

## Coagulopathy and COVID-19

Ferdinando Luca Lorini\*, Maria Di Matteo, Paolo Gritti, Lorenzo Grazioli, Alberto Benigni, Lucia Zacchetti, Isabella Bianchi, Fabrizio Fabretti, and Luca Longhi

*Dipartimento di Anestesia e Terapia Intensiva, Ospedale Papa Giovanni XXIII, Bergamo, Italia*

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SARS-CoV-2 infection is associated with frequent thrombotic events, at the micro and macro-vascular level, due to the perpetuation of a state of hypercoagulability. The so-called ‘COVID-19 associated coagulopathy’ (CAC) represents a key aspect in the genesis of organ damage from SARS-CoV-2. The main coagulative alterations described in the literature are represented by high levels of D-dimer and fibrinogen. Although CAC has some common features with disseminated intravascular coagulation and sepsis-induced coagulopathy, there are important differences between these clinical pictures and the phenotype of CAC is unique. The pathogenesis of CAC is complex and is affected by the strong interconnection between the inflammatory system and coagulation, in the phenomenon of immunothrombosis and thrombo-inflammation. Several mechanisms come into play, such as inflammatory cytokines, neutrophils, the complement system as well as an alteration of the fibrinolytic system. Finally, an altered platelet function and especially endothelial dysfunction also play a central role in the pathophysiology of CAC. Heparin has several potential effects in CAC, in fact in addition to the anticoagulant effect, it could have a direct antiviral effect and anti-inflammatory properties. The high incidence of thrombo-embolic phenomena despite the use of antithrombotic prophylaxis have led some experts to recommend the use of anticoagulant doses of heparin, but at present the optimal anticoagulant regimen remains to be determined.

SARS-CoV-2 infection, especially in its severe form, has been associated with frequent venous and arterial thrombotic events, due to the perpetuation of a state of hypercoagulability. Currently, the so-called ‘COVID-19 associated coagulopathy’ (CAC) is considered one of the key aspects of the pathophysiology of SARS-CoV-2 infection. If in the initial stages the vascular damage and the activation of coagulation are localized at the pulmonary micro-vascular level, when the disease progresses there is a systemic involvement that can result in thrombosis at the level of other organs distant from the lung, with consequent multi-organ dysfunction.

In the early phase of the pandemic, several publications have highlighted a very high incidence of thrombo-embolic events (VTE).<sup>1</sup> Thrombotic events affect up to one-third of

patients with COVID-19, and are associated with greater disease severity and increased mortality. Thrombotic manifestations affect not only the venous side but also the arterial one. Furthermore, alongside the macro-vascular complications, COVID-19 appears to be strongly associated with micro-vascular thrombosis.<sup>2</sup> Pulmonary micro-vascular thrombotic phenomena had already been described in cases of severe non-COVID-19 acute respiratory distress syndrome (ARDS), but this feature appears to be much more pronounced in COVID-19 ARDS with a frequency of micro-thrombi nine times higher.<sup>2</sup>

An accurate estimate of the true incidence of VTE in patients hospitalized for COVID-19 remains elusive and different studies have reported incidences of 4.8-85%. Several systematic reviews and meta-analyses underline the great variability of the data. A recent meta-analysis demonstrated an estimated overall incidence of VTE of 17%, with

\*Corresponding author. Email: llorini@asst-pg23.it

higher values in the intensive care unit (ICU) population, if a screening protocol for VTE was adopted, or distal deep vein thrombosis (DVT) and sub-segmental pulmonary embolisms (PE) were also included in the analysis.<sup>3</sup> According to this analysis, bleeding complications would be recorded in 7.8% of patients, a frequency influenced by the use of higher doses of anticoagulation.<sup>3</sup>

The discrepancy found in some studies between the incidence of DVT and PE, the presence of non-occlusive filling defects on CT angiography, as well as their segmental or sub-segmental localization has led several authors to believe that these lesions were not 'emboli' rather, '*in situ* micro-vascular thrombosis' secondary to severe COVID-19 lung inflammation.<sup>4</sup>

The main changes in coagulation described in patients with COVID-19 suggest the presence of a hyper-coagulation state triggered by the deep and complex inflammatory response to the virus, which has been called 'thrombo-inflammation'. The practically constant coagulation alteration in COVID-19 patients, particularly those with severe disease, is the elevation of the D-dimer. Other clotting parameters found in CAC include elevated fibrinogen levels, mild platelet count reduction, and, in some cases, prolonged prothrombin time (PT).

