrade endothelial glycocalyx components, such as TFPI and TM, making ECs highly thrombogenic. Platelets play a key role in this process. They directly bind to NETs and activate.[210] Upon activation, platelets secrete pro-inflammatory cytokines, such as IL-1β,[211] VEGF,[212] and PolyP from their dense granules, thereby activating the contact pathway and increasing fibrin generation. Through this process, invading pathogens become trapped and eliminated

8.9.4 Immunothrombosis Dysregulation

Engelmann and Massberg [207] first described an intrinsic effector pathway of innate immunity, termed immunothrombosis, triggered by pathogens and injured cells to limit the detrimental effects of invading pathogens (Fig. 8.6).

As long as immunothrombosis is controlled, it could be considered a beneficial mechanism of innate intravascular immunity. However, when uncontrolled, immunothrombosis causes a sustained and dysregulated activation of coagulation, leading to micro- and, ultimately, macrothrombosis, a phenomenon termed thromboinflammation [208, 209]. In an illuminating perspective, Bonaventura and associates [210] suggested that endothelial dysfunction and immunothrombosis represent the key pathogenic mechanisms in COVID-19. In this disease, immunothrombosis may explain both COVID-19-associated hyperinflammation and coagulopathy, which, along with endothelial dysfunction, well couples with the clinical picture of COVID-19-associated ARDS. Autopsy studies have revealed co-localized

thrombosis and inflammation within the pulmonary vasculature of COVID-19 patients [211]. Mitchell [212] identified the convergence of thrombosis and inflammation (thromboinflammation) in COVID-19-associated lung injury. Thromboinflammation occurs commonly in a broad range of human disorders. Microvascular thrombosis with associated inflammation is well recognized in the context of sepsis and ischemia-reperfusion injury [213]. From an evolutionary standpoint, thrombosis and hemostasis are connected. Thrombosis evolved as a part of the innate immune system as a means of isolating invading pathogens [212]. Platelets play a role both in the immune system by binding microorganisms and activating neutrophils to produce neutrophil extracellular traps (NETs) [214] and in the hemostatic-coagulative system.

Activation of coagulation and inflammation converge in COVID-19 [212]. Platelets, neutrophils, and coagulation cascade team up to contain the virus. Nevertheless, when deregulated, this process can aggravate tissue damage by inducing vessel occlusion and hypoxia [215]. Central to exaggerated immunothrombosis or thromboinflammation is the loss of the normal antithrombotic and anti-inflammatory functions of ECs, leading to dysregulation of coagulation, complement and platelet activation, and leukocyte recruitment in the microcirculation [213]. Preexisting endothelial dysfunction due to risk factors (old age, diabetes, obesity, hypertension) makes the endothelium more vulnerable to SARS-CoV-2 assault, offering the virus a breeding ground to be poorly controlled by the normal host defense mechanisms. In COVID-19, the tri-cellular aggregate EC-platelet-leukocyte may thus represent the "guilty unit" of microcirculatory perfusion alterations in the lungs and other organs (Fig. 8.7). COVID-19 histopathological studies revealed the presence of mononuclear cell infiltrates around occluded capillaries and neutrophils trapped within the fibrin-thrombi. The thrombotic bulk in the pulmonary microcirculation is rich in inflammatory cells, platelets, and NETs, a sign of thromboinflammatory burst. Postmortem and in vivo (see later) demonstration of microvascular dysfunction in severe COVID-19 patients strongly confirms that the events leading to atypical ARDS in this setting consist of "microvascular COVID-19 lung vessel obstructive thromboinflammatory syndrome" (MicroCLOTS) [216].

8.9.4.1 Triggers for Immunothrombosis Deregulation

- *Damage/dysfunction of pulmonary vascular endothelium*: The severe damage inflicted by SARS-CoV-2 to the endothelium compromises its normal antithrombotic functions by disrupting fundamental mechanisms, including PGI2 and NO (inhibitors of platelet activation), TFPI, and the protein C-activator thrombomodulin. The exposure of TF by the destroyed endothelial layer triggers thrombin generation. Of importance, TF expression is also upregulated by HIF.
- *Thrombin burst*: Thrombin is the cornerstone of the coagulation cascade. Thrombin is primarily generated by tissue factor (TF) exposed after EC damage, but is also released by activated monocytes (blood-borne TF) during inflammation. Hence, TF is a key driver of immunothrombosis. Once generated, thrombin plays a role in thrombosis by activating platelets with granule release and expression of phospholipids (contact pathway activation); it activates EC

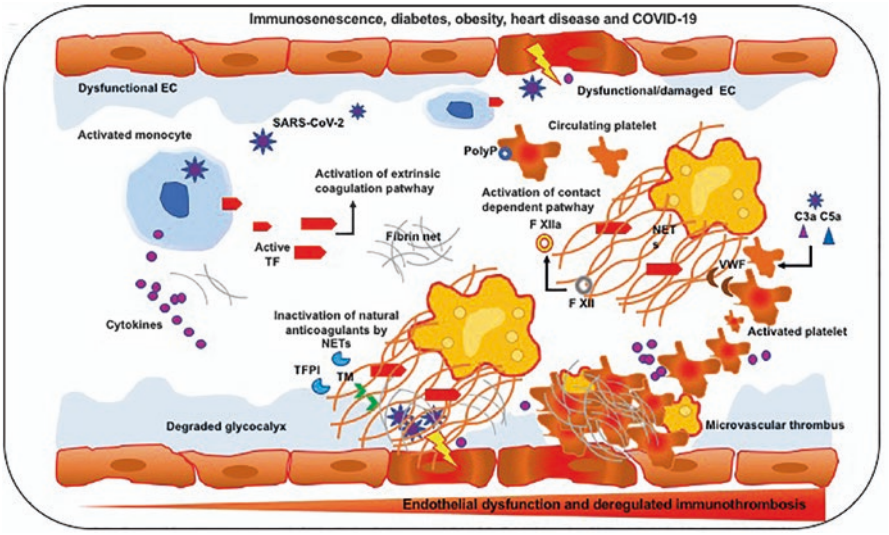

Fig. 8.7 The crossroads of inflammation and coagulation in severe COVID-19. Preexisting endothelial dysfunction due to risk factors (aging, diabetes, obesity, hypertension) makes the endothelium more vulnerable to SARS-CoV-2 assault. In these conditions, endothelial dysfunction and exaggerated immunothrombosis may lead to sustained and dysregulated activation of coagulation, resulting in hyperinflammation and microthrombosis. *ECs* endothelial cells, *TF* tissue factor, *NETs* neutrophil extracellular traps, *VWF* von Willebrand factor, *TFPI* tissue factor pathway inhibitor, *TM* thrombomodulin, *IL-1β* interleukin-1β, *VEGF* vascular endothelial growth factor, *PolyP* polyphosphate

through protease-activated receptor-1 (PAR1) cleavage leading to VWF release from WPB. Finally, thrombin generates fibrin by fibrinogen proteolysis. In its inflammatory role, thrombin stimulates expression of P-selectin on EC surface, thus promoting leukocyte recruitment and adhesion. In turn, the activated leukocytes release multiple inflammatory cytokines. Ranucci and associates [217] reported that severe patterns of COVID-19 ARDS are characterized by increased thrombin generation. At follow-up, thrombin generation was significantly reduced in survivors, whereas it increased in non-survivors. In severe COVID-19 patients, Jin and associates [218] found a high thrombin activity reflected by the high levels of thrombin-antithrombin complexes. In COVID-19 the importance of thrombin in the development of thromboembolic complications has been widely described, although no uniform treatments with anticoagulants (i.e., heparin) to contrast it have been established yet.

– *Hypofibrinolytic state:* A number of studies identified impaired fibrinolysis in COVID-19 patients [217, 219–221]. Fibrinolysis shutdown together with thrombin burst appears to play an important role in the COVID-19-associated coagulopathy and in the development of macro- and microthrombosis.
– *Platelet activation*: The major activator of platelet is thrombin, and platelet activation underlies thrombus formation. Platelets support the thrombotic process by

activating the contact pathway through polyphosphate expression. In addition, platelets are activated by C3a and C5a of the complement system [222].
- Beyond hemostasis, activated platelets also produce multiple inflammatory cytokines, which recruit further leukocytes to the growing thrombi. Furthermore, platelets bind, activate, and induce leukocytes to form NETs.
- *The role of NETs*: NETs appear to play an important role in deregulated immunothrombosis [223]. NETs are both pro-inflammatory and thrombogenic as they trap TFPI on the surface of ECs, thereby inhibiting their normal fibrinolytic activity. Their blood levels are markedly increased in COVID-19 ARDS patients [211, 224]. Middleton and associates [223] identified a NET formation increase in COVID-19, supported by autopsies which confirmed NET-containing microthrombi with neutrophil-platelet infiltration in the lungs, heart, and kidney. Therefore, NET-induced immunothrombosis may represent a target for therapeutic interventions.

8.9.4.2 Medical Interventions for Immunothrombosis

Since the mechanisms underlying immunothrombosis dysregulation in COVID-19 are multifactorial, potential therapeutic approaches should target both the components (i.e., antithrombotic drugs and anti-inflammatory drugs). Several trials addressing safety and efficacy of such treatments are in progress [210].

8.10 Biomarkers of Endothelial Damage and Immunothrombosis in COVID-19

Increased numbers of circulating ECs have been described in severe COVID-19 patients, and their presence correlated with soluble vascular cell adhesion molecule and soluble intercellular adhesion molecule, supporting the presence of endothelial dysfunction [225].

Increased levels of endothelial glycocalyx degradation products, such as syndecan-1 and hyaluronic acid, have been demonstrated in COVID-19 patients [226, 227]. Stahl and associates [206] found increased syndecan-1 and sTie-2 concentrations in the blood, indicating the shedding of important structural components of the glycocalyx, thus confirming endothelial injury in COVID-19. In a cohort of 20 COVID-19 patients, Potje and associates [228] demonstrated increased plasma concentrations of IL-6 and IL1-β, associated to increased lipid peroxidation and glycocalyx components compared to plasma from healthy subjects. Furthermore, plasma from COVID-19 patients induced glycocalyx shedding in cultured human umbilical vein endothelial cells and disrupted redox balance, and such perturbations were inhibited by treatment with low-molecular-weight heparin.

Before COVID-19, biomarkers of endothelial injury have been investigated in the setting of sepsis and ARDS. VWF antigen, Ang-II, and biomarkers of inflammation, especially IL-8 and soluble TNF receptor, identified septic patients with a higher mortality [229].

In a cohort of 68 COVID-19 patients, Goshua and associates [230] demonstrated that endothelial and platelet activation markers were significantly elevated in intensive care unit (ICU) compared with non-ICU COVID-19 patients, including VWF antigen (565% in ICU patients vs. 278% in non-ICU patients; $P < 0.0001$) and soluble P-selectin (15.9 ng/mL vs. 11.2 ng/mL; $P = 0.0014$). In this study, mortality significantly correlated with VWF antigen ($r = 0.38$; $P = 0.0022$) and soluble thrombomodulin ($r = 0.38$; $P = 0.0078$) among all patients. Increased levels of VWF have been reported in both critical and noncritical COVID-19 patients [231–233]. Endothelial damage may lead to abnormally high levels of VWF which can exceed the VWF-cleaving protease ADAMTS-13 activity, resulting in the formation of large VWF multimers [234, 235]. The relative deficiency of ADAMTS-13, due to reduction of cleavage activity or degradation, is described in severe inflammatory conditions with high levels of IL-6 [236] as well as in COVID-19 patients [237] and has been associated with poor prognosis. In COVID-19, the relative reduced activity of ADAMTS-13 may lead to insufficient cleavage of the already increased VWF resulting in enhanced platelet-vascular wall interaction causing thrombotic microangiopathy. Rovas and associates [238] measured circulating levels of a variety of endothelial and glycocalyx-associated markers, together with the assessment of glycocalyx dimension and red blood cell velocity by SDF in the sublingual microcirculation. Several markers of endothelial dysfunction were increased and correlated with disease severity in COVID-19. In particular, mechanically ventilated COVID-19 patients showed higher (i.e., thinner glycocalyx layer) values of perfused boundary regions (PBR), a reliable estimate of glycocalyx damage, compared to less severe, non-ventilated patients. In accordance, highly elevated plasma levels of hyaluronic acid and syndecan-1 were markedly increased with the need for mechanical ventilation in COVID-19 patients. In the same study, circulating levels of TM and ADAMTS-13 significantly increased and decreased with disease severity, respectively. The vasodilating and permeability factor VEGF-A resulted to be markedly increased in COVID-19 patients and correlated with disease severity. Finally, PBR (AUC 0.75, $P = 0.01$), ADAMTS-13 (von Willebrand factor-cleaving protease; AUC 0.74, $P = 0.02$), and VEGF-A (AUC 0.73, $P = 0.04$) showed the best discriminatory ability to predict 60-day in-hospital mortality. In another study [239], VEGF-D which promotes angiogenesis and lymphangiogenesis [240] was identified as the most important indicator related to the severity of COVID-19.

Mancini and associates [241] observed a moderate ADAMTS-13 reduction in the more severe COVID-19 cases, with about one-third of patients in the high-intensity care unit presenting ADAMTS-13 activity levels below 50 IU/dL and VWF antigen levels above 150 IU/dL, thereby confirming an important prothrombotic status. Furthermore, the authors found a significant increased VWF antigen-to-ADAMTS-13 activity ratio, strongly associated with COVID-19 severity ($P < 0.001$). The imbalance in VWF/ADAMTS-13 axis may enhance the hypercoagulable state in COVID-19 and heightens the risk of microthrombosis.

Higher levels of soluble P-selectin, a marker of endothelial and platelet activation, were observed in severe COVID-19 ICU patients than in non-ICU patients,

whereas increased levels of thrombomodulin have been associated with increased mortality risk [230].

In addition, thrombin-antithrombin complexes and prothrombin fragments 1.2, both sensitive markers of thrombin generation and microthrombosis, are significantly higher in severe COVID-19 [217].

NETs are markers of disease severity in COVID-19 [223, 224]. Compared with controls, COVID-19 patients have higher levels of myeloperoxidase-DNA complexes, which are biomarkers of circulating NETs. In accordance, Leppkes and associates demonstrated that markers indicating NET turnover are consistently increased in COVID-19 and that such NETosis is linked to disease severity [242].

Smadja and associates [243] measured circulating levels of Ang II, creatinine, D-dimer, and CRP at admission in 40 consecutive COVID-19 patients and found angiopoietin 2 as the best predictor for ICU direct transfer and ICU outcome.

All the aforementioned findings strongly support the concept that endothelial dysfunction is central to COVID-19, and reinforce the hypothesis of a COVID-19-associated endotheliopathy and immunothrombosis leading to microcirculatory dysfunction.

8.11 Microcirculatory Dysfunction in COVID-19: The Evidence

Endothelial dysfunction seems to be the central unifying event in the pathogenesis of COVID-19. Endothelial dysfunction may be both the cause or the result of the hyperinflammation, coagulopathy, and hypoxemia, in a vicious, self-perpetuating circle. Such endotheliopathy may cause microcirculatory dysfunction in many organ systems throughout the body, and could underlie the deterioration of oxygen transport in the microcirculation of COVID-19 patients. Postmortem studies from COVID-19 patients revealed regular presence of widespread microthrombosis, capillary congestion, and areas of increased capillary density in different organ systems [168, 244, 245]. The relevance to investigate the nature of microcirculatory alterations induced by COVID-19 has been understood. To date, however, a limited number of studies investigated the sublingual microcirculation in COVID-19 patients [238, 246–252]. A first evaluation conducted by Damiani and associates [246] in 12 patients with COVID-19 pneumonia showed that sublingual microvascular capillary densities were inversely correlated with D-dimer levels, suggesting the impact of microthrombosis on the microcirculatory function. Rovas and associates [238] conducted a comprehensive analysis of sublingual microcirculation in 23 moderate-to-critical COVID-19 patients, compared to 15 healthy controls. They characterized and quantified microcirculatory alterations by assessment of both SDF imaging and endothelial and glycocalyx markers of dysfunction. Small capillary (4-6 μm) density and RBCv were reduced, compared to controls. Then, COVID-19 patients on mechanical ventilation showed significantly higher PBR values compared to non-ventilated patients (2.44 μm vs. 2.16 μm, $P = 0.002$) and controls (2.44 μm vs. 2.24, μm, $P = 0.008$), respectively. High levels of

circulating markers' glycocalyx shedding, such as syndecan-1 and hyaluronan, corroborated such findings. In a prospective observational study [249] Kanoore Edul and associates demonstrated that sublingual microcirculation in severe COVID-19-ARDS patients was characterized by decreases in PPV (0.96 ± 0.03) and flow quality (MFI: 2.79 ± 0.10 and RBCv: 1124 ± 161 μm/s) along with high vascular densities (TVD: 21.9 ± 3.9 and PVD: 21.0 ± 3.5 mm/mm^2), compared to normal values [136]. In a multicenter study including 38 mechanically ventilated COVID-19 patients with moderate-to-severe ARDS [250] Favaron and associates explored the sublingual microcirculation and found that COVID-19 patients showed elevated values of TVD, FCD, capillary hematocrit, capillary-to-systemic hematocrit ratio, and RBCv in comparison with the microcirculatory parameters of healthy volunteers. In addition, they reported normal values of PPV. Interestingly, such microcirculatory compensatory effects were present in less compromised COVID-19 patients as assessed by the SOFA score, and were absent in patients with SOFA scores ≥10. Finally, the authors found increased numbers of leukocytes and RBC aggregates in the microcirculation, which are likely related to the virus-induced inflammation and hypercoagulability.

The reported increased capillary density in COVID-19 is consistent with the action of a microcirculatory compensatory mechanism to increase oxygen extraction, in reaction to the hypoxemia secondary to the COVID-19-associated hyperinflammation and hypercoagulatory states.

In physiologic conditions, nearly 30% of microvessels are shut but can be recruited under the condition of increased oxygen requirements, as happens during ascent to high altitudes [253].

The hypoxemic state, indeed, is a well-recognized powerful trigger for capillary recruitment and also angiogenesis [253, 254]. Angiogenesis has been described in severe COVID-19 patients [168, 255]. Therefore, the reported increased capillary density is likely to be an adaptive response to hypoxemia, a physiologic compensatory reaction to augment the oxygen-extraction capacity by decreasing diffusion distances in the microcirculation. Last, microthrombosis is another well-known stimulus for vascular growth [243], accounting for both the increased capillary density and the impaired microcirculatory flow.

Of great importance is that the reported microcirculatory compensatory mechanisms to hypoxemia in COVID-19 disagree with the microcirculatory alterations reported in conventional sepsis. Here, both the components of microcirculatory oxygen delivery (capillary density and quality of flow parameters) are impaired. Furthermore, to date, evidence of loss of hemodynamic coherence between micro- and macrocirculation in severe COVID-19 patients is weak, as most of the patients did not show signs of conventional sepsis or shock, at least at the time of microcirculation assessments.

In the light of current evidences in literature, the sublingual microcirculatory alterations in COVID-19 are discrepant. Such differences in the results deserve some considerations: (i) the different technologies (SDF or IDF imaging) and the methods used for analysis of microcirculatory variables (manual, semiautomated, or software-assisted analysis) may lead to nonhomogeneous results, drawing

discordant conclusions between studies; (ii) the heterogeneity regarding the timing (time gap between diagnosis/ICU admission) of measurements between studies and within patients in the same study may catch some crucial findings while losing others; (iii) the different inter- and intra-study characteristics of patient population in terms of preexisting risk factors, and stage of COVID-19 in terms of severity of hypoxia at the time of microcirculation assessment, may be a bias; and (iv) the differences in sample size/power between studies are of paramount impact on interpreting the results.

Focusing on the role of microcirculatory dysfunction in the development of organ damage in COVID-19 shares with sepsis a common goal: the development of targeted medical approaches and tailored interventions aimed to limit endothelial damage and, thus, progression of this treacherous disease.

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Clinical Manifestation of COVID-19-Associated Coagulopathy

9

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9.1 Introduction

COVID-19 infection primarily causes pneumonia and respiratory failure, but also damage to other organs. This is likely related to thrombo-inflammation. In fact, tissue factor is activated by the inflammatory stimulus, and it is thought to act as the trigger factor for activation of the coagulation cascade which, if uncontrolled, causes endothelial injury and microvascular clot formation. Similar RNA viruses such as Ebola virus, Lassa, and dengue fever virus cause coagulopathy with a marked tendency to hemorrhagic signs. On the contrary, coronaviruses do not cause hemorrhagic complications that often. During the severe acute respiratory syndrome in 2002 caused by SARS-CoV-1, altered platelet count and prolonged aPTT were reported, but few bleeding complications [1, 2]; on the contrary, a considerable number of patients developed deep vein thrombosis and pulmonary embolism [3]. In this chapter we review the clinical presentations of COVID-19-associated coagulopathy that the clinician should be aware of during the management of this disease.

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9.2 COVID-19-Associated Thrombosis

Venous thromboembolism (VTE) is the prominent feature of COVID-19 coagulopathy. The clinical presentation of VTE includes both deep vein thrombosis (DVT) and pulmonary embolism (PE) which occur when a clot migrates from the vein wall (usually from a leg) and travels to the pulmonary circulation [4]. VTE is a well-known complication in hospitalized patients [5] where all the pathogenetic mechanisms described by Virchow in 1884 coexist: stasis, blood hypercoagulability, and endothelial cell dysfunction and inflammation. Especially this very last feature is typical of COVID-19, where the so-called cytokine storm appears to be responsible for the thrombo-inflammatory responses and subsequent tissue injury that drive the prothrombotic changes resulting in microvascular thrombosis and overt VTE [6].

The coagulation alterations of COVID-19 are reflected by elevated fibrinogen and D-dimer [7, 8] which, respectively, represent the substrate of thrombus formation and its ultimate dissolution. In the context of an acute inflammatory response, however, the elevation of D-dimer can also be traced back to the inflammation itself, as can be speculated by some reports that highlight the coexistence of high D-dimer and hypofibrinolysis in viscoelastic tests [9].

Concerning the broader concept of VTE, its prevalence in the COVID-19-hospitalized population is confirmed to be high, and most importantly it is thought to add up a five- to sixfold higher risk of death [10, 11]. Of note, we must keep in mind that the incidence of VTE may be flawed by the screening method. A systematic review and meta-analysis on 66 studies estimated the overall VTE prevalence at 14.1% (95% confidence interval [CI] 11.6–16.9) that increased to 40.3% (95% CI 27.0–54.3) in studies where ultrasound screening was routinely performed, while it resulted to be only 9.5% (95% CI 7.5–11.7) without screening [12]. Another meta-analysis on 48 studies found that the pooled incidence of VTE was 17.0% (95% CI, 13.4–20.9); again, when diagnosis was made with systematic screening the incidence rose to 33.1% and was only 9.8% in studies based solely on clinical diagnosis [13]. Also in view of the above reason, it is difficult to make comparisons with the pre-COVID era in which the prevalence of VTE in ICU ranged from 5.4 to 23.6% in the presence of prophylaxis and from 13 to 28% in the absence of prophylaxis [14].

From studies with a control group, nonetheless, we can affirm that COVID-19 increases the risk of VTE. In a study by Helms et al. conducted in four French ICUs the authors compared the occurrence of thromboembolic events in COVID-19 ARDS patients with non-COVID-19 ARDS subjects using propensity-matched techniques: they found that the COVID-19 studied population developed significantly more thrombotic complications, which mainly consisted of pulmonary embolism (11.7 vs. 2.1%) [15].

9.2.1 Deep Vein Thrombosis

The incidence of DVT in COVID-19-hospitalized patients is convincingly noteworthy and confirmed by autoptic studies that revealed DVT in 58% of the patients in whom venous thromboembolism was not suspected before death [16]. Cui et al. reported, in a series of 81 patients, a prevalence of DVT of 25%; remarkably, thromboprophylaxis was not the standard of care in that study [17]. However, the high prevalence of DVT was confirmed in later reports even during the administration of thromboprophylaxis. Ierardi et al. in a series of 234 patients described an overall incidence of DVT of 10.7% and 13.8% in critically ill patients. This trend towards a higher prevalence of DVT in more severe patients is confirmed by Middeldorp et al., who described an overall prevalence of DVT of 13%, which rose to 32% in the intensive care unit (ICU) population (proximal leg DVT was again the most frequent clinical presentation in that study) (10). At last, Lodigiani et al. found a cumulative rate of thrombosis of 21% (6.6% in the general wards and 27.6% in the ICU) [18], which was also confirmed by a recent meta-analysis [13].

9.2.2 Pulmonary Embolism

What is more striking is the high incidence of pulmonary embolism in COVID-19 patients. A meta-analysis on 27 studies found a pooled incidence rate of PE of 16.5% (95% CI: 11.6, 22.9) which was more frequently found in patients who were admitted to ICU (24.7% vs. 10.5% in those not admitted to ICU). Remarkably, DVT was present only in 42.4% of patients with PE, indicating that more than half of the patients with PE lacked signs of ongoing DVT [19]. Of note, this is confirmed in postmortem studies in which the thrombosis of small and midsized pulmonary arteries is found in various degrees in the absence of clinical suspicion of VTE antemortem [20].

In a study by Klok et al. conducted in ICU, the estimated cumulative incidence of a composite outcome of symptomatic PE, DVT, ischemic stroke, myocardial infarction, and/or systemic arterial embolism was 49% (95% CI 41–57%) after a median follow-up of 14 days [11]. In that study the majority of thrombotic events were PE (87%) despite the fact that all patients received systematic pharmacological thromboprophylaxis. Interestingly, 29% of the cases of PE were limited to subsegmental arteries and did not involve segmental or more proximal arteries. This, along with autopsy findings of thrombosis and microangiopathy in the small vessels and capillaries of the lungs [21], has led some authors to question whether the observed pulmonary vessel occlusions are “emboli” that develop due to the arrival of DVT thrombus in the arterial lung vasculature (pulmonary embolism) or rather local thrombi that may form in the lung vessels as a consequence of strong activation of inflammatory processes (pulmonary thrombosis) resulting in pulmonary

endothelial dysfunction and damage [22–24]. Interestingly, this could have a practical therapeutic consequence because if the latter hypothesis is confirmed, to prevent pulmonary thrombosis not only anticoagulants are needed but maybe also anti-inflammatory drugs.

9.2.3 Diagnosis of COVID-19-Associated Thrombosis

The diagnostic workup of suspected VTE usually includes the sequential application of a clinical decision rule and D-dimer testing [4]. In the presence of a normal D-dimer further assessments may be safely withheld due to the high negative predictive value. However, in the COVID-19 context this might not be valid because D-dimer is almost always elevated due to the very high degree of inflammation. In fact, Dujardin et al., in a study on 127 ICU patients, reported that the accuracy of D-dimer in predicting VTE is high only when combined to another marker of inflammation as the C-reactive protein (AUC of 0.83, $P < 0.05$) [25]. The predicted probability of VTE with a D-dimer >15 μg/mL in combination with a CRP > 280 mg/dL was 98%. Remarkably, the ability of a D-dimer level of <3.0 μg/mL to rule out VTE (negative predictive value) in this study was only 67%. Indeed, due to this limit, D-dimer alone is not recommended to guide clinical practice for VTE diagnosis in COVID-19 [26, 27]. Guidelines recommend the use of non-contrast-enhanced thorax computed tomography (or high-resolution computed tomography—HRCT) for the diagnosis, severity assessment, and follow-up of COVID-19 infection [28]; however, computed tomography pulmonary angiography (CTPA) is required to confirm the diagnosis of VTE. In some conditions (e.g., renal failure) the use of contrast might be contraindicated, but unfortunately scintigraphy is not a valid alternative in severe patients because of high false-positive results. In fact, it may hold diagnostic value only in case it deploys a perfusion defect that is located in different sites from CT findings of focal opacities. A valid alternative to the abovementioned techniques in severe patients is the versatile use of bedside echography, which can diagnose a VTE using color Doppler; at the same time, B-mode observation of the dilation of the right heart chambers (or other indirect measures of VTE) can suggest PE.

In conclusion, VTE is a common clinical presentation of COVID-19 coagulopathy and is plays a role in patient outcome. In this context, coagulation markers like D-dimer may be of scarce clinical support for the diagnosis of VTE because of their low specificity. Thus, a complete clinical assessment is required to carry out an accurate diagnosis. Further understanding of COVID-19 pathogenesis will clarify which therapeutic option might show the highest benefit.

9.2.4 Arterial Thrombosis

Another clinical manifestation of COVID-19 coagulopathy—which may contribute to considerable morbidity and mortality—is represented by arterial thrombosis. This type of thrombosis traditionally occurs in patients with cardiovascular risk

factors. In COVID-19 this may share another mechanism more specifically related to the inflammation and the host immune response induced by the virus itself. Arterial thrombosis has been observed in other viral infections [29] but the prevalence in the COVID-19 setting, even in the presence of thromboprophylaxis, appears to be higher, as it is the risk associated with death [30].

Although in COVID-19 a causal relation between the viral infection and arterial thrombosis cannot be precisely established, a rather consistent number of patients present at the hospital with an arterial thrombosis or they develop this complication during the hospital course, with an estimated prevalence of 11%. A retrospective study on 3334 consecutive hospitalized COVID-19 patients in four hospitals in New York City reported that 533 (16.0%) patients developed any type of thrombosis of which the majority (365 patients, 11.1%) were arterial (mainly myocardial infarction, and few ischemic strokes and systemic thromboembolism) with a higher risk in ICU patients [31]. A lower proportion of arterial events were reported in other studies. In a cohort study in a tertiary hospital in Lombardy (Italy), thromboembolic events occurred in 28 of 362 patients (7.7%): ischemic stroke was diagnosed in 9 (2.5%) and acute coronary syndrome/myocardial infarction was diagnosed in 4 (1.1%) patients. Interestingly, for the majority of these patients this represented the primary reason for hospitalization [18]. In another study performed in three Dutch hospitals of 184 ICU patients, 68 patients developed VTE but only 7 arterial thrombotic events (5 ischemic strokes and 2 systemic arterial embolism) [11]. Similarly in a large cohort of 1419 COVID-19 patients treated in a university hospital in Madrid, Spain, only 14 patients (1%) developed a systemic arterial thrombotic event (3 acute coronary syndromes and 8 cerebrovascular events) [32]. In another retrospective study in a single hospital in Paris of 531 COVID-19 patients admitted in 1 month, only 30 (5.6%) experienced arterial thrombotic events [30]. This study introduces another important aspect of arterial thrombosis in COVID-19, that is, the atypical presentation patterns that in that case were thrombosis of the aorta, upper limb, or renal arteries or cerebral micro-vasculopathy in 7 (23.3%) of the cases. Several other cases of atypical presentations are reported, such as the occlusion of radial artery catheters [33] or peripheral artery extremity occlusion [34], and one study confirmed that COVID-19 is highly associated with catheter-related thrombosis [35].

As mentioned above the mechanism of COVID-19 arterial thrombosis has not been determined. COVID-19 is considered a systemic vascular disease affecting multiple organs. In fact, SARS-CoV-2 targets the angiotensin-converting enzyme 2 host receptor to enter the endothelial and epithelial cells and leads to endotheliitis [36]. The consequent vasculitis caused by the immune complexes inside the smooth muscle cells of blood vessels is supposed to induce a severe inflammatory state exacerbated by a cytokine release syndrome. However, whether these changes are the result of a viral cytopathic process or of an autoimmune reaction to the infection is still unclear [37]. Also, myocardial injury has been observed in COVID-19, which might be attributed to endotheliitis of small epicardial and intramyocardial vessels; on the contrary, the direct peri/epicardial nerve injury and consecutive inflammatory cardiac neuropathy caused by the virus could explain arrhythmias [38].

Another mechanism involved in arterial thrombosis could be platelet activation. Platelets are essential to hemostasis; they are implicated in thrombosis and they also contribute to vascular inflammation [39]. Moreover, they are a major source of inflammatory mediators and in the context of viral infection [40] they can interact with microbes and viruses as well. Although the platelet count in COVID-19 patients is rarely diminished, it has been shown that their platelet thrombus formation is altered [41]. A study by Zaid et al. showed that platelets were hyper-activated, contained SARS-CoV-2 RNA molecules, had enhanced adhesion properties, and were a source of inflammatory cytokines in patients with COVID-19 [42]. Whether administering antiplatelet agents to COVID-19 patients may be beneficial is debated with promising results from some studies [43, 44] and no benefit from others [45]. However, larger studies are required to better understand the role of platelets in this context.

Lupus anticoagulant and antiphospholipid antibodies may be frequent in patients with COVID-19, and this may in part explain arterial thrombosis [46, 47]. However, antiphospholipid antibodies are common in the general population [48] and false-positive lupus anticoagulant testing may be found in patients with COVID-19 given the marked elevation in C-reactive protein levels seen in patients with significant pulmonary or systemic inflammation.

In summary, arterial thrombosis is one of the clinical manifestations of COVID-19 coagulopathy. It might affect patients that have preexistent cardiovascular risk factors, but the virus itself may act as a second hit and exacerbate the clinical manifestations. Attention must be paid to atypical presentation like ischemia of cannulated vessels; thus the adequacy of perfusion after catheterizations should be meticulously checked.

9.2.5 Disseminated Intravascular Coagulation

Disseminated intravascular coagulation (DIC) has been reported as a clinical presentation of COVID-19. According to ISTH 2001 definition, DIC is “an acquired syndrome characterized by the intravascular activation of coagulation with loss of localization arising from different causes that can originate from and cause damage to the micro-vasculature, which if sufficiently severe, can produce organ dysfunction” [49]. In the presence of a clinical condition known to be associated with DIC, the diagnosis is made assigning a score to the various alterations of coagulation markers (basically a decrease of platelets and/or fibrinogen and an increase of PT and/or aPTT and D-dimer): in case the score is higher or equal to 5, the definition of overt DIC is met. To identify DIC at an earlier phase, a new definition of DIC induced by sepsis has been recently proposed (SIC) which excluded the evaluation of D-dimer and fibrinogen, while included the SOFA score in the calculation [50]. As a matter of fact, the coagulation alterations that are observed in COVID-19 comprise prolongation of PT and aPTT and a strong increase in D-dimers, but platelets are generally not affected: this is the reason why a definitive diagnosis of DIC is seldom met. A study published at the beginning of the pandemic on 183 consecutive

patients with a COVID-19 pneumonia reported a rather low mortality (11.5%) than later reports, but DIC appeared in most of the deaths [51]. The authors found that 71.4% of the non-survivors met the ISTH DIC criteria for overt DIC (with D-dimers and PT prolongation as the main contributors to the DIC score), while only 0.6% of the survivors met the criteria. Interestingly, the authors studied the dynamic changes and found that D-dimers in particular increased along the course of the disease in non-survivors. However, DIC in COVID-19 has not been frequently reported in later studies. In the work by Deng et al., only 6.4% of patients who died met the ISTH DIC criteria [52], and in another study only 2.1% of the patients [18]. A recent paper on 150 severe COVID-19 patients in ICU, despite an elevated number of thrombotic complications, reported zero cases of DIC [15]. Trajectories of COVID-19 coagulation markers were also analyzed by Paparella et al. [53], but none of the studied patients met the criteria for overt DIC during the ICU stay. D-dimer and fibrinogen levels were very elevated but, on the contrary, platelets, PT, and PTT were within the normal range; moreover, their trajectories over the whole follow-up did not show any sign of consumption. As noted above, thrombocytopenia is not a significant finding, at least initially, in COVID-19 [54]. Supporting this evidence that COVID-19 coagulopathy is rarely a consumptive coagulopathy (probably only in the late irreversible stage of the disease), Huang et al. reported a platelet count of less than 100×10^9/L in only 8% of ICU and 4% in non-ICU patients at admission [55]. Yin et al. compared the platelet count between COVID-19-associated ARDS patients and non-COVID-19 ARDS patients and reported minor clinical differences in platelet counts [56].