Increased D-dimer is the most common laboratory alteration in hospitalized COVID-19 patients. A high D-dimer, although not specific and associated with several medical conditions, in the context of COVID-19 has been consistently reported to be a negative prognostic factor, associated with both increased disease severity and high mortality.<sup>5</sup>

In some preliminary reports, a higher incidence of disseminated intravascular coagulation (DIC) was described in deceased patients than in survivors (71.4% vs. 0.6%)<sup>5</sup>, but subsequently the incidence of DIC was shown to be very low at the time of initial presentation. Although COVID-19-associated coagulopathy has some common features with both DIC and sepsis-induced coagulopathy (SIC), there are several important differences among these clinical pictures.<sup>6</sup> In CAC, in fact, high levels of D-dimer are found, but also high levels of fibrinogen and only minimal alterations in platelet count and PT compared to SIC and DIC, thus missing the typical characteristic of 'consumption coagulopathy'. High levels of pro-inflammatory cytokines (CKs) are known to induce macrophage activation syndrome/haemophagocytic lymphohistiocytosis (MAS/HLH) resulting in a prothrombotic coagulation state. Although the pathophysiology of MAS/HLH appears similar to that of COVID-19, the CK levels found in COVID-19 are much lower, while the endothelial alteration is more marked. Sometimes CAC can take on characteristics that resemble those of thrombotic micro-angiopathy and some authors have found the presence of antiphospholipid syndrome. Although the pathogenesis of these thrombotic disorders partly overlaps, CAC represents a coagulopathy in its own right (Table 1).<sup>6</sup> Iba *et al.*<sup>6</sup> proposed a definition of CAC based on the presence of at least two of the following criteria in patients with established COVID-19: increased D-dimer (>2 times the upper limit of normal), decreased platelet count (<150 × 10<sup>9</sup>/L), PT > 1 or international normalized ratio >1.2, presence of thrombosis. Furthermore,

the presence of one of the following conditions would identify patients at risk for CAC: increased fibrinogen, increased von Willebrand factor (>2 times the upper limit of normal), and presence of high titer antiphospholipid antibodies. Finally, although CAC is a distinct condition from DIC, it can evolve and predispose to DIC as the severity of COVID-19 increases.<sup>6</sup>

Some experts have proposed a 3-stage classification of CAC based on pulmonary changes, the epicentre of the coagulation changes found in COVID-19. In the first stage, patients have mild symptoms, pulmonary micro-thrombi are found only at the micro-vascular and peripheral level so they cannot be detected on CT angiography. Stage 2 identifies patients admitted to ICU, in whom there are alterations in the ventilation-perfusion ratio secondary to thrombotic phenomena evidenced by angio-CT and DVT may coexist. Critical patients on invasive mechanical ventilation or extracorporeal support fall into the 3rd stage and, in addition to pulmonary thrombotic phenomena, have systemic micro/macro-thrombi and may develop DIC.<sup>7</sup>

Standard coagulation tests often fail to identify the pro-coagulant status of COVID-19 patients, as most conventional coagulation parameters are within limits, only the D-dimer and fibrinogen are significantly elevated. Instead, viscoelastic tests (VET), evaluating the characteristics of both formation and lysis of the clot, allow to identify the hyper-coagulation state of COVID-19 patients characterized by an increase in clot strength and a reduction in fibrinolysis up to the so-called 'fibrinolytic shut-down'.<sup>8</sup> Less consistently, some studies have also reported a shorter time of clot formation. Observable changes in VTEs have been associated with an increased risk of thrombo-embolic complications and increased mortality in COVID-19.<sup>8</sup> The hyper-coagulation state described in the VETs would seem to persist over time, without returning to normal values even on discharge from the ICU.

The pathogenesis of CAC is complex, involving not only the coagulation system, but also the immune system, endothelium, and platelets. SARS-CoV-2 can cause micro-/macro-thrombotic phenomena with different mechanisms including CK storm with activation of leukocytes, endothelium, and platelets resulting in up-regulation of tissue factor (TF), activation of coagulation, generation of thrombin and fibrin formation; in addition, an imbalance among the type 1 plasminogen activator inhibitor (PAI-1), TF inhibitors, and activated protein C would stimulate the generation of fibrin, limiting fibrinolysis.

There is a bidirectional cross-talk between inflammation and coagulation in which the two systems cooperate in order to block the spread of the pathogen. This interconnection between the two systems has been called 'immuno-thrombosis', which if uncontrolled causes an unregulated activation of the coagulation cascade resulting in a prothrombotic state known as 'thrombo-inflammation'.

Pneumocytes infected with the virus cause excessive infiltration of the lung parenchyma by monocytes, macrophages, and neutrophils, which then produce high levels of inflammatory chemokines and CKs, such as interleukin (IL)-1 $\beta$ , IL-6, the tumour necrosis factor- $\alpha$  causing a hyper-inflammatory response known as a 'cytokine storm', found

**Table 1** Characteristics of COVID-19 associated coagulopathy compared to other forms of coagulopathy

	Platelets	D-dimer	PT/aPTT	Fibrinogeno	ATIII	C'	Ab anti PL	CK
CAC	↑ o ↓	↑	↔ o ↑	↑	↔	+	+	↑
DIC/SIC	↓	↑	↑	↔ o ↓	↓	-	-	↑
HLH	↓	↔	↔	↔	↔	-	-	↑
APS	↓	↔	↔/↑	↔	↔	-	+	-
aHUS	↓	↔ o ↑	↔	↔	↔	+	-	-
TTP	↓	↔ o ↑	↔	↔	↔	-	-	-

CAC, COVID-19 associate coagulopathy; DIC, disseminated intravascular coagulopathy; SIC, sepsis-induced coagulopathy; HLH, hemophagocytic lymphohistiocytosis; APS, antiphospholipid antibody syndrome; aHUS, atypical hemolytic uremic syndrome; PTT, purombic aPTTemprotic syndrome; purombic aPT purombic time, activated partial thromboplastin time; ATIII, third antithrombin; C', complement activation; Ab antiPL, antiphospholipid antibodies; CK, cytokines. ↑, increase; ↓, decrease; ↔, normal; +, present; -, absent.

in majority of severe cases.<sup>9</sup> The CK storm is able to directly and indirectly activate the coagulation cascade which in turn causes the production of pro-inflammatory mediators with a positive reinforcement mechanism. The endothelial cells damaged by the CK storm recall platelets and release TF, activating the extrinsic coagulation pathway, moreover monocytes and macrophages also express TF in hyper-inflammatory conditions. Elevated levels of IL-6 have been found in COVID-19 patients admitted to ICU. The correlation between the levels of IL-6 and those of fibrinogen confirms the interconnection between the inflammatory response and coagulation.

The first autopsy reports on COVID-19 have suggested a role of neutrophils in CAC due to the presence of microvascular thrombi containing numerous neutrophils, in some cases partially degenerated to form 'neutrophil extracellular traps' (NETs).<sup>10</sup> NETs are fragments of DNA released by neutrophils in order to trap pathogens that activate intravascular coagulation both through the extrinsic and intrinsic pathways (increasing the expression of TF and the activation of factor XII) and trap platelets, activating them. SARS-CoV-2 can directly and indirectly induce the formation of NETs, contributing to the COVID-19 pathology in several ways: direct cytotoxic effect against endothelial and epithelial cells, activation of coagulation and platelets with consequent formation of micro-thrombi and microvascular damage in different organs by perpetuating the production of autoantibodies.<sup>10</sup> Furthermore, the levels of NETs appear to be a marker of severity in COVID-19.

Closely connected to the role of neutrophils and the phenomenon of the so-called 'NET-osis' is the complement system, which plays a fundamental role in the hyperinflammation and thrombotic micro-angiopathy characteristic of COVID-19. SARS-CoV-2 is able to activate the complement in different ways, ultimately generating the fractions C3a, C5a, and the sC5b-9 complex. Subsequently C3a induces platelet activation, while the C5a fraction and thrombin, deriving from activated platelets, induce neutrophils to express TF and produce NETs. In turn, NETs are pro-coagulants and induce endothelial activation and TF expression. The interaction between the complement system and NETs therefore contributes substantially to generate the vicious circle that leads to immunothrombosis.<sup>10</sup> Following the unregulated activation of the complement,

there would be a sort of atypical uremic-haemolytic syndrome that would contribute to COVID-19 endotheliopathy, as supported by the correlation between complement activation markers and endothelial damage markers.