Independently by the available DIC scores, COVID-19 coagulopathy goes hand in hand with the severity of the disease and it is associated with organ dysfunction and higher mortality [8]. The pathogenesis of coagulation alteration is still under investigation, but it might be related to the excess production of inflammatory cytokines, damage-associated molecular patterns, apoptosis, and vascular endothelial damage as in other infections. In fact, COVID-19 coagulopathy could share some common features with the suppressed fibrinolytic type DIC [24]. In that case, coagulation activation is severe but fibrinolytic activation is mild, as typically seen in bacterial sepsis. Consequently, the coagulation cascade is activated as a host defense to limit the spread of the pathogens. In this situation plasminogen activator inhibitor (PAI-1) is markedly increased and fibrinolysis is strongly suppressed, dissolution of multiple microthrombi is more difficult, and, as a result of microcirculatory impairment, severe organ dysfunction may occur. It should be noted that in this type of DIC the degree of D-dimer increase is not directly correlated with the severity of the pathological condition and patients with proven sepsis and normal D-dimers may even show the highest mortality rate [57]. In suppressed fibrinolytic type DIC, bleeding complications are relatively mild, which is—clinically—the case of COVID-19, even if the high levels of D-dimers observed in COVID-19 contrast with this previous type of DIC. The several reports of viscoelastic test results in COVID-19 patients helped providing an insight on the pathophysiology of COVID-19 coagulopathy: in those studies fibrinolysis was never reported; rather an impairment of fibrinolysis was found to be associated with organ dysfunction or

thromboembolic events [58–62]. Interestingly, adding a viscoelastic test to a D-dimer measurement would help in understanding how much of the D-dimer is from clot breakdown and how much is the non-thrombotic D-dimer (i.e., inflammatory marker). Another interesting issue is that antithrombin and other natural anticoagulants are almost never consumed in COVID-19 coagulopathy [43, 51, 53, 63], in contrast with bacterial sepsis-associated DIC.

In conclusion, more and more evidences emerged pointing that COVID-19 coagulopathy has the feature of an inflammation-driven prothrombotic state. Whether this fits the classification of DIC is mainly semantic. Instead, what is more important is that the clinician should be aware of the variable clinical presentation of COVID-19-associated coagulopathy in order to promptly prevent, diagnose, and treat complications.

9.3 COVID-19-Associated Bleeding

The clinical manifestations associated with SARS-CoV-2 infection mainly consist of venous thrombosis, either involving deep veins or manifesting as pulmonary thromboembolism [64]. These, in turn, have brought to the widespread practice of increasing the dose of thromboprophylaxis to a partial-to-full anticoagulation regimen, in order to reduce the clinical risk derived from thromboembolic events [6]. In general, COVID-19 infection has also been linked to an increased risk of bleeding, either caused by the derangement of the coagulation system itself or promoted by an enhanced dose of thromboprophylaxis administered to infected patients.

The incidence of COVID-19-related bleeding varies widely across different studies, and this is probably caused by the lack of identification of specific mechanisms involved in the increase in bleeding risk (e.g., hemorrhagic events are not necessarily related to a fall in platelet count, reduced fibrinogen concentration, or prolongation of standard coagulation tests) [51, 64]. Interestingly, even during the SARS-CoV-1 outbreak in 2002 only a minor incidence of standard coagulation test alterations were observed, in contrast with hemorrhagic viral infections as Ebola, where the high incidence of bleeding events is connected to vast derangements of the mentioned tests [65]. The principal site of bleeding connected to COVID-19 is thought to be the lung, where the infection itself might provoke intrapulmonary micro-hemorrhages [66]. Whether this could be at the core of a systemic coagulopathy is still under investigation. On the other hand, many confounders are often present when analyzing the COVID-19-infected population, since the intake of oral anticoagulants and antiplatelet therapy is common in the studied subjects. At last, as previously introduced, anticoagulation regimens might play a pivotal role in the occurrence of hemorrhagic events. Therefore, whether the occurrence of hemorrhagic events represents a true increase in bleeding risk directly related to COVID-19 infection and immune activation, to the use of anticoagulation, or to illness severity remains unclear [67].

In a recent study by Al-Samkari et al. [64] on 400 hospitalized COVID-19 patients (of whom 144 critically ill), the overall bleeding rate was 4.8% (95% CI,

2.9–7.3), while major bleeding occurred in 2.3% (95% CI, 1.0–4.2) of admitted patients. Of note, the rate of major hemorrhagic events increased in the critically ill subgroup, 5.6% (95% CI, 2.4–10.7), which nonetheless seems to be in line with previous reports in non-COVID patients [68]. Gastrointestinal (GI) bleeding accounted for the majority of the events, followed by hemoptysis and bleeding from multiple cannulation sites. Specifically, GI bleeding in COVID-19 patients has been linked to intestinal mucosa hypoxia and injury. Its occurrence is thought to account for 4–13.7% of bleeding events, especially in the critically ill subpopulation [69]. Intracranial hemorrhage (see Section 4.2, Hemorrhagic Stroke) was detected in one fatal case in the abovementioned paper [64]. In this study, at least 86% of the subjects received standard-dose anticoagulation (enoxaparin 40 mg daily, or BID if BMI >40 kg/m^2), while 18% of the studied population was anticoagulated at an intermediate-to-full dose (at least enoxaparin 40 mg BID). Interestingly, bleeding events were associated to a mild increase in PT (while aPTT was found to be normal). Moreover, bleeding was not linked to a decrease in platelet count (which remained in the normal range), or to a reduction in fibrinogen count (which, on the contrary, resulted to be increased probably due to the hyperinflammatory state). Finally, an increase in D-dimer was associated with the occurrence of both thrombotic and hemorrhagic events.

Another study by Shah et al. [67] in 187 patients (89.3% requiring intensive care admission) investigated the incidence of hemorrhagic events in the COVID-19 population. In this work, 8% of studied patients experienced a bleeding event, and more than half of them were classified as major. Bleeding occurred at a median (IQR) of 15 (6–25) days following ICU admission: again, GI bleeding was the most frequent event (51.9% of the episodes), followed by intracranial hemorrhage (30%). Genitourinary bleeding and epistaxis were reported less frequently. Prolonged aPTT, thrombocytopenia, antiplatelet therapy, renal replacement therapy, and therapeutic heparin anticoagulation were all linked to an increased risk of hemorrhagic events. Moreover, the patients that experienced bleeding events were predominantly male, had higher SOFA score (9) [5–12], and were in need of a higher degree of intensive care support. Of note, in this study all patients underwent low-molecular-weight heparin (LWMH) thromboprophylaxis. Instead, therapeutic anticoagulation with LWMH was commenced for image-proven thrombosis or on the basis of a strong clinical suspicion, and it was monitored by anti-Xa levels with a target of 0.3–0.7 U/mL.

At last, a systematic review and meta-analysis on the incidence of venous thromboembolism and bleeding among hospitalized patients with SARS-CoV-2 have recently focused on this specific issue [13]. In this study by Jiménez et al., a pooled sample of 1411 patients with reported information related to bleeding was investigated [15, 70, 71]. The pooled incidence of bleeding was 7.8% (95% CI, 2.6–15.3), while it resulted to be 3.9% (95% CI, 1.2–7.9) for major bleeding. From the analyzed data, the authors conclude that the highest pooled estimate incidence of hemorrhagic events was reported for patients receiving intermediate- or full-dose anticoagulation (21.4%), compared to those patients who underwent usual prophylaxis. In contrast with the previous results, the lowest incidence on bleeding events

was reported in the only prospective study included in the meta-analysis [15] where only 4/150 (2.7%) of the included patients experienced hemorrhage. Notably, in the latter study, two of the four patients who developed hemorrhagic complications had trauma shortly before intensive care admission, while a third patient was on extracorporeal membrane oxygenation (ECMO). Moreover, 70% of the included patients underwent standard thromboprophylaxis, while 30% received therapeutic anticoagulation [15].

According to the study by Jiménez et al. [13], a higher incidence of bleeding events in hospitalized (and especially critically ill) COVID-19 patients undergoing intermediate-to-full anticoagulation regimens has been acknowledged by several recent papers [72, 73], and this regimen has also been mildly discouraged by recent guidelines [74]. Interestingly, despite the rationale of counteracting the increased tendency towards thrombosis due to SARS-CoV-2 infection with an augmented heparin administration, and regardless of the probable enhancement of the bleeding risk in these patients, it seems from the latest results that the adoption of intermediate- to full-dose anticoagulation fails to improve hospital survival or days free of organ support, when compared to usual care thromboprophylaxis [75].

9.4 Central Nervous System Manifestations

9.4.1 Ischemic Stroke and Venous Sinus Thrombosis

9.4.1.1 Ischemic Stroke

Since the very beginning of the pandemic, SARS-CoV-2 infection has been known for its involvement of the respiratory system and its severe complications in terms of respiratory failure and need of intensive care support. Alongside pulmonary manifestations, acute ischemic stroke (AIS) has been recognized as one of the major neurological manifestations of COVID-19 patients [76]. Past studies indicate that acute infection (both of bacterial and viral etiology), and especially respiratory-related infections, represents an independent risk factor for stroke [77]. Notwithstanding an incomplete picture of the pathophysiological mechanisms behind AIS in COVID-19, the prothrombotic tendency of the coagulation system during SARS-CoV-2 infection—already involved in DVT and pulmonary thromboembolism—is thought to play a pivotal role also in AIS [77]. This primarily systemic mechanism of hyperinflammation/thrombogenic tendency is thought to be flanked by a specific tropism of the virus for the neurological system, fostered by the expression of the ACE2 receptor in the brain. There, the virus could replicate the typical hyperinflammatory response which has been described in the lung tissue, and thus it could lead to endothelial dysfunction—with local release of interleukin-6 and tissue factor (TF) in the macro- and microvascular net of the central nervous system [78]. Consequently, the incidence of AIS in COVID-19 subjects could derive from a two-hit mechanism, where a local release of thrombogenic factors is superimposed to a systemic prothrombotic diathesis.

The incidence of AIS in COVID-19 patients is thought to range between 0.9% and 2.7%, for an overall pooled incidence of 1.2% among 4466 subjects included in the analysis reported by Tan et al. [77]. In this study, the main patient characteristics associated with the development of AIS were arterial hypertension, diabetes mellitus, and hyperlipidemia, not differently from pooled COVID-19 populations investigated elsewhere. Likewise, elevated D-dimers and fibrinogen concentration were found in these cohort of COVID-19 patients. It is important to underline that the risk factors for the development of AIS are often shared between COVID-19 and non-COVID-19 patients, resulting in a difficult identification of the neurological events specifically caused by SARS-CoV-2 infection. Notably, the time to manifestation of signs connected to AIS was 10 ± 8 days from the beginning of COVID-19 symptoms (e.g., fever, cough, dyspnea), while the occurrence of AIS as first presentation of COVID-19 is reported in a minority of cases [78]. The National Institute of Health Stroke Scale (NIHSS) score at presentation was 19 ± 8 (moderate severity) [77]. This clinical picture fits with the radiological finding of large vessel involvement in COVID-19-related AIS (40.9% of COVID-19 IS studied patients), with a multifocal presentation in up to 15% of them. The mentioned data are in line with previous numbers showing large vessel involvement in COVID-19-related AIS, especially in younger subjects (<50 years of age) [79], but seem even underestimated if compared to more recent reports where large vessel involvement could reach 80% of AIS presentations [80]. In another report on 174 COVID-19 patients hospitalized for AIS, the median NHISS score was 10 (IQR 4–18), which resulted to be significantly higher with respect to a propensity score-matched population of non-COVID-19 AIS subjects [81]. Again, large vessels represented the culprit lesion in a vast percentage of the subjects. At last, in a recent large review and meta-analysis on cerebrovascular events in the COVID-19 population, the overall incidence of cerebrovascular events resulted to be 1.4% (108,571 subjects included), where 87% of them was represented by ischemic stroke [80]. Concerning the COVID-19 population, the subjects who developed neurological complications were older and had a higher severity of infection; nevertheless, when compared to noninfected subjects, COVID-19 patients affected by cerebrovascular events resulted to be younger and had higher NIHSS score, higher frequency of large vessel occlusion, and higher in-hospital mortality rate (OR = 5.21; 95% CI: 3.43–7.90). Altogether, these results underline a more severe clinical presentation and consequently worse outcomes in terms of neurological disability and mortality for AIS in the COVID-19 population.

9.4.1.2 Venous Sinus Thrombosis

Despite the majority of central neurological manifestations related to SARS-CoV-2 infection being associated with the development of AIS, cases of venous sinus thrombosis (VST) have been reported [82–84]. Again, while risk factors for VST were identified in the majority of patients who developed this cerebrovascular complication, this is not true for the whole population analyzed in a recent review by Fraiman et al., leaving space for a possible direct role of SARS-CoV-2 in the pathophysiology of VST [84]. In this work, the incidence of VST in a COVID-19 pooled

population resulted in 5.1% of all cerebrovascular events. The most frequent neurological manifestations of VST were headache and/or altered mental status, variably associated with focal signs or symptoms. D-dimer, CRP, and ferritin elevation were reported in the majority of included cases, similarly to other COVID-19 patients developing thrombotic complications. While the clinical manifestations and treatments of AIS and VST may differ, the pathophysiology that stands behind these two cerebrovascular complications in COVID-19 is probably shared, owing to hyperinflammation and prothrombotic tendency of its central mechanism.

9.4.2 Hemorrhagic Stroke

As for systemic bleeding, hemorrhagic manifestations involving the central nervous system are significantly less frequent than ischemic ones. Nevertheless, due to the striking prognostic weight and the mortality rate connected to hemorrhagic stroke (HS), a direct link between SARS-CoV-2 infection and incidence of HS has been sought.

Among a large pooled population of COVID-19 subjects studied by Nannoni et al. [80], HS contributed to 11.6% of all cerebrovascular complications. In this study, out of 102 patients with intracerebral hemorrhage, 44.1% presented with lobar hematoma, and in 18.5% the volume of hematoma led to intracranial herniation. Specific risk factors for the incidence of HS in the COVID-19 population were not identified; in the same way, molecular mechanisms that could favor the development of HS in the infected population are still under investigation [85]. A possible explanation involves again the ACE2 receptor, which is represented in the central nervous system, as abovementioned. In fact, when SARS-CoV-2 binds to ACE2 receptors, the ability of ACE2 to lower blood pressure is reduced, so that a local tendency towards hypertension—together with the known inflammatory insult—could promote bleeding and thus HS [78].

Besides the possible direct and indirect role of SARS-CoV-2 in the etiology of HS, the use of systemic thromboprophylaxis and anticoagulation in this population has been called into question. Indeed, the tendency towards the administration of an increased dose of heparin to counteract the thrombogenic coagulative profile of the infected subjects has raised many doubts on whether this could result in a surge of bleeding complications (see Sect. 9.3, COVID-19-Associated Bleeding). Dogra et al. retrospectively analyzed a cohort of 33 hospitalized COVID-19 subjects who developed HS during their stay [86]. Radiographic evidence of hemorrhage was detected on day 17 (IQR 8–23). Various regimes of systemic prophylaxis and anticoagulation were used (namely enoxaparin, unfractionated heparin, and argatroban), dependently on clinical indication (e.g., diagnosticated thrombosis, reduced glomerular filtration). Five out of 33 HS (15.2%) had parenchymal hemorrhages with mass effect and herniation, with a mortality of 100%. Notably, all the five patients were on full anticoagulation regimen, and 80% of them have had supramaximal anti-Xa activity within 72 h prior to HS development. Moreover, 33% of the studied population was found to have a platelet concentration $< 150 \times 10^3/\mu L$

prior to intracerebral bleeding. The latter results, together with the data coming from studies that focused on the incidence of thrombotic and hemorrhagic events in the hospitalized COVID-19 population [13, 64, 71], warn clinicians about the risks connected to the use of anticoagulation regimens which are not yet standardized. Moreover, where the administration of intermediate-to-full anticoagulation meets a clinical rationale, thorough monitoring of standard coagulation tests, platelet concentration, anti-Xa activity, viscoelastic tests (where available), and renal function is of utmost importance in order to avoid bleeding complications.

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Radiologic Imaging of Thromboembolic Complications in COVID-19

10

Mara Falco and Pier Paolo Campanino

10.1 Introduction

All the previous chapters have contributed to the characterization of the COVID-19-associated coagulopathy, its clinical patterns, and possible prophylactic or therapeutic approaches. The most relevant and common clinical consequence of this coagulopathy is the onset of subclinical or clinically relevant thromboembolic events. It is therefore of paramount importance for the clinicians taking care of COVID-19 patients to correctly utilize the possible diagnostic tools for a prompt diagnosis of these complications.

Among the different techniques, radiologic imaging is certainly the most relevant and valuable tool to provide clinical and diagnostic information rapidly available to the clinicians. Within the radiologic imaging techniques, computed tomographic angiography (CTA) is certainly the most accurate. However, and given the logistic difficulties in the setting of the COVID-19 pandemic storm, a routine use of CTA is out of question. Therefore, the clinicians should be guided by a number of clinical and laboratory signs to avoid an excessive as well as a limited request for CTA. The purpose of this chapter is to highlight, for separate anatomical districts, the available imaging features and when to perform the different imaging techniques.

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10.2 Pulmonary Embolism

The most common and dangerous thromboembolic complication of COVID-19 is pulmonary embolism (PE). A meta-analysis on 27 studies found a pooled incidence rate of PE of 16.5% which was more frequent in patients admitted to the ICU (24.7%) than in ward patients (10.5%) [1].

10.2.1 The Role of Computed Tomographic Angiography (CTA)

Conventional chest radiography is not useful for the diagnosis of PE. Venous ultrasound and computed tomographic (CT) venography are reliable in detecting peripheral vein thrombosis, while pulmonary angiography and catheter pulmonary angiography are employed only for interventional procedures. CTA nowadays represents the first choice among imaging techniques for detecting PE [2, 3].

The great and widespread availability of CT scanners and well-established technical protocols make this diagnostic tool effective and fast in diagnosing or excluding the suspected acute PE, representing a fundamental support to clinicians to promptly treat the embolic pathology. CTA has a high sensitivity and specificity, with PIOPED II trial [4] demonstrating a sensitivity of 83% and a specificity of 96%. Moreover, CTA not only depicts clots in the pulmonary arteries but can also evaluate the right ventricle-to-left ventricle diameter ratio. This value is a strong predictor for adverse clinical outcomes in patients with acute PE [5, 6].

The well-known algorithm combining clinical probability, D-dimer testing, and computed tomography results to be effective to manage PE and to suggest whether or not to perform a CTA examination [7]. However, in the setting of COVID-19, some of the concepts included in the diagnostic algorithm of PE have been challenged and will be discussed later on in this chapter [8].