In COVID-19 patients, there is an alteration of the fibrinolytic system secondary to the increase in angiotensin II and secondarily to PAI-1 which by blocking the plasminogen activators (t-PA and u-PA) induces a state of hypofibrinolysis and thrombosis.<sup>11</sup> Fibrin deposits in the lungs cannot be degraded and removed, compromising gas exchange. The increase in D-dimer has been misinterpreted as a marker of increased fibrinolysis in COVID-19, in fact only 0.02-0.2% of the fibrinogen mass is degraded to D-dimer. The elevated D-dimer levels reflect increased fibrin deposition (micro-thrombosis) but not increased fibrin degradation (fibrinolysis).<sup>11</sup> The combination of increased fibrin deposits and hypofibrinolysis results in multi-organ damage and has been associated with thrombotic phenomena.

One of the central alterations in the pathophysiology of CAC is endothelial dysfunction.<sup>12</sup> SARS-CoV-2 can directly infect endothelial cells causing apoptosis and necrosis as well as endothelial dysfunction with reduction of physiological antithrombotic and vasodilatory properties, release of pro-inflammatory and prothrombotic substances. Damaged endothelial cells release multimers of von Willebrand factor, the latter can reach levels up to 3-4 times normal, exceeding the degradation capacity by ADAMTS13 with consequent activation of coagulation and platelets. Endothelial damage and dysfunction induced by SARS-CoV-2 ultimately lead to vasoconstriction, inflammation, and thrombosis.<sup>12</sup>

SARS-CoV-2 induces alterations in the gene expression of platelets resulting in important functional alterations: although in normal numbers, platelets are hyperactive and have increased interactions with leukocytes. Platelet hyperactivity leads to an increased release of  $\alpha$ -granules and CK, which contributes to inflammation and thrombosis. The increased interaction between platelets and leukocytes favours the formation of thrombus, and on the other hand, contributes to the consumption of platelets.<sup>12</sup>

Beyond the pathogenesis, the presence of a coagulation disorder in COVID-19 is evident; therefore, it is necessary to use a prophylactic or therapeutic anticoagulant therapy.

The role of heparin in COVID-19 unfolds on several levels. In fact, in addition to the anticoagulant effect, heparin could have an antiviral effect, inhibiting the entry of SARS-CoV-2 into cells, and an anti-inflammatory effect, counteracting the action of CK. The fears generated by the high incidence of VTE despite the use of antithrombotic prophylaxis have led some experts to recommend the use of anticoagulant doses of heparin. However, the potential benefits of this approach must be balanced against the risk of bleeding. A recent review on the management of anticoagulation in COVID-19 analysed more than 80 randomized controlled trials (RCTs), but at present the optimal anticoagulation regimen remains to be determined.<sup>13</sup> The heterogeneity of the different clinical studies, as well as the absence of high-quality RCTs preclude clear conclusions and make these recommendations weak, with limited evidence.

Analysing the main guidelines on anticoagulation, they all agree with the need to administer thrombo-prophylaxis in patients hospitalized for COVID-19.<sup>14</sup> Some guidelines suggest an increase in dosage in patients admitted to ICU or with additional risk factors. Preliminary data suggest a benefit in terms of mortality and organ function deriving from the use of heparin with anticoagulant dosage in patients with moderately severe disease, not hospitalized in ICU, therefore the adaptation of the NICE guidelines in their latest edition. In fact, the use of anticoagulant doses of heparin has been analysed by three large international clinical trials, ACTIV-4 (NCT04505774), REMAP-CAP (NCT02735707), and ATTACC (NCT04372589), which have harmonized their protocols for faster achievement of results.<sup>15</sup> The primary goal was a combination of hospital mortality and organ support-free days at 21 days in patients in hospital (moderate COVID-19) or in ICU (severe COVID-19). Preliminary analysis of these data shows that therapeutically dosed heparin would not improve outcome or mortality in critically ill patients, indeed it could be harmful.<sup>15</sup> In contrast, in patients with moderate COVID-19, therapeutic anticoagulation appears to reduce the need for organ support in both patients with high D-dimer and in those with low D-dimer. The possible explanation for the difference between critically ill and non-critically ill patients could lie in the fact that the inflammatory and coagulation alterations of the patient with severe COVID-19 are too deep and ingrained to be normalized.