10.2.2 Technical Aspects

Modern multidetector CT scanners (16, 32, 64, 128 rows of detectors and over) allow entire acquisition of the thoracic volume by proper collimation in thin slices (<1 mm) in short time (<10 s) [9]. The introduction of iterative reconstruction software improves the noise/signal ratio with benefit to postprocessing low-dose images. This minimizes respiratory artifacts, especially in emergency environment, and reduces the dose of contrast medium and radiation by using low tube voltage [10]. The bolus injection of iodine contrast agent is properly timed by automated bolus tracking of the pulmonary artery trunk (Fig. 10.1), or optimized by biphasic time-enhanced curves: dose of contrast medium tailored to patient weight or better to body mass index. The goal is to visualize the pulmonary circulation without venous contamination for more precise evaluation of clot extension and burden on axial images and multiplanar reconstructions. High-quality images are important to detect subsegmental embolism that otherwise could be overlooked by the

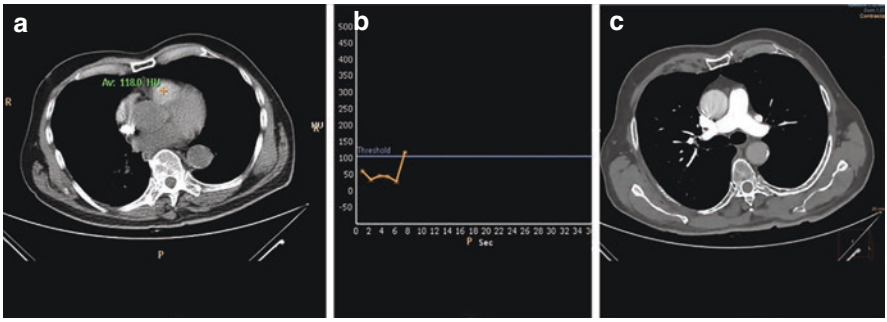


Fig. 10.1 Automated bolus tracking of the pulmonary artery trunk. **Panel a**: region of interest positioned at the origin of pulmonary artery. **Panel b**: threshold of the selected density for timed acquisition. **Panel c**: optimal result in the opacification of the pulmonary artery

radiologists [11, 12]. This is an interesting challenge for CTA to evaluate small vessel injury and chronic embolism in COVID-19 disease. Dual-energy CT scanners can detect clots and perform pulmonary perfusion study in one sitting, representing a promising diagnostic choice as a surrogate of nuclear medicine like the classical ventilation/perfusion scintigraphy or the more recent single-photon emission computed tomography [13].

10.2.3 CTA in the COVID Era

In patients with COVID-19 pneumonia elevation of D-dimer levels is generally confirmed as the strongest risk factor to suspect acute PE [14]. However, it must be recognized that D-dimer is not specific for PE, as it increases in any case of even subclinical thrombosis at any anatomical district. Therefore, differently from the general patient population, elevated D-dimer levels are not the first choice to suspect an acute PE [8]. D-dimer is often elevated in COVID-19 patients, without necessarily being related to acute PE. Microvascular thrombi in different regions may be responsible for this. Acute PE should be considered when a patient exhibits hemodynamic instability or poor gas exchange that is not fully explained or is out of proportion to the stage, duration, and rate of progression of COVID-19 infection.

D-dimer probably maintains its function in raising the suspicion for PE when an abrupt elevation is found in concomitance with clinical signs.

There is a limited value of non-contrast chest CT in the diagnostic process of PE. However, different parenchymal patterns may be observed at different stages of severity of PE [15–17]. Pulmonary infarct in acute PE manifests on CT as wedge-shaped, peripheral opacity commonly with a “reverse-halo” or “atoll” appearance consisting of central ground glass and a rim of consolidation. These findings are distinct from chronic PE which includes mosaic perfusion, band-like opacities, and bronchial dilation in abnormal areas [2].

In COVID-19 pneumonia non-contrast CT has been evaluated to detect early signs related to thromboembolic disease but there are non-univocal findings. Some studies showed that crazy-paving pattern and/or air bronchogram were significantly associated with PE [15]. In other studies, there was no significant difference between acute and non-acute PE patients concerning lung lesions (ground glass opacity: 85% vs. 97%; consolidation: 69% vs. 68%; crazy paving: 38% vs. 37%; linear reticulation: 69% vs. 78%) [18]. In conclusion, in the light of today's knowledge non-contrast chest CT alone even if suggestive for suspicion of PE is not sufficient to prompt a subsequent CTA.

The localization of pulmonary thromboembolic disease revealed by CTA examinations is more frequently segmental, subsegmental (Fig. 10.2), and lobar (Fig. 10.3) with a predominant extension to one up to three lobes; less frequently four to six lobes are involved. This pattern can be more commonly associated with signs of right-heart dilation and dysfunction with respect to patients negative for PE [19]. In the series of Espallargas and associates [17], PE predominantly affected segmental arteries and the right lung, especially its upper lobe. With respect to the morphology of thromboembolic disease in COVID-19 pneumonia, the classic findings of acute PE in CT are confirmed, including "polo mint sign" (central filling defect within a vessel surrounded by contrast material) (Fig. 10.4), "railway sign" (observed parallel to the vessel long axis) (Fig. 10.5), and the so-called saddle embolus (large amount of thromboembolic material draped over the pulmonary trunk bifurcation). Other signs are eccentric or mural filling defect, complete occlusion resulting in vascular enlargement, or dilatation in areas of lung opacity [17, 20, 21]. Figures 10.6 and 10.7 are typical images of the previously addressed patterns.

At a clinical level, it is difficult to suggest a flowchart that from standard chest X-rays (routinely used in COVID-19 pneumonia) leads to non-contrast CT (not

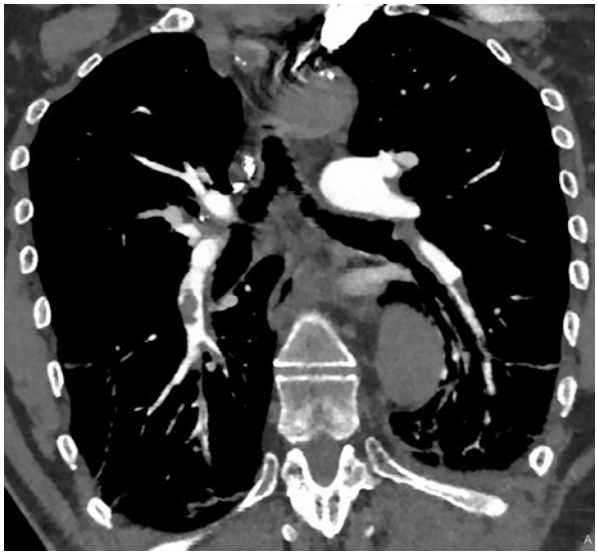


Fig. 10.2 Lobar, segmental and subsegmental bilateral pulmonary embolism

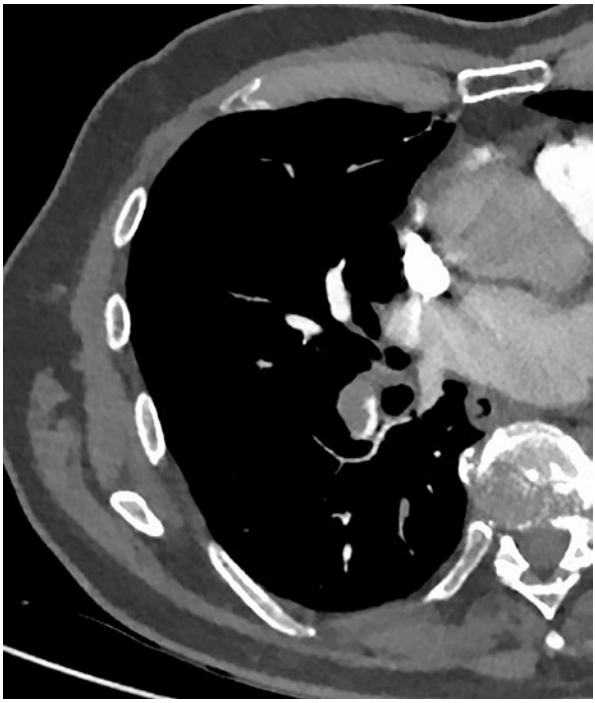


Fig. 10.3 Right inferior lobar pulmonary embolism

routinely used by many institutions) finally arriving to CTA (that should be dedicated to PE diagnosis). Certainly, routine CTA is not feasible; additionally, a specific study [22] conducted to evaluate the systematic employment of CTA in patients referred to emergency department did not provide clear evidence that there is a benefit to routinely perform CTA as first-line imaging modality in patients suspected for COVID-19 pneumonia. In conclusion, it is reasonable to suggest to follow a combination of biomarkers (D-dimer) [23], clinical signs of hemodynamic deterioration, echocardiographic finding of right ventricular dysfunction, and deterioration of lung gas exchanges, to request a CTA examination.

Figure 10.8 depicts a flowchart to guide clinicians in the choice of requesting a lung CT or CTA.

Ward patients should receive a CT to stage the disease in case of worsening pulmonary gas exchanges and/or escalating need for respiratory support (noninvasive ventilation). Suspicion of PE, based on acute increase in D-dimer, right ventricular dysfunction, hemodynamic deterioration, and other signs included in the currently available PE scoring systems, should prompt a CTA.

ICU patients under mechanical ventilation should receive a CT scan at admission and serial CT to follow the parenchymal evolution of the disease. Extracorporeal membrane oxygenation (ECMO) candidates should receive a CTA to assess the possible existence of a PE, which could trigger the decision to implant a

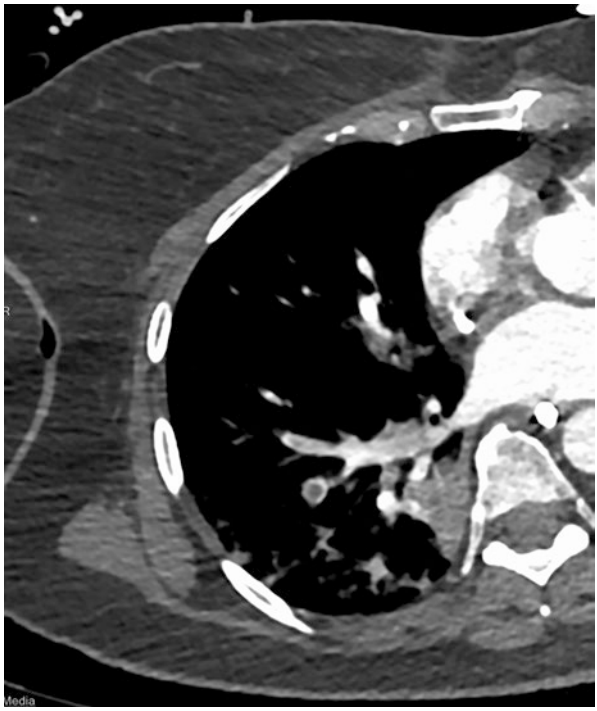
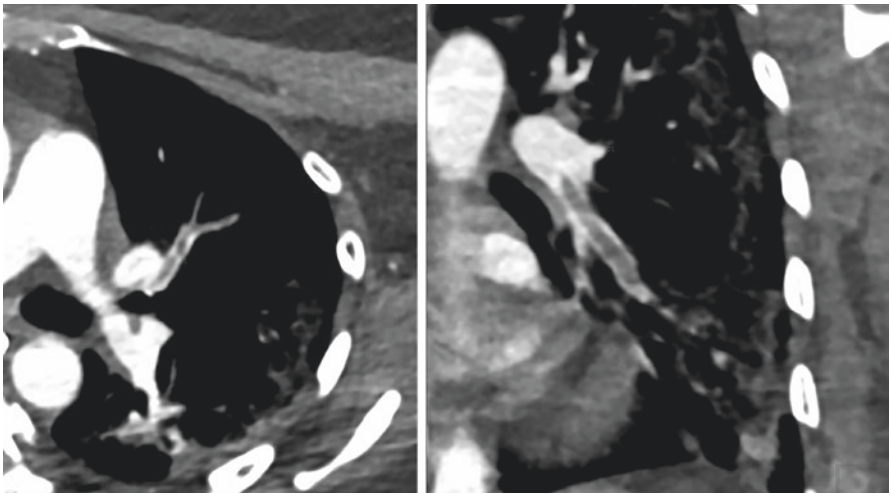


Fig. 10.4 "Polo mint" sign: within a segmental vessel a central filling defect surrounded by contrast material is visible



Axial view Coronal view

Fig. 10.5 "Railway sign": the clot appears as a defect parallel to the long axis of the vessel bordered by contrast material

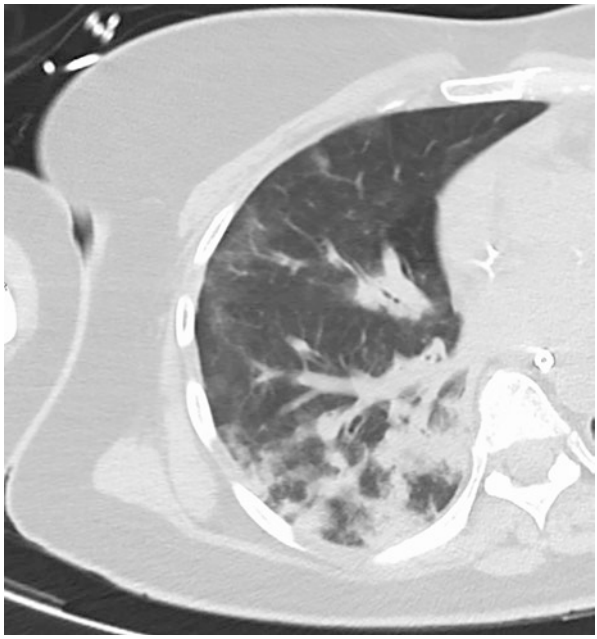


Fig. 10.6 Unenhanced CT scan showing lung opacities and vessel enlargement

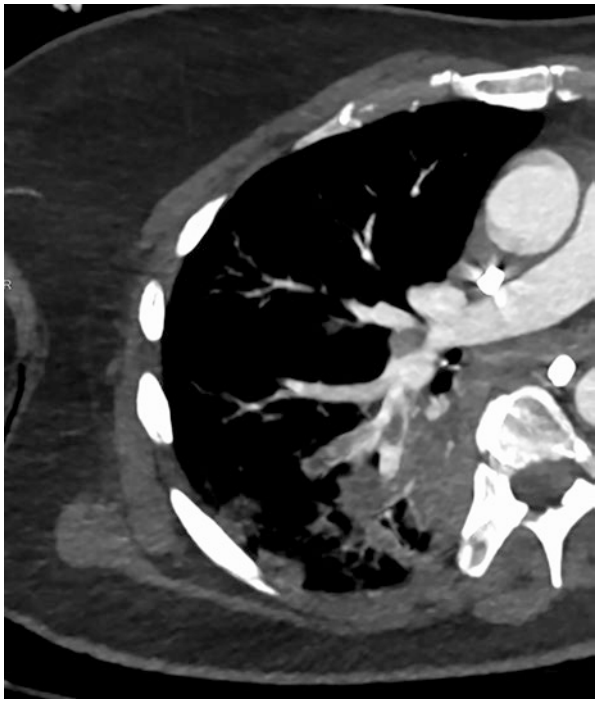


Fig. 10.7 Same case on CT angiography: multiple mural or eccentric defects are visible within segmental arteries

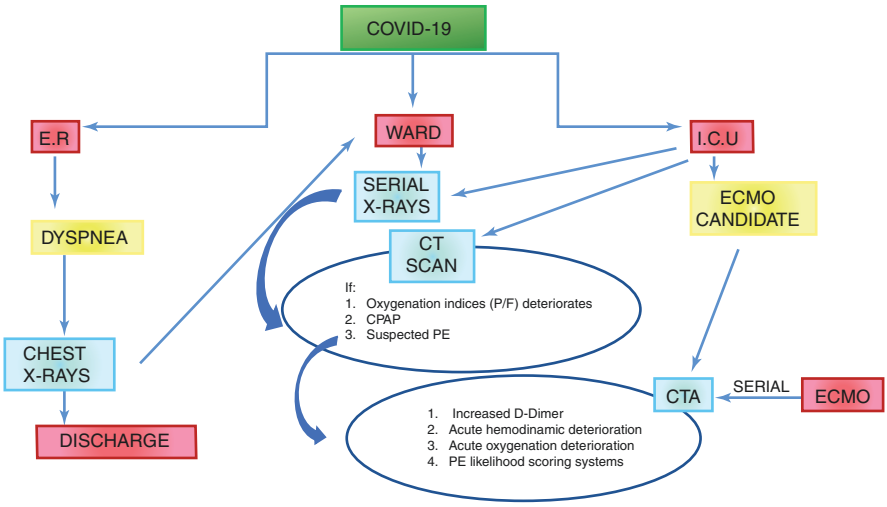


Fig. 10.8 Flowchart for CT-based pulmonary embolism diagnosis in COVID-19 pneumonia. *CT* computed tomography, *CTA* computed tomographic angiography, *ECMO* extracorporeal membrane oxygenation; ER: emergency room, *ICU* intensive care unit

veno-venous or a venoarterial ECMO configuration. Finally, patients on ECMO should receive serial CTA to the course of both the parenchymal and vascular course of the disease.

10.3 Extrapulmonary Thrombosis and Thromboembolism

The results from the previously described studies indicate that in patients with COVID-19, radiologists should maintain a high index of suspicion for thromboembolic complications.

In a recent study by Cui and associates [24] which enclosed 81 critically ill patients, the incidence of venous thromboembolism (VTE) was 25% with a mortality rate of 40% in that subset of patients.

In a similar Dutch study [25], conducted on 184 intensive care unit patients with confirmed COVID-19 pneumonia, thrombotic complications were found in 31% (VTE in 27% and arterial thrombotic events in 3.7%), the most common of which was PE.

10.4 Abdominal Organs

Although patients typically present with respiratory illness, up to 40% of patients with COVID-19 present with abdominal symptoms, which include diarrhea, vomiting, and acute abdominal pain [26].

Additionally, they commonly develop elevated liver enzymes and biliary stasis.

In patients presenting to the emergency department with nonspecific gastrointestinal symptoms such as abdominal pain, the first-line diagnostic exam is usually represented by ultrasounds (US); abdominopelvic CT is indicated to evaluate possible sources of aspecific infection or in case of suspected organ ischemia. Doppler US can be performed in patients with suspected abdominal venous or arterial thrombosis but abdominopelvic CTA study is mandatory whenever US and/or clinical examination raise a high suspicion for intestinal ischemia, perforation, solid organ injury, or infarct.

Of notice, some patients with COVID-19 initially present with symptoms of abdominal pain although not accompanied by any detectable abdominal finding at CT. This peculiar pattern may be ascribed to a referred pain arising from the basal regions of the lung, particularly those located close to the diaphragmatic pleura, as happens in basilar pneumonia.

10.4.1 Gastroenteric Tract

Gastroenteric involvement in COVID-19 can present as gastritis, enteritis, colitis, or combinations of them, as a direct consequence of viral infection or viral induced inflammation. In viral enterocolitis it is common to observe alterations of the perivisceral mesenteric fat, which appears thickened and soaked due to virus-related immunoreaction and cytokine cascade (Fig. 10.9).

Patterns of mesenteric ischemia are described. This complication could manifest with an early, intermediate, or late presentation according to the gravity of clinical presentation and coagulation pattern of the patients.

Acute mesenteric ischemia due to thromboembolism is not uncommon in COVID-19. It is therefore mandatory to suspect, diagnose, and manage this severe complication.

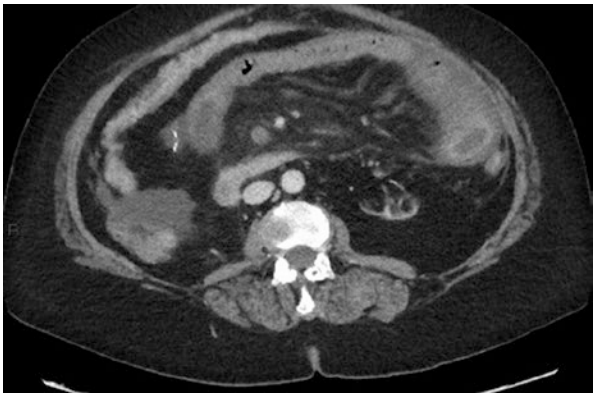


Fig. 10.9 Intravenous contrast-enhanced CT images of abdomen showing small amount of ascites and mesenteric congestion as indirect sign of enterocolitis

Abdominal and pelvic CT findings of mesenteric ischemia include wall thickening and edema, fluid intestinal lumen distention, and mucosal hyperenhancement.

Parietal thickening is commonly due to submucosal edema, also responsible for ribbonlike appearance of the wall fold. In the very early phase mesenteric ischemia presents in CT imaging as contracted gasless bowel that evolves in paper-thin bowel wall and, in the later phase of ischemia in lumen dilation, non-enhanced parietal wall and pneumatosis. This pattern is sometimes complicated by intra-portal or intra-mesenteric vein bubbles of gas. Aggressive mechanical ventilation in severely ill COVID-19 patients may induce a visceral pneumatosis pattern.

When untreated, bowel infarct evolves in bowel perforation, identified in CT scan by parietal discontinuity and fluid-gas perivisceral collection. In advanced phase abscesses close to wall perforation can be found. The so-called cupola sign, saddlebag sign, or lucent liver sign lays down for pneumoperitoneum.

The diagnosis should be excluded or confirmed through an abdominopelvis CTA that depicts the vascular mesenteric district and its possible thrombotic filling defects in the arterial or venous lumen (Figs. 10.10 and 10.11).

It is common to observe multiple parietal and perivisceral alterations suggestive for bowel ischemia with no CTA evidence of intra-arterial thrombi. Autopsy findings highlighted patterns of microthrombosis of the vascular distal mesenteric beds and of submucosal arterioles that cannot be detected at CT imaging due to technical limitations.

Hyperdense material distending the lumen of the pathological bowel tract allows to identify hemorrhagic evolution of bowel ischemia.

The causes of ischemia are more frequently arterial, either embolic (40–50%) or in situ thrombosis of a narrowed vessel, the latter being more common in the elderly (>70 years).

Mesenteric venous occlusion is a less common cause of ischemia (5–10%) and usually occurs in much younger population [27].

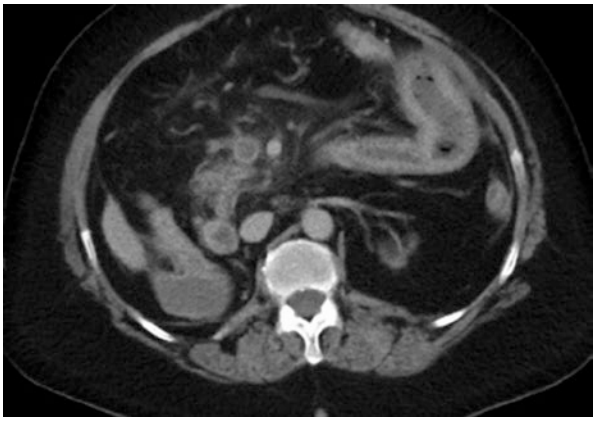


Fig. 10.10 Axial contrast-enhanced CT images of the abdomen showing filling defect in the superior mesenteric vein and mesenteric fat congestion

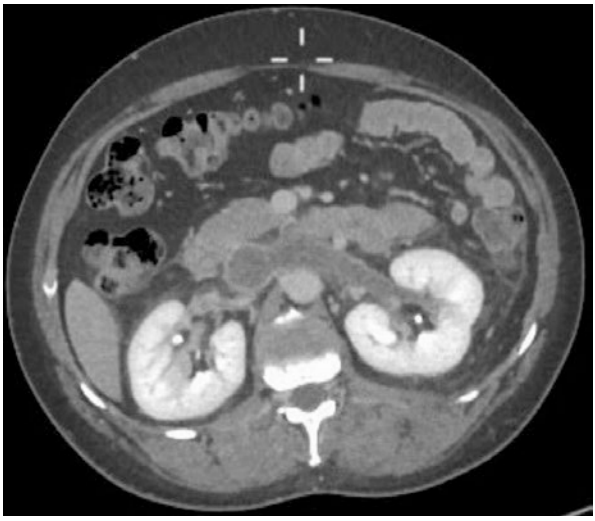


Fig. 10.11 Axial contrast-enhanced CT images of the abdomen showing complete long segment filling defect in the left renal vein and in inferior vena cava and lumen dilatation, indicative of vein thrombosis

10.4.2 Liver and Other Solid Organs

In a recent study on 141 COVID-19 patients, who underwent abdominopelvic CT scan, 18% presented solid-organ infarcts [28]. Doppler US depicts decreased vascularization within the parenchyma and contrast-enhanced CT confirms hypoattenuated wedge-shaped area in solid organs corresponding to infarct.

Vascular thrombosis was seen as a filling defect within one or few of the supplying vessels at dynamic contrast-enhanced CTA.

The liver is the most frequently damaged organ in COVID-19 outside of the respiratory system. The mechanisms of hepatic injury are not completely clear, and may be multifactorial and related to direct viral infection that results in an immune-related biliary or hepatic cells damage.

Current data show that 15–50% of COVID-19 patients have abnormal levels of hepatic enzymes and approximately 50% of patients have elevated levels of γGT [29].

The range of hepatic manifestations of COVID-19 yields mild and transient to moderate, even if severe liver damage may occur. One of the major potential causes of liver damage and elevation of liver enzymes is microthrombosis within hepatic sinusoid, related to the well-known COVID-19-induced coagulopathy [26].

US, CT, and magnetic resonance imaging (MRI) are considered modalities of choice for the evaluation of liver and biliary dysfunctions. Their findings include periportal edema, increased incidence of hepatic steatosis (likely related to the known association between infection and obesity), biliary stasis, and in few cases ascitic effusion. However, these findings may be marginal and nonspecific in COVID-19.

Complications related to micro- and macrothrombosis may not be immediately evident at Doppler US, contrast-enhanced CT, or MRI. Bubbles of gas in the portal vein lumen, extending peripherally, are related to portal venous gas resulting from bowel ischemia.

Absent color at Doppler US in vascular hepatic system is indicative for hepatic thrombosis and may correspond to filling defects within affected vessel in contrast-enhanced CT or MRI, depending on the acuity of the thrombosis [30].

To date, only few imaging findings have been described in CT scans of solid organs, including multifocal liver infarcts, and focal lack or splenic perfusion. Infarcts are related to microangiopathy or systemic coagulopathy and cardiac thromboembolism.

These findings are incidentally diagnosed during CT abdominal scan, not being associated with symptoms related to spleen alterations or infarcts.

Splenic parenchymal congestion, hemorrhage, lack of lymphoid follicles, and atrophy were all evident and described at autopsy in patients who died of COVID-19 infection [31].

10.4.3 Urogenital Tract

Acute kidney injury in COVID-19 patients is quite common, occurring in approximately 20–40% of those admitted to hospital, particularly to intensive care unit [32].

Apart from patterns related to hemodynamic compromise and prolonged mechanical ventilation, that are common in many severely ill patients, there are two different pathophysiologic mechanisms of COVID-19-related renal injury. The first one involves tubular necrosis through interstitial inflammation and glomerulopathy. In this case US may show loss of corticomedullary differentiation.

In case of renal infarction, hypoperfusion of renal parenchyma and trigonal shaped areas of decreased perfusion or contrast enhancement may be observed in contrast-enhanced CT or MRI. These alterations may be multifocal, monolateral, or bilateral. It is important to remember that contrast media cannot be used in case of renal failure or impaired renal function. This makes US the first-line imaging modality of choice in COVID-19 patients with suspicion of vascular renal damage [33].

10.5 Other Districts

10.5.1 Brain

There is increasing evidence of different neurological manifestations in COVID-19 patients such as stroke (6–9%), altered mental status (15%), epilepsy, disturbed consciousness, headache, and encephalopathy [34].

Multiple pathological factors seem to be involved in the onset of neurological alterations. They include gross cerebral thromboembolism, endothelial vascular cell

inflammation resulting in hemato-encephalic barrier disruption, a hypoxic status induced by impaired lung gas exchange, virus cell penetration, and activation of the cytokine cascade.

COVID-19 patients presenting neurological symptoms may undergo basal CT scan of the head to identify indirect signs of ischemia, hemorrhage, or brain infection.

If an infarct is suspected CTA could be performed to identify the vessel involved and evaluate the indication for endovascular treatment or thrombolysis. If a cerebral infarction has been ruled out by CT scan, a non-enhanced MRI should be performed, using the short protocol composed of critical sequences such as diffusion-weighted and corresponding apparent diffusion coefficient (ADC) mapping and axial T2 FLAIR sequences. The use of paramagnetic contrast media should be reserved to cases of high suspicion of encephalitis with the aim of highlighting leptomeningeal enhancement.

A large spectrum of imaging findings was reported in COVID-19-associated encephalitis. A recent important study [35] with a large cohort of patients revealed a high prevalence of monolateral MRI imaging findings in the mesial temporal lobe due to autoimmune encephalitis.

Acute hemorrhagic leukoencephalitis and acute diffused encephalomyelitis present at MRI imaging as diffuse white matter lesions and associated hemorrhagic foci. This pattern was described in 30% of COVID patients subjected to MRI while in 24% large confluent areas of white matter hemorrhage were found [36].

Supratentorial hyperintensity at FLAIR sequences or T2 hyperintensity in splenium, corpus callosum, and cerebellar peduncles was explained as postinfectious demyelination, posterior reversible encephalopathy syndrome, or metabolic or toxic encephalopathy.

The majority of COVID-19 patients described as the first symptoms a loss of taste, loss of smell, or both. These are to be considered neurological manifestations, explained by the viral central nervous system invasion through a retrograde neuronal route with direct damage to olfactory and gustatory receptors [37].

Whenever the non-enhanced CT of the head shows a hypo-attenuation of the cerebral parenchyma, a CTA of the intracranial vessels is mandatory to rule out a large vessel occlusion. At this examination, embolic infarcts manifest as multiple areas of hypo-attenuation in white, gray, and transitional areas (Figs. 10.12–10.14).

Cases of venous sinus thrombosis are being increasingly reported during pandemic. Clinicians need to maintain a high index of suspicion while treating COVID-19 patients with persistent headache irrespective of the presence of other neurological symptoms. Venous infarcts should be suspected at CT when they are bilateral, depicted in non-arterial territory and in the presence of hemorrhage. Imaging of venous sinus thrombosis includes demonstration of thrombus as a loss of flow ("signal void") in baseline MRI images or hyper-attenuation within a sinus or large cortical vein in non-enhanced CT scan.

If needed, the diagnosis can be confirmed at CT venography or MR venography where filling defects appear in the venous ramus or sinus affected (Fig. 10.15).

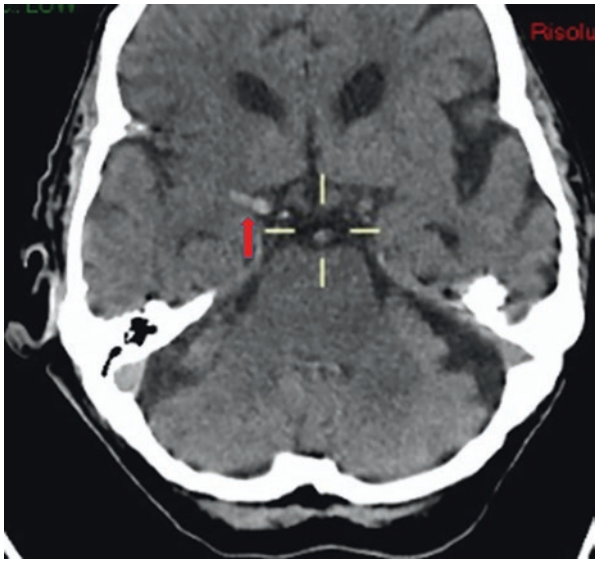


Fig. 10.12 Axial unenhanced CT images in a proximal segment of the right middle cerebral artery obtained 3 h after the onset of right hemiparesis and aphasia show areas of hyperattenuation (arrow) suggestive of intravascular thrombi

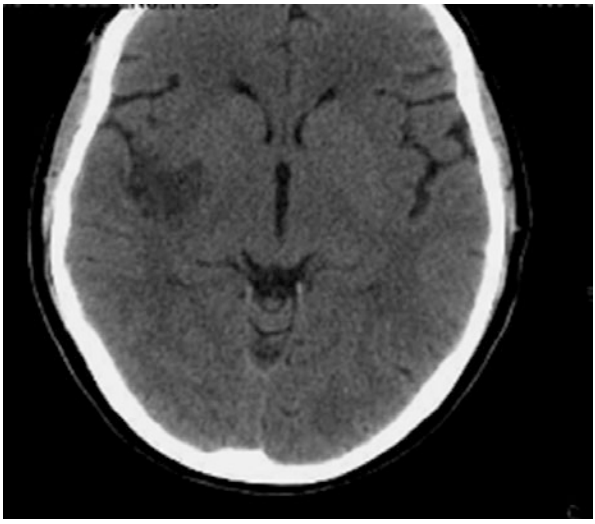


Fig. 10.13 Axial unenhanced CT image, obtained 3 h after the onset of left hemiparesis, shows hypoattenuation and obscuration of the posterior part of the right lentiform nucleus and a loss of gray matter–white matter definition in the lateral margins of the right insula

10.5.2 Thoracic Aorta

Aortic mural thrombus is a rare condition, usually associated with vessel wall abnormalities such as vasculitis, atherosclerosis, and dissection. Primary aortic thrombosis without predisposing local factor is really uncommon.

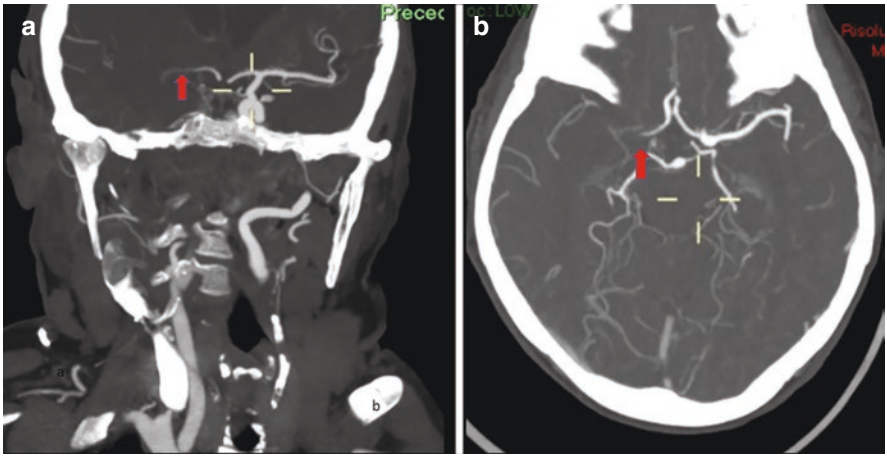


Fig. 10.14 Coronal (**a**) and axial (**b**) reformatted images from CT angiography showing the apparent absence of the same vessel segment (arrows). The presence of an intravascular thrombus in this location was confirmed by comparing the reformatted images with the non-enhanced images and row images

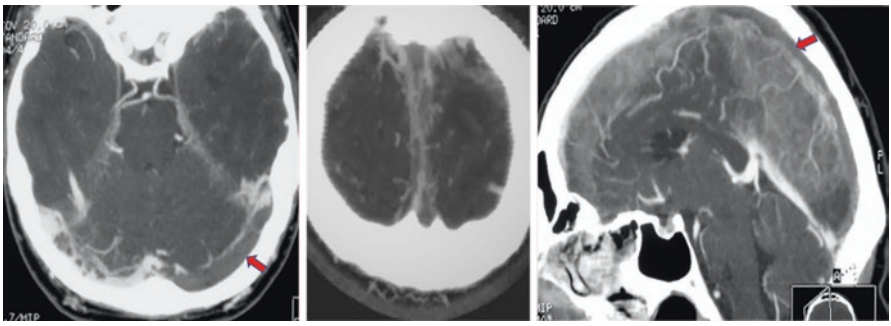


Fig. 10.15 Axial and sagittal 2D MIP CT image showing the empty delta sign in the superior sagittal sinus with enlargement, and a vascular defect of the adjacent cortical vein. Note thrombosis of the superior sagittal sinus and of the left transverse sinus (arrow)

COVID-19-related hypercoagulability is a possible cause, likely related to endothelial inflammation [38].