The balance between thrombotic and haemorrhagic complications in patients with COVID-19 appears extremely complex and the optimal anticoagulant regimen is not yet known and numerous studies are underway to provide answers to the many still open questions.

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## References

- Al-Samkari H, Karp Leaf RS, Dzik WH, Carlson JCT, Fogerty AE, Waheed A, Goodarzi K, Bendapudi PK, Bornikova L, Gupta S, Leaf DE, Kuter DJ, Rosovsky RP. COVID-19 and coagulation: bleeding and thrombotic manifestations of SARS-CoV-2 infection. *Blood* 2020;136:489-500.
- Fox S, Akmatbekov A, Harbert J, Li G, Quincy Brown J, Vander Heide R. Pulmonary and cardiac pathology in African American patients with COVID-19: an autopsy series from New Orleans. *Lancet Respir Med* 2020;8:681-686.
- Jiménez D, García-Sánchez A, Rali P, Muriel A, Bikdeli B, Ruiz-Artacho P, Le Mao R, Rodríguez C, Hunt BJ, Monreal M. Incidence of VTE and bleeding among hospitalized patients with coronavirus disease 2019: a systematic review and meta-analysis. *Chest* 2021;159:1182-1196.
- O'Donnell J, Peyvandi F, Martin-Loeches I. Pulmonary immunothrombosis in COVID-19 ARDS pathogenesis. *Intensive Care Med* 2021;30:1-4.
- Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost* 2020;18:844-847.
- Iba T, Warkentin T, Thachil J, Levi M, Levy J. Proposal of the definition for COVID-19-associated coagulopathy. *J Clin Med* 2021;10:191.
- Thachil J, Cushman M, Srivastava A. A proposal for staging COVID-19 coagulopathy. *Res Pract Thromb Haemost* 2020;4:731-736.
- Hartmann J, Ergang A, Mason D, Dias J. The role of TEG analysis in patients with COVID-19-associated coagulopathy: a systematic review. *Diagnostics* 2021;11:172.
- Savla S, Prabhavalkar K, Bhatt L. Cytokine storm associated coagulation complications in COVID-19 patients: pathogenesis and management. *Expert Rev Anti Infect Ther* 2021;1-17.
- Zuo Y, Kanthi Y, Knight J, Kim A. The interplay between neutrophils, complement, and microthrombi in COVID-19. *Best Pract Res Clin Rheumatol* 2021;35:101661.
- Creel-Bulos C, Sniecinski R. Fibrinolysis shutdown and thrombosis in a COVID-19 ICU. *Shock* 2021;55:845-846.
- Gu SX, Tyagi T, Jain K, Gu VW, Lee SH, Hwa JM, Kwan JM, Krause DS, Lee AI, Halene S, Martin KA, Chun HJ, Hwa J. Thrombocytopenia and endotheliopathy: crucial contributors to COVID-19 thromboinflammation. *Nat Rev Cardiol* 2021;18:194-209.
- Talasz AH, Sadeghipour P, Kakavand H, Aghakouchakzadeh M, Kordzadeh-Kermani E, Van Tassel BW, Gheymati A, Ariannejad H, Hosseini SH, Jamalkhani S, Sholzberg M, Monreal M, Jimenez D, Piazza G, Parikh SA, Kirtane AJ, Eikelboom JW, Connors JM, Hunt BJ, Konstantinides SV, Cushman M, Weitz JI, Stone GW, Krumholz HM, Lip GYH, Goldhaber SZ, Bikdeli B. Recent randomized trials of antithrombotic therapy for patients with COVID-19: JACC state-of-the-art review. *J Am Coll Cardiol* 2021;77:1903-1921.
- Leentjens J, van Haaps T, Wessels P, Schutgens R, Middeldorp S. COVID-19-associated coagulopathy and antithrombotic agents-lessons after 1 year. *Lancet Haematol* 2021;S2352-3026:00105-00108.
- Goligher E, Bradbury C, Mcverry B. Therapeutic anticoagulation in critically ill patients with Covid-19-preliminary report. *medRxiv* 2021. doi: 10.1101/2021.03.10.21252749.