The symptoms of the disease are nonspecific. Unexplained chest and abdominal pain should alert the clinicians to order a thorough workup, including hematologic tests, and imaging examination should be implemented at the first instance. CTA scanning is recommended as a first-choice examination because of advantages such as convenience and high sensitivity. Radiologists play a nodal role in the diagnosis and treatment because the site, size, and shape of thrombus (sessile or pedunculated) drive the subsequent management, which ranges from medical therapy to endovascular treatment or open-chest surgery.

10.5.3 Musculoskeletal District

Subclinical musculoskeletal manifestations have been reported in COVID-19 patients (fatigue, muscle pain), even if a few cases describe rhabdomyolysis as late manifestation of COVID-19 complication. This disorder manifests with myalgia, fatigue, and urine pigmentation due to the elevation of myoglobin, often resulting in acute kidney injury.

US and CT findings are poor and nonspecific and with a late onset. MRI imaging may support the diagnosis and assist in individuation of severity and extent of muscle injury.

Two types of imaging presentation are described. Type one is characterized by hyperintense signal in T2-weighted and STIR sequences and homogeneous enhancement post-contrast media infusion. Type two manifests with nonhomogeneous hyperintense signal in T2 and rim enhancement post-contrast media administration [39].

In case of severe disease it is possible to put in evidence areas of colliquation or areas of muscular necrosis. In that case the risk of deep or superficial thrombosis should be considered.

10.6 Special Clinical Settings

10.6.1 Pediatrics

COVID-19 is less common in pediatric patients than in adults. Data regarding the clinical features and epidemiological characteristics of pediatric infection remain therefore limited.

According to data derived from a group of hospitalized children, COVID-19 pediatric symptoms are less severe when compared with those of older patients [40]. Common symptoms involve the upper respiratory tract such as pharyngitis, tonsillitis, otitis media, or sinusitis. Most children who required intensive care support had preexisting clinical conditions.

In a systematic review article Hoang and associates [41] reported that most pediatric symptomatic patients have normal chest radiography and, when performed, diffuse mild ground glass opacity at CT scan.

Vascular abnormalities were described in children who presented with symptoms of hyperinflammatory shock or in pediatric multisystem inflammatory syndrome (PMIS).

In children with PIMS signs of cardiac dysfunction were seen, such as myocarditis, pericardial effusion, and coronary artery aneurysms.

Coronary artery aneurysms were best detected either at echocardiography or at coronary CT with contrast media administration. Abnormalities ranged from mild single artery dilation to large aneurysm (from 4 to 7.7 mm diameter) affecting multiple coronary arteries [42].

10.6.2 Pregnancy

As pregnancy is a physiological prothrombotic state, pregnant women may be at increased risk of developing coagulopathic and/or thromboembolic complications associated with COVID-19.

Since most CT pulmonary angiographic examinations are performed based on clinical suspicion rather than systematic screening, the incidence of pulmonary embolism may be somewhat underestimated, especially in cases of small segmental or subsegmental pulmonary embolism. However, the choice of submitting a pregnant woman to ionized radiations is of course challenging. For this reason, the role of D-dimer elevation and other clinical signs assumes a higher relevance than in the remaining population. D-dimer levels are however increased in pregnant women, and specific cutoff values for pregnant women with COVID-19 remain elusive.

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Anticoagulation in COVID-19

11

Nicole P. Juffermans and Marcella C. Muller

11.1 Introduction

The high incidence of thrombosis in patients with COVID-19 as well as the observation that thrombotic complications or high D-dimer levels are associated with adverse outcomes [1] has prompted publication of guidelines and consensus documents from a number of societies in the spring of 2020 on anticoagulant management strategies. At that time, there were several observational studies reporting a particularly high prevalence of thrombotic complications in the critically ill, despite the use of systematic thromboprophylaxis [2, 3]. In addition, the community was starting to appreciate that coagulation abnormalities seemed to be inflammatory driven. Thereby, it is not surprising that despite the absence of sound evidence, these guidelines, based on expert opinion, mostly suggested to base the prophylactic LMWH dose on the severity of the disease, generally suggesting an intermediate dose in the critically ill [4, 5].

However, whether intensified anticoagulant treatment is effective in terms of improving the outcome of COVID-19 was not clear at the time these guidelines appeared. Although intensified anticoagulant treatment sounds reasonable, it has also been argued that intravascular thrombosis is beneficial in severe infection. Pulmonary thrombosis occurring in parts of the lung that are most affected and hence less well ventilated limits blood flow in these lung parts, thereby decreasing shunting. Thrombosis in other parts of the body may be a way to limit dissemination of virus.

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Since then, a number of studies have informed us on the optimal anticoagulant management of COVID-19, which we summarize in this chapter. We also summarize the ongoing trials on this topic. Anticoagulant therapy for COVID-19 is a highly dynamic research topic and knowledge will be highly improved in the coming years. Obviously, the current certainty of evidence continues to evolve.

11.2 Efficacy of Increased Prophylactic Dose on the Incidence of Thrombosis and Outcome

11.2.1 Observational Studies

A retrospective analysis of Chinese patients that appeared early in the pandemic showed a survival benefit for patients with high D-dimers receiving heparin versus those not receiving heparin, the majority of whom received LMWH in prophylactic dose [6]. These kind of data helped raise awareness of the high incidence of thrombotic complications when the pandemic was hitting Europe, resulting in the clinical practice of increasing doses of prophylactic LMWH. An observational study in ~1500 patients in eight centers in the Netherlands comparing thrombosis incidence in the first wave with that in the second wave demonstrated a lower thrombosis incidence in ICU patients, associated with a trend towards reduced mortality [7]. The reduction in mortality in ICU patients in this study cannot directly be attributed to increased LMWH dose, as COVID treatment including steroids and antiviral medication was also intensified. Findings in ward patients were seemingly in contrast, as mortality was also reduced, but a *higher* thrombosis incidence was found. The authors attributed this higher incidence to survival bias and detection bias.

An observational study in 852 patients from 28 European centers also reported a high use of intensified dosing of thromboprophylaxis in clinical practice. Using multivariate modelling, intensified thromboprophylaxis was associated with reduced ICU mortality, although thromboembolic complications were not concomitantly reduced, suggesting absence of a causal link. There was no increased burden of hemorrhagic complications [8].

Taken together, observational studies may point towards a particular benefit of intensified prophylaxis in the most severely ill, although confounding and bias hamper conclusions.

11.2.2 Trials on Prophylactic Dose

An intermediate dose of 1 mg/kg enoxaparin was compared to a standard dose of 40 mg enoxaparin as thromboprophylaxis in a randomized superiority trial in 562 patients with COVID-19 admitted to 10 ICUs in Iran [9]. Modification according to body weight and creatinine clearance was applied. The primary outcome (a composite of thrombosis, treatment with extracorporeal membrane oxygenation, or mortality) did not differ between groups.

Thereby, these results do not suggest a benefit from the routine use of intermediate-dose prophylactic anticoagulation in ICU patients. Of note however, this study reported a 4% incidence of thrombosis, which is considerably lower than that reported in other critically ill patient cohorts. This may be related to a lack of systematic screening. Alternatively, patients may not have been severely ill, as only 20% of these patients were on invasive mechanical ventilation, suggesting that this cohort may not reflect COVID-19-related ARDS. As patients were randomized irrespective of D-dimer levels and confidence intervals were wide, this study cannot exclude the possibility of benefit or harm for specific patient subgroups.

11.3 Efficacy of Anticoagulant Treatment on Thrombosis and Outcome

11.3.1 Observational Studies

In the first wave, a large cohort of ~2800 US hospitalized patients with COVID-19 was analyzed on the effect of anticoagulant treatment on in-hospital mortality, using a Cox proportional hazards model adjusted for age, sex, ethnicity, body mass index, and cardiovascular risk factors. 28% of patients received systemic anticoagulant treatment during their hospital course, the indication of which is not reported. In this study, therapeutic anticoagulation was associated with increased survival, particularly in mechanically ventilated patients [10].

A similar study performed at the same time in the USA analyzed ~1000 hospitalized patients, comparing those receiving anticoagulation with those not receiving anticoagulation using propensity score matching of baseline characteristics [11]. In the whole group, there was no difference in outcome. However, among patients requiring invasive mechanical ventilation, empiric therapeutic anticoagulation was associated with lower mortality, albeit at the cost of higher incidence of bleeding.

A meta-analysis was performed on observational studies that appeared since then. 16 studies, mostly of low quality, were pooled in a random effect model. The analysis suggested that anticoagulation was associated with lower mortality, although heterogeneity was large [12].

11.3.2 Trials on Preemptive Anticoagulant Treatment

A small phase IIb trial performed early in the pandemic randomized 20 mechanically ventilated patients to therapeutic or prophylactic dose of enoxaparin, showing an increase in ventilator-free days in the therapeutic group when compared to the prophylactic group [13].

In 2020, a collaboration between three large multiplatform RCTs took place, which were all investigating the same research question. The ATTACC trial (antithrombotic therapy to ameliorate complications of COVID-19) involves 58 sites in Canada, the USA, Brazil, and Mexico. The REMAP-CAP trial (randomized embedded

multifactorial, adaptive platform trial on community-acquired pneumonia) involves 290 sites in Canada, the USA, the UK, Ireland, the EU, Saudi Arabia, Australia, New Zealand, Nepal, India, and Pakistan. The ACTIV-4a trial (accelerating COVID-19 therapeutic interventions and vaccine) involves 60 sites in the USA and Spain. These platforms were independent but harmonized their study protocols to obtain common primary and safety outcomes while applying the same superiority and futility rules.

Patients with severe COVID-19, defined as the requirement for organ support with high-flow nasal cannula, noninvasive ventilation, invasive ventilation, vasopressors, or inotropes, were randomized to receive therapeutic anticoagulation (as per hospital policy) with heparin or pharmacological thromboprophylaxis as per local usual care. The primary outcome was being alive and free of organ support.

Preliminary, non-adjudicated data on thrombotic and bleeding outcomes have been made public [14]. In patients with severe COVID-19 (requiring ICU admission), the trial was stopped because the predefined criteria for futility were met. Incidence of thrombosis was decreased with 5.7 vs. 10.3%. Despite this supposed benefit, therapeutic anticoagulation did not improve hospital survival or days free of organ support compared to usual care, while bleeding risk was slightly increased.

Thereby, empiric anticoagulation does not seem favorable in the critically ill and is not recommended. Pending availability and review of the finalized multiplatform trial data, guideline panels have not changed their recommendation regarding intensified thromboprophylaxis. However, an individualized assessment of the patient's risk of thrombosis and bleeding is important when deciding on anticoagulation intensity, although risk assessment models to estimate thrombotic and bleeding risk in hospitalized patients have not yet been validated in patients with COVID-19.

11.4 Observational Studies on Antiplatelet Treatment on Thrombosis and Outcome

Given that platelets are activated, there is a rationale to prescribe antiplatelet therapy. No randomized trials are yet available on the efficacy of antiplatelet therapy in COVID-19.

The largest observational study on this subject was performed in hospitalized as well as ambulant patients in a US healthcare system during the first wave, comparing outcomes among those who were and were not receiving antiplatelet medication for unrelated indications at the time of COVID-19 diagnosis using propensity-matched analysis. The study found no statistically significant difference in survival or time-to-mechanical ventilation between the two groups [15]. In March 2021, a systematic review was performed, identifying 6 studies with nearly 6000 patients [16]. After pooling of results of two studies, results of an unadjusted analysis revealed an association of increased mortality in COVID-19 patients on antiplatelet agents. Adjustment was done to account for comorbidities such as cardiovascular disease, diabetes, or other factors that can lead to increased risk of taking antiplatelet medication and increased risk of higher mortality rate not directly associated with the

efficacy of antiplatelet agents. This adjusted analysis showed no harm of antiplatelet agents and also no benefit. However, even after propensity matching or adjustments, confounding by indication cannot be ruled out, as studies were retrospective in nature.

11.5 Observational Studies on Antifibrinolytic Treatment

Fibrinolysis is severely impaired in critically ill COVID-19 patients, which may provide rationale for (low-dose) antifibrinolytic therapy. Limited case series have described the effects of low-dose tPA for patients with ARDS due to COVID-19, showing an initial improvement in P/F ratio, although these improvements were mostly transient [17]. Bleeding complications were not reported.

11.6 Ongoing Trials

Anticoagulation in COVID-19 is heavily researched. In April 2021, a comprehensive review summarizing all ongoing randomized controlled trials on optimal anticoagulant therapy in COVID-19 with different disease severity was published [18]. Of over 80 trials, only 4 have released results at that time, all of which are discussed in this chapter.

Trials are evaluating all kinds of regimes, doses, and agents, including heparin (both systemic and inhaled), direct oral anticoagulants (DOACs), aspirin, P2Y12 inhibitors, dipyridamole, prasugrel, dociparstat, nafamostat, and a combination of these drugs. Six RCTs include the use of tissue plasminogen activator (tPA). Most trials exclude pregnant women, bleeding patients, and patients with renal impairment. This large research activity is likely to inform us on optimal management in the coming years.

11.7 Monitoring Anticoagulant Treatment

Monitoring of the appropriateness of the level of anticoagulation of unfractionated heparin is a particular challenge in COVID-19. Numerous variables can influence activated partial thromboplastin time (aPTT) measurements. The aPTT may be prolonged in some COVID-19 patients due to a consumptive coagulopathy in the most severely ill, and possibly due to the presence of a lupus anticoagulant. However, a general observation is that aPTT may also be diminished, which may be due to high levels of factor VIII that is shed by the highly activated endothelium.

Another challenge is the possible development of heparin resistance in the setting of an acute-phase response. Heparin resistance is defined as the requirement of high doses of unfractionated heparin (>35,000 units/day) to achieve a therapeutic range. This phenomenon occurs due to the ability of heparin to bind to various acute-phase proteins as well as to an activated endothelium. In COVID-19, a low antithrombin level does not appear to be a major factor in the occurrence of heparin resistance. A

practical solution for managing appropriate anticoagulation in the face of heparin resistance is to measure both aPTT and a concomitant anti-factor Xa heparin level.

Another solution may be to switch to LMWH. Half-life of LMWH is prolonged in patients with renal impairment. Also, there is no antidote, rendering this agent impractical in critically ill patients who often are in need of invasive procedures, such as gaining central venous access. Of note, prophylactic LMWH doses have been used in critically ill patients with impaired renal function without adverse effects [19]. However, full anticoagulant LMWH dose may confer a higher bleeding risk.

Alternatively, viscoelastic testing may be useful to monitor UFH therapy in the setting of an acute-phase response. In vitro, blood of healthy volunteers incubated with UFH and subjected to different ROTEM tests demonstrated a linear correlation between UFH level and clotting time (CT) in the ROTEM® test with a correlation coefficient of 0.92 [20]. Also in patients, correlation between anti-Xa and CT was good [21]. Whether these assays perform well in COVID-19 remains to be elucidated.

11.8 Bleeding Complications of the Anticoagulant Management in COVID-19

Comprehensive assessment of the thrombotic and hemorrhagic event rates is critical in the assessment of the disease course for COVID-19 and for considering strategies to mitigate patient outcomes. However, bleeding as an outcome is subjective and not easily captured in retrospective studies. To date, there is only one prospective study that has investigated bleeding events in COVID [22]. In this study in a single hospital in France analyzing patients during the first wave, only 4 out of 150 patients (2.7%) experienced major bleeding, of which 1 patient was under anticoagulant treatment. This study and other observational studies have been pooled in a meta-analysis from five studies with a total of 1600 patients [23]. It was found that pooled incidence of major bleeding was 3.9%. Again, heterogeneity was high. Not surprisingly, pooled incidence estimate of any bleeding was higher for patients receiving intermediate- or full-dose anticoagulation (21.4%).

This high incidence is somewhat in contrast to data from recent RCTs. In pooled data from the three RCTs comparing preemptive anticoagulation to standard of care, bleeding occurred in 3.1% vs. 2.4% in the critically ill and in 1.6% vs. 0.9% in ward patients. In the INSPIRATION trial comparing intermediate prophylactic dose to standard of care, bleeding occurred in 4.3 vs. 1.7%.

Thereby, the risk of bleeding from intensified empiric anticoagulant treatment appears to be slightly increased.

11.9 Duration of Prophylaxis or Anticoagulant Treatment in COVID-19 Patients

Reports have discussed pulmonary embolism as a reason for readmittance in patients discharged after COVID. It is not known, however, whether thromboprophylaxis is required post-discharge.

In a report on the incidence of post-discharge thrombosis in 1877 COVID patients not taking thromboprophylaxis after discharge from the hospital, episodes of thrombosis were diagnosed within 42 days, giving a post-discharge rate of 4.8 per 1000 discharges, which was only slightly higher than in the previous pre-COVID year (3.1 per 1000 discharges). Other reports showed similar event rates [24–26]. However, trials formally evaluating the need for extended thromboprophylaxis are still required, as this knowledge gap results in differential recommendations in guidelines from different societies [27].

The same holds for patients with COVID-related thrombosis; it is not known what the optimal duration of treatment is. For COVID patients with a thromboembolic complication but without other risk factors for thrombosis, guidelines generally recommend to treat for 3 months, similar to infection-provoked thrombosis due to other causes [27].

11.10 Conclusion

As thrombotic complications in COVID-19 are associated with in-hospital mortality, thromboprophylaxis should be given to all hospitalized patients with COVID-19. Risk of thrombosis is related to COVID-19 disease severity, but from this observation, it does not follow that intensity of anticoagulation should be increased for the most severely ill. Preemptive full anticoagulation may not be beneficial in the critically ill in terms of mortality. Several trials are ongoing which will inform us on optimal coagulation management in COVID-19 in the coming years. Until then, treatment regimens from non-COVID-19 guidelines can largely be adapted.

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ECMO in COVID-19: Bleeding and Thrombosis

12

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12.1 Introduction

The pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an ongoing medical problem worldwide [1, 2]. Most COVID-19 patients present with mild or moderate symptoms, but a small patient population has severe disease manifestations resulting in respiratory failure, myocarditis, septic shock, and multiorgan failure. Approximately 15–31% of patients hospitalized with severe acute respiratory syndrome coronavirus 2 pneumonia develop coronavirus-induced acute respiratory distress syndrome (ARDS) [3–6]. In this subgroup, and despite maximal cardiopulmonary support and invasive mechanical ventilation, mortality remains high [7, 8].

Extracorporeal membrane oxygenation (ECMO) could offer lifesaving rescue therapy when maximal conventional strategies fail [9]. The most common clinical scenario in patients requiring ECMO is ARDS refractory to standard lung-protective ventilation strategy, prone positioning, and neuromuscular blockade [10, 11]. Veno-venous ECMO (V-V ECMO) is the modality of choice and criteria commonly adopted for implantation are $PaO_2/FiO_2 < 150$ mmHg and/or arterial blood pH <7.2 and $PaCo_2 > 60$ mmHg [12, 13] (Fig. 12.1).

In addition to respiratory compromise indications (Table 12.1), patient's cardiovascular function may be severely depressed, such as in severe myocarditis or

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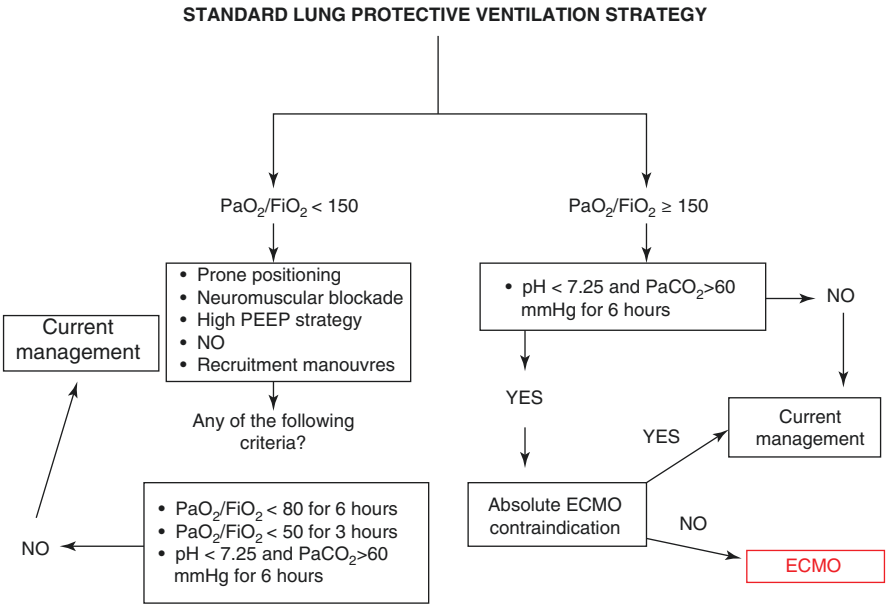

Fig. 12.1 Algorithm for acute respiratory distress syndrome management of ARDS (adapted from Abrams and associates [10]). *ECMO* extracorporeal membrane oxygenation, *NO* nitric oxide

Table 12.1 Extracorporeal membrane oxygenation indications and contraindications in COVID-19 patients

Indications	Contraindications
Refractory hypoxemia despite prone positioning and adequate PEEP	Multiple comorbidities
ARDS requiring vasoactive drugs	Immunocompromised status
Evidence of no more than one organ failure with minimal comorbidities	Severe global developmental delay
	Intracranial bleeding
	Severe irreversible brain damage
	Severe multiple-organ failure
	Mechanical ventilation >10 days

Adapted from Shekar and associates [86]
ARDS acute respiratory distress syndrome, *PEEP* positive end-expiratory pressure

sepsis-related cardiomyopathy. These latter conditions may need a venoarterial ECMO as primary support [14–16].

In the first phase of the COVID-19 infection outbreak, ECMO therapy was not used in significant numbers, and the early and small series of patients reported on excessive mortality rates [17–19]. Initially, the Extracorporeal Life Support Organization (ELSO) did not support the use of ECMO for COVID-19 patients with severe cardiopulmonary failure [20]. Afterwards, Paris-Sorbonne University

Hospital Network documented a 60-day mortality rate of 31% in COVID-19 patients treated with ECMO [21]. This encouraging result was confirmed by a multicenter study by the ELSO, in which the estimated in-hospital mortality 90 days after ECMO initiation was 37.4% (38% in ARDS cohort), consistent with previous mortality rates in non-COVID ECMO patients with ARDS [22, 23]. Pre-pandemic ELSO registry reported indeed 40% mortality in V-V ECMO and 55% in V-A ECMO. COVID-19 data on V-A ECMO are still limited and less clear. Another multicenter retrospective study conducted in five European countries (France, Germany, Italy, Sweden, UK) documented an in-hospital and 6-month mortality rate of 53% in patients who underwent ECMO of any configuration, much higher than that in previous studies [24]. This study showed that bleeding and thromboembolism are frequent complications among ECMO-treated patients with COVID-19 (stroke 14.4%, pulmonary embolism 13.6%, deep vein thrombosis 11.4, need for red blood cell transfusion 79%).

In EOLIA trial, the frequency of bleeding complications in non-COVID ECMO was 53% [11]. ELSO registry data from 2014 to 2019 reported lower rate of bleeding complications (24%) but the rate of circuit-related complication (pump failure, oxygenator failure, needs for circuit change) was 25% [25, 26]

Thrombotic and bleeding complications are common during V-V ECMO [27]. Bleeding still remains a major extracorporeal life support (ECLS) complication, including the intracerebral bleeding as the most dreaded. This strongly impacts short-term outcomes and was reported in 3.8% in the last ELSO database. Likewise, bleeding occurred in 29% of patients affected by A-(H_1N_1) influenza [28]. Given the significant impact of bleeding, the strict anticoagulation target decreased over the last decade, therefore giving room to the other side of the coin, represented by the thromboembolic events. The real incidence of thromboembolic events, which accounts for 50%, might be highly underestimated in clinical practice and only discovered during postmortem examinations, as Rastan and associates showed in their autoptic experience [29]. Obviously, the most common finding is the partial vein thrombosis of the cannulated vessels which was identified in 9.5% of 127 patients requiring ECLS for ARDS [30].

Under this light, the novel SARS-CoV-2 is defined as MicroCLOTS (Microvascular COVID-19 lung vessels obstructive thrombo-inflammatory syndrome) which underlines the evidence supporting a key role of vascular inflammation and microthrombosis in the pathophysiology of COVID-19 [31]. This evidence is well shown in the current literature data.

In a recent ELSO registry collecting 1,035 patients with COVID-19 who received ECMO support, central nervous system hemorrhage occurred in 6%, whereas central nervous system infarct occurred in only 0.7%. Yet, a large experience from the Paris ECMO-COVID-19 investigators collected 302 patients, showing in 43% major bleeding event (which included 12% of intracranial hemorrhage [ICH]), in 18% pulmonary embolism, and in 3% ischemic stroke [22].

Shaefi and associates studied a population of 190 patients treated with ECMO and admitted to 68 hospitals across the United States [32]. They described bleeding

events in 27.9% of patients, and among them 4.2% suffered of ICH. Differently, thrombotic events were identified in 22.6% of cases: 18.4% belonged to deep vein thrombosis and only 1.6% were pulmonary embolism and ischemic stroke. In another European multicenter experience, Biancari and associates found pulmonary embolism in 13.6% and deep vein thrombosis in 11.4%, whereas red blood cell transfusion was required in 79.5% of patients [24]. Furthermore, Durak and associates investigated 77 patients supported with ECMO who developed thrombotic and hemorrhagic events in 71% of cases. Forty-one percent were thromboembolic events (among them, 29% developed a pulmonary artery embolism) and 59% were bleeding complications [33].

It has become evident that severe inflammatory state secondary to COVID-19 leads to a unique derangement of hemostasis recognized as COVID-19-associated coagulopathy (CoAC) [34–36]. Proposed underlying mechanisms include an excessive immune response-mediated cytokine storm, endothelial pathology, intussusceptive angiogenesis, and hypercoagulability [37–39]. This procoagulant pattern may be associated with microthrombosis and macrothrombotic events, leading to an increase in morbidity and mortality [40–42].

ECMO therapy itself leads to pathophysiological changes in both immune and hemostatic system. In critically ill patients requiring extracorporeal support, the leading causes of mortality and morbidity remain thrombosis and bleeding; excessive bleeding is the most common reason for premature separation from ECMO [43–45]. Among COVID-19 patients requiring ECMO, much less is known regarding the resulting risk profile for the development of both thromboembolic and hemorrhagic complications due to unknown interaction between hypercoagulability of COVID-19 and ECMO support [46]. Inflamed lung connective tissue and pulmonary endothelial cells may result in microthrombus formation and contribute to the high incidence of thrombotic complications in severe COVID-19. ECMO could aggravate the activation of the coagulation cascade and consumption of clotting factors, causing additional coagulation abnormalities.

12.2 ECMO-Associated Coagulopathy

Supraphysiological shear stress and interactions between blood and nonendothelial surfaces during ECMO result in coagulation and fibrinolytic pathway activation and a complement-mediated inflammatory response (Table 12.2). The extensive interaction between foreign surface and plasma proteins produces a layer represented by fibrinogen, albumin, and γ-globulins, on the surface of the circuit and oxygenator. Fibrinogen strongly triggers platelet adhesion to its receptors. Simultaneously, factor XII is activated to factor XIIa, leading to generation of pre-kallikrein and high-molecular-weight kininogen and activation of factor XI and factor X. Activated factor XI and factor X elicit prothrombin activation to thrombin. Damaged endothelial surface releases tissue factor, the most powerful trigger for thrombin generation. Thrombin plays a major role in inflammatory response; it stimulates the expression of P-selectin and E-selectin by endothelial cells, increasing neutrophil adherence

and activation. Thrombin generation induces also platelet activation; adherent platelets release their granular content including chemokines, pro-inflammatory cytokines, and hemostatic factors. The long-lasting characteristics of ECMO run (days or weeks) induce a condition of chronic thrombin generation, the main trigger for both hemorrhagic and thromboembolic complications. Systemic anticoagulation is intended to control thrombin generation. This procoagulant state is counterbalanced by an excessive fibrinolytic response mediated by plasmin. Plasmin cleaves fibrin, releasing fibrin degradation products. Hyperfibrinolysis could be one of the factors leading to hemorrhage [45–49].

Despite the improvements in materials and configurations of ECMO circuits [50], hemorrhagic and thromboembolic complications remain the most frequent causes of death [51]. The alterations in blood circulation and the interactive effects between the COVID-19 patient and ECMO circuit are reflected by a prothrombotic pattern of variable magnitudes (Fig. 12.2).

Table 12.2 Hemostatic system abnormalities related to extracorporeal membrane oxygenation

Activation	Consumption
Blood-foreign material interface	***Thrombin mediated***
• *FXIIa, kallikrein*	
Tissue factor release	***Plasmin mediated***
• *Tissue injury*	
• *Monocyte related*	
Fibrinolysis activation	***Inflammation mediated***
• *Tissue plasminogen activator release*	• *Elastase*
• *Urokinase-type plasminogen activator release*	• *Complement*
• *Intrinsic activation*	• *Leukocyte-platelet complexes*
• *Heparin*	

Determinants of activation and consumption of coagulation cascade

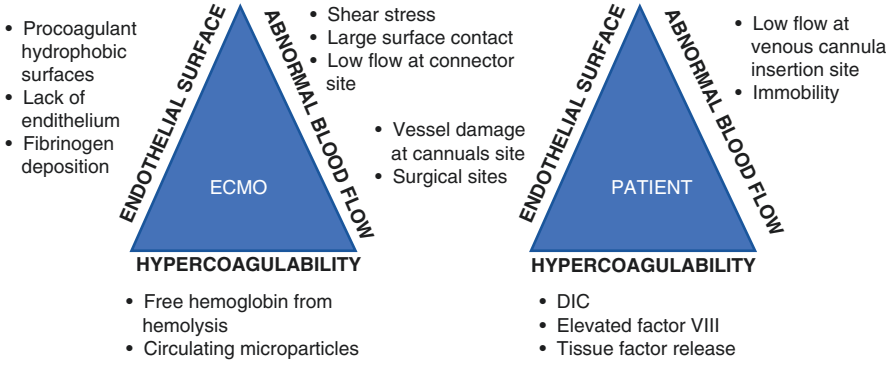

Fig. 12.2 Prothrombotic changes described by Virchow's triad with respect to ECMO circuit and patient factors. *DIC* disseminated intravascular coagulopathy

Table 12.3 ECMO-mediated coagulopathy: prothrombotic and pro-hemorrhagic determinants

Prothrombotic factors	Pro-hemorrhagic factors
Acquired antithrombin deficiency	Consumption of coagulation factors
Protein C-S complex consumption	Low fibrinogen levels
Tissue factor pathway inhibitor consumption	Thrombocytopenia
Endothelial dysfunction	Platelet dysfunction
Heparin-induced thrombocytopenia	Hyperfibrinolysis
Endotoxins	Acquired von Willebrand disease
Inadequate anticoagulation	Excessive anticoagulation

Thrombus deposition in the membrane oxygenator, in the circuit or, rarely, inside the pump, can lead to oxygenator failure and thromboembolism (stroke, mesenteric infarction, and peripheral arterial thrombosis). The causes of thrombosis and bleeding are multifactorial (Table 12.3).

Microclot formation has been identified as a determinant of ischemic organ dysfunction [51]. Contact with foreign surfaces activates the complement system inducing the synthesis of pro-inflammatory (IL-6, IL-8, TNF-α) and anti-inflammatory (IL-10) cytokines. Pro-inflammatory cytokines exacerbate endothelial dysfunction and increase vascular permeability. During ECMO run, heparin-induced thrombocytopenia (HIT) represents a quite common prothrombotic condition caused by circulating heparin-platelet factor 4 complex antibodies [52–54]. Treatment of suspected or confirmed HIT includes removing all exposure to heparin; options for alternative anticoagulation include direct thrombin inhibitors as well as fondaparinux and danaparoid [55, 56].

Acquired deficiency of antithrombin in ECMO is the result of hemodilution and consumption due to the use of heparin. Supplementation of AT may be necessary to restore adequate anticoagulation [57–59].

At the same time, even the causes of bleeding in ECMO are multifactorial and many cellular interactions leading to adequate hemostasis may be disturbed in these patients. Thrombocytopenia is common in critically ill patients and it is consistently associated with increased bleeding and mortality [60, 61]. During ECMO, platelets are constantly exposed to activation, resulting in recurrent dysfunction and reduction in number [62]. Activation of fibrinolysis may be either a primary plasmin-mediated process or a secondary thrombin-mediated activation. Hyperfibrinolysis should be suspected if bleeding is associated with very high level of D-dimer and relatively normal platelet level [63]. Exposure to elevated shear forces from the ECMO circuit leads to a loss of large multimers of the von Willebrand factor with a consequent defect of the platelet adhesion to the disrupted endothelium. Acquired von Willebrand syndrome has been correlated to an increase in bleeding typically from the respiratory tract, mucosal surfaces, and puncture sites [64].

12.3 COVID-19-Associated Coagulopathy

CoAC is characterized by a prothrombotic state, clinical hypercoagulopathy, and high incidence of thromboembolism (Table 12.4). Several studies identified a procoagulant profile at both standard and viscoelastic tests [65–67]. The main findings are summarized in Fig. 12.3.

Table 12.4 COVID-19-associated coagulopathy characterization by standard laboratory and ELISA tests, and viscoelastic point-of-care tests

Standard test	Viscoelastic test
Fibrinogen ⇑	R or CT value ⇓
D-dimer ⇑	K angle ⇑
PF 1 + 2 ⇑ (thrombin generation ⇑)	MA or MCF ⇑ (clot firmness ⇑)
tPA ⇓ (fibrinolysis shutdown)	Lysis 30 ⇓ (fibrinolysis shutdown)
PAI 1−2/PAP⇑ (fibrinolysis shutdown)	
PAI 1−2 ⇑ (fibrinolysis shutdown)	

CT clotting time, *MA* maximum amplitude, *MCF* maximum clot firmness, *PAI* plasminogen activator inhibitor, *PAP*: plasmin-antiplasmin complex, *PF* prothrombin factor, *tPA* tissue plasminogen activator

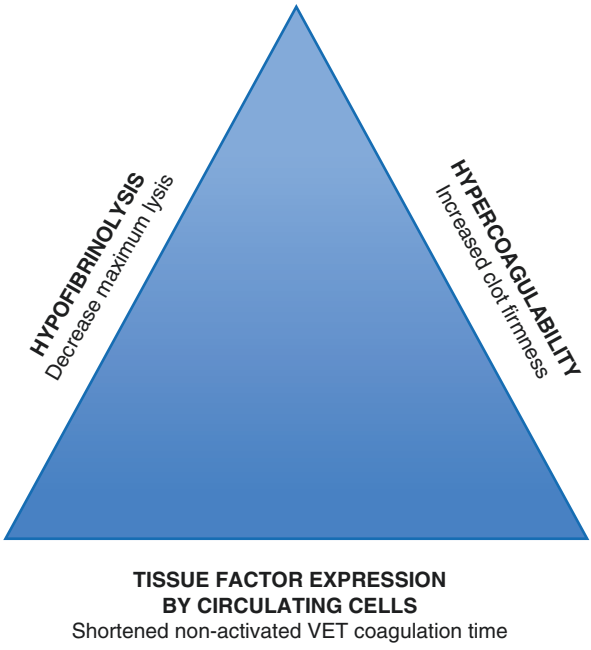

Fig. 12.3 Patterns of hemostasis at viscoelastic tests (VET)

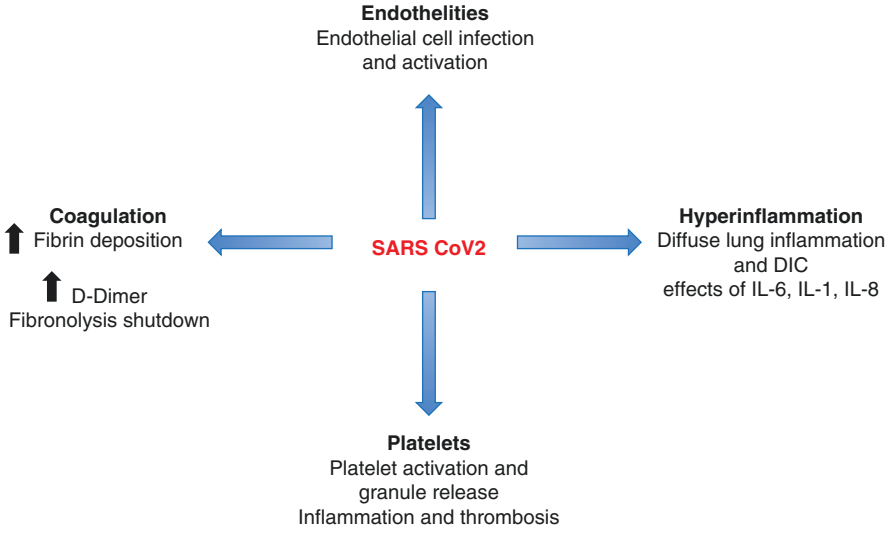

Fig. 12.4 Effects of COVID-19 on coagulation system. *DIC* disseminated intravascular coagulopathy

SARS-CoV-2 binds ACE2 receptors on human cells and may attack the endothelium through the renin-angiotensin system, leading to its activation. Endothelial dysfunction due to inflammation and hypoxia is the principal trigger for CoAC and results in excessive thrombin generation and fibrinolysis shutdown (Fig. 12.4) [68, 69]. Healthy endothelial cells regulate vascular tone and permeability and prevent leukocyte and platelet adhesion with nitric oxide production. Infected endothelial cells lose their physiological functions, especially the antithrombotic activity. COVID-19 patients manifest a rise in inflammatory biomarkers like IL-6, and a decrease in lymphocyte count, both associated with poor outcome [70, 71]. The excess production of pro-inflammatory cytokines, also known as cytokine storm, increases endothelial damage and stimulates thrombosis. CoAC is associated with a high incidence of VTE (24%) and PE (15%); thromboprophylaxis is recommended in all hospitalized COVID-19 patients, but optimal drug and dose are actually under investigation [72]. In the intensive care unit (ICU) population, the reported incidence of thromboembolic complication is higher, ranging from 21 to 69% [73–76].

Recently, Thachil and associates proposed a classification of stages of COVID-19 coagulopathy. This classification considers lungs as the center of hemostatic abnormalities and uses available and common diagnostic biomarkers (Table 12.5) [77].

Stage 1 is represented by patients with mild symptoms, hospitalized in non-ICU wards, in whom pulmonary microthrombi are localized at the peripheral microvasculature. Stage 2 includes patients with lung ventilation/perfusion mismatch and thromboembolism at the tomographic scan. These patients may need ICU support. Stage 3 includes critically ill patients with pulmonary or extrapulmonary thrombotic manifestations who need mechanical ventilation or ECMO. At this stage,

Table 12.5 Stages of COVID-19-associated hemostatic abnormalities (adapted from Thachil and associates [77]): time-dependent changings from early to late phase

Factor	Stage 1	Stage 2	Stage 3
Fibrinogen	Increased	Increased	Decreased
Platelet count	Normal	100–150 × 10^9/L	<100 × 10^9/L
D-dimers	2–3 times > ULNR	3–6 times > ULNR	>6 times ULNR
Inflammation	Local	Systemic	Hyperinflammation
DIC	No	No	May be present
Plasminogen activity	Increased	Moderately increased	Relative deficiency

DIC disseminated intravascular coagulation, *ULNR* upper limit of normal range

patients may develop disseminated intravascular coagulopathy (DIC) and bleeding events.

Two different studies reported similar overall bleeding rates (respectively, 8% and 7.6%) in critically ill COVID-19 patients, higher than in non-COVID population. Intrapulmonary microhemorrhage and gastrointestinal hemorrhage are the predominant sites of bleeding [78, 79]. These findings can represent a true increase in bleeding risk due to immune mechanism related to COVID-19 as well as a result of anticoagulation.

12.4 Thrombosis and Bleeding on ECMO in COVID-19: Diagnosis and Treatment

The hemostatic profile of COVID-19 patients also requiring ECMO is affected by timing.

The course of the disease evolves from the initial phase in which virus-related endothelial dysfunction and fibrinolytic shutdown concur in determining inflammatory and coagulation disarrangement typical of ECMO, leading to an overexpression of the hemostatic system in a prothrombotic sense. Later on, whole-body micro- and macro-clots lead to a consumption coagulopathy that involves circulating factors and platelets, synergistically combining with systemic anticoagulation for extracorporeal circulation, leading to a pro-hemorrhagic pattern.

As the ECMO run proceeds, consumption of coagulation factors and von Willebrand acquired disease increase the bleeding risk. The hyperinflammatory state associated with COVID-19 disease can first exasperate the prothrombotic response at the start of extracorporeal support; subsequently, a transition to a pro-hemorrhagic state is possible, similar to a DIC-like disease [80, 81].

As previously described, hemorrhagic stroke during ECMO in COVID-19 patients can be not infrequent: timing for diagnosis remains crucial and it is even more so in this highly selected population to optimize resources in a hostile environment. Usman and associates [82] in their series reported 40% of hemorrhagic strokes (parenchymal or sub-arachnoid hemorrhage) with a mortality rate of 75%. Notably, the authors reported no significant differences in anticoagulation levels that remained in therapeutic ranges both for who experienced the event and those

who did not, but they found higher levels of fibrinogen and more circuit thromboses that required replacement in patients with stroke.

These data are different with respect to non-COVID ECMO for ARDS, as previously reported by Lorusso and associates (ICH 3.6%) [83], by the CESAR investigators (4% neurologic injuries) [23], and in the EOLIA trial (2.4% hemorrhagic stroke) [11].

Recently, the ELSO has created a live COVID ECMO dashboard, and the report recorded less than 1% of stroke and 5% ICH [84].

Hyperfibrinolytic response may have a role in ICH in the late stage of COVID-19 disease, in which progressive microclot accumulation triggers plasmin activation: even if in viral infection and inflammatory context D-dimers can also be altered, and fibrin clot cleavage into fibrin degradation products may represent an indicator of altered hemostatic response in a population characterized by hyperactivity in fibrin net synthesis, exacerbated by extracorporeal membrane oxygenation.

On this basis Seelhammer and associates [85] supported the use of antifibrinolytic agents to counteract a thromboelastographic evidence which accounts for 7% of their daily assays. Titration of tranexamic acid by thromboelastography has been described to mitigate this condition in a high-risk population according to previous experiences in non-COVID population.

A diagnosis of stroke may be suspected based on bedside findings of focal neurologic deficits: unless the patient is kept awake on ECMO, these signs are subtle in patients treated with heavy sedation and neuromuscular blockade agents. Once suspected, a formal emergency neurologic consultation and a computed tomography (CT) scan should be implemented to define the cost-benefit ratio of anticoagulation levels and trigger partial deviations from the institutional protocol.

Recent reports have identified an increase in the incidence of prolonged activated partial thromboplastin time (aPTT) at baseline in COVID-19, with the majority of these cases being positive for lupus anticoagulant and without evidence of bleeding but conversely higher risk of thrombosis. Therefore, the recent ELSO Guidelines suggest targeting to higher level of anticoagulation during ECMO in COVID-19 patients and evaluating if the hypercoagulable status may benefit from antiplatelet agents, even in the absence of clear data for recommendation [86].

Seelhammer and associates [87] reported their experience for ECMO anticoagulation in COVID-19 with the direct thrombin inhibitor bivalirudin, endorsing its use. The potential benefit for this population includes the ability to exert its effect by directly attaching to and inhibiting freely circulating and fibrin-bound thrombin. Additionally, bivalirudin anticoagulation is not influenced by plasmatic levels of antithrombin, and is characterized by a reliable pharmacokinetics because of its largely (but not completely) organ-independent clearance. Furtherly, HIT is prevented by avoidance of heparin.

Bivalirudin drawbacks in COVID-19 patients with ECMO mainly correlate to the evidence that many of them need continuous renal replacement therapy: this may affect blood drug concentration with fluctuations that lead to under- or overdosing; the latter can be dangerous given that no antidote is available.

Immune-mediated HIT2 represents a potential additional risk in COVID-19 patients undergoing ECMO and anticoagulated with unfractionated heparin; even the general incidence on ECMO is reported to be around 0.36% [88].

Phan and associates [89] reported an ECMO COVID-19 case of HIT2 confirmed by anti-PF4/heparin antibodies, complicated by two subsequent ECMO circuit thromboses requiring oxygenator change and treated with rivaroxaban, an oral factor Xa direct inhibitor (15 mg twice daily by nasogastric tube), in conjunction with an adsorbent cartridge in hemoperfusion to remove PF4/heparin antibodies. The patient was then switched to argatroban anticoagulation for an additional 42 days of ECMO without thrombotic or hemorrhagic events, then weaned, and discharged.

The group of Daviet and associates [90] described a series of ECMO COVID-19 cases showing multiple deep venous thromboses, intracardiac thrombosis, and thrombosis of pump and artificial lung membrane, treated with conversion to argatroban. The authors reported a quite impressive incidence of HIT in COVID ECMO compared to their previous publication in non-COVID ECMO (21% vs. 2%), and hypothesized that the extensive use of pre-ECMO antithrombotic prophylaxis with low-molecular-weight or unfractionated heparin in COVID-19 patients could be a possible cause.

Although the major thromboembolic and hemorrhagic complications of COVID-19 patients can occur inside the patient, the presence of ECMO certainly makes it a trigger but at the same time a "victim."

The effect created through the interaction between endothelium, blood, and materials promotes a redundant response that we have seen to be related to the timing of the disease.

Given that most ECMO implants are performed in the early stages, the most evident effects on ECMO circuits are those related to thrombosis of one or some of its parts.

Bemtgen and associates [91] reported in their ECMO V-V population 63.6% of thrombotic events in COVID-19 versus 18.2% in their historical non-COVID comparison cohort. COVID-19 patients had a higher probability of circuit replacement, a significantly longer aPTT, and a significantly higher level of D-dimers.

Therefore, the standard target aPTT could be an unreliable indicator of the degree of anticoagulation in COVID-19; however, the authors' conclusion was that the indication for more extensive anticoagulation would have required larger studies.

Similar results were reported by Ahmadi and associates [92] in their series, finding an increased ECMO circuit replacement due to thrombosis that exposed to a greater risk of unsatisfactory outcome.

The early detection of possible thromboembolic and hemorrhagic complications in ECMO represents a fundamental step for COVID-19 ECMO patients. Despite the significant workload required by COVID-19 management, diagnostic imaging is of paramount importance for early diagnosis.

Ripoll and associates [93] showed their experience in the diagnostic evaluation process. They enrolled 30 ECMO patients for respiratory insufficiency associated with COVID-19, implanted in other centers and centralized, who finally underwent CT scans: in 13 a thrombotic event was diagnosed; 4 of these had major bleeding

complications (subarachnoid hemorrhage and pulmonary hemorrhage), and 5 lately developed ICH (4 cases) and subcapsular hepatic bleeding combined with ICH (1 case). Of notice, the time of stay within the therapeutic range of anticoagulation has not been shown to be predictive for thrombosis or bleeding.

Parzy and associates [94] reviewed a cohort of 13 COVID-19 patients who underwent ECMO, with CT scan analysis: all of them (100%) suffered at least one thromboembolic event.

Their findings showed 76.9% isolated venous thrombosis associated with cannulation, 15.4% with isolated pulmonary embolism, and 7.7% with both.

Of those with cannulation-associated thrombosis, 53.8% involved the jugular cannulation site, 76.9% the femoral site, and 46.2% both. Pulmonary embolism was present in 23.1% and the same percentage was associated to HIT with deep venous thrombosis of the femoral axes and about 66% experiencing pump or oxygenator thrombosis.

In agreement with the findings of other authors, also in this study the aPTT ratio was on average higher than the historical cohort as well as the percentage of patients who exceeded an aPTT ratio of 1.8.

All these findings demonstrate that, given that cannulation of peripheral veins is essential for ECMO support, in this population at high risk of thrombosis, especially in the early phase of the disease which generally corresponds to the ideal timing of V-V ECMO implantation, careful monitoring is mandatory. Mechanical obstruction (Fig. 12.5) due to the presence of cannulas both at the level of the inferior and superior vena cava should be carefully evaluated because it can dangerously contribute to the stagnation or reduction of the venous drainage flow in the areas around the cannula, in particular of the inferior vena cava characterized by an anti-gravitational path and by the presence of venous valves.

ELSO Guidelines for COVID-19 raise awareness among clinicians on this aspect, helping to provide recommendations on the most suitable cannulation configurations: as an example, dual-lumen cannulas are not recommended and "should be avoided if possible as they take relatively longer time to insert, and are associated with higher risk of thrombotic complications and malpositioning requiring repeat echocardiography with associated increased resource utilization and personnel exposure."

Bleeding on ECMO is a multifactorial complication: acquired von Willebrand syndrome also represents a possible cofactor in hematological disorder leading to severe bleeding on ECMO.

During extracorporeal circulation, high shear stress is known to be causative for destruction of large multimers of von Willebrand factors impairing platelet binding to endothelium and stabilization of factor VIII.

ECMO exposes the artificial surfaces to fibrinogen competitive adsorption in large quantities that in the early presentation of COVID-19 cannot be cleaved due to fibrinolytic shutdown. As a consequence, laminar flow within the circuit may be heavily altered and the shear stress on blood component inevitably increases: the

process of destruction of von Willebrand multimers thus begins its course and takes place subtly until clinical signs of bleeding appear.

Furtherly, in COVID-19, a high pump flow is often necessary to counteract hypoxia; this determines even more shear stress despite large cannulas: additionally, negative drainage pressure effects are often underestimated.

Hayakawa and associates [95] reported a case of acquired von Willebrand disease combined with DIC and treated with cryoprecipitate without modifying the administration of anticoagulant.

The same findings have been clearly described by Kalbhenn and associates [96] in their series of COVID-19 ECMO patients. Platelet count dropped in all cases and aggregometry resulted to be pathological; von Willebrand-collagen binding-to-von Willebrand-antigen ratio decreased as well as factor XIII, with an evident loss of large multimers.

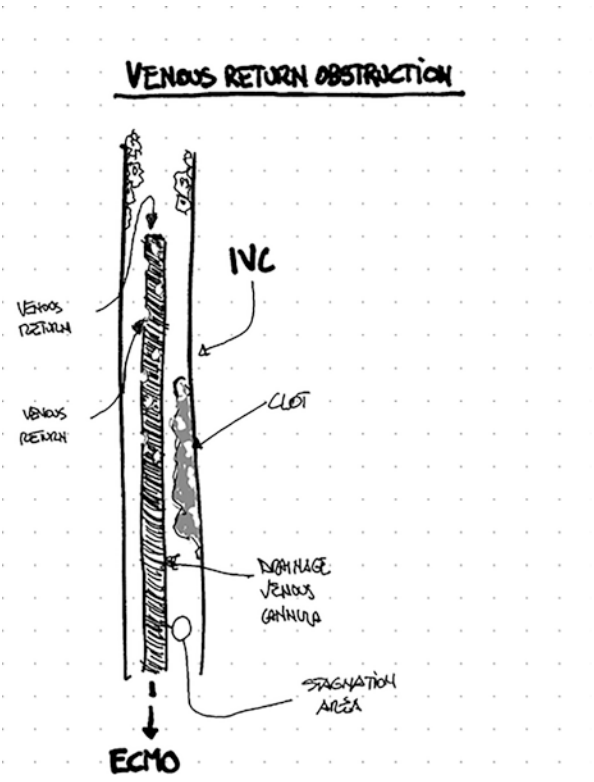

Fig. 12.5 Blood stagnation, drainage cannula, and inferior vena cava (IVC) involvement in clot formation process

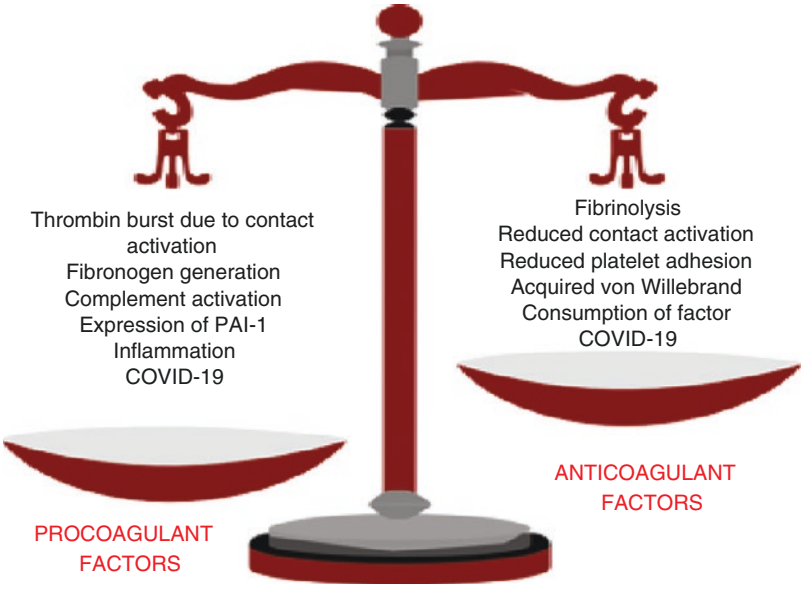

Fig. 12.6 Effect of hyperinflammatory state on coagulation in COVID-19 patients receiving ECMO: unbalanced overexpression procoagulant factor. *PAI* plasminogen activator inhibitor

12.5 Conclusions

All the previous findings clearly point out the high variability of bleeding and thrombotic events in COVID-19 patients supported with ECMO (Fig. 12.6). ECMO and COVID-19 itself are associated with certain, often synergistic, changes in hematological and inflammatory status of the patients. Therefore, the efficacy of ECMO and the related complications are largely dependent on centers' experience. On the other hand, the mechanisms behind this demanding biological interaction are still unknown and a matter of discussion.

In this scenario, COVID-19 patients requiring ECMO support represent a stimulating challenge for practitioners.

In the first stage of ECMO support the procoagulant effect is prevalent; coagulation cascade is activated by the contact phase and the inflammatory response leads to an upregulation of prothrombotic and fibrinolytic pathways. Need for anticoagulation can make situation worse and coexistence of bleeding and thrombotic events requires a careful management. Focus on ideal anticoagulation strategy and its best monitoring becomes mandatory.

Consumption of the determinants of primary and secondary hemostasis is an aggravating factor and adds a consensually detected bleeding risk.

During the ECMO course, the cannulation setup certainly plays a role in determining possible thrombogenic areas: the need for large access and reinfusion sites for the high flows necessary for minimal oxygenation collides with the mechanical obstruction exerted in the venous vessels.

COVID-19 is a dynamic disease, and ECMO is a dynamic technique. Their mutual interaction represents the real challenge for the ECMO team.

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