Review

Thromboinflammation in COVID-19 acute lung injury

William Beau Mitchell

Hemostasis and Thrombosis, Division of Hematology/Oncology, Children's Hospital at Montefiore, Bronx, NY, USA

Educational aims

The reader will be able to appreciate:

- Thromboinflammation is a component of COVID-19 acute lung injury.
- Thromboinflammation has a multifactorial aetiology.
- There are multiple potential therapies for thromboinflammation, some already in clinical trials, that may benefit COVID-19 acute lung injury.

ABSTRACT

Since the initial description in 2019, the novel coronavirus SARS-Cov-2 infection (COVID-19) pandemic has swept the globe. The most severe form of the disease presents with fever and shortness of breath, which rapidly deteriorates to respiratory failure and acute lung injury (ALI). COVID-19 also presents with a severe coagulopathy with a high rate of venous thromboembiolism. In addition, autopsy studies have revealed co-localized thrombosis and inflammation, which is the signature of thromboinflammation, within the pulmonary capillary vasculature. While the majority of published data is on adult patients, there are parallels to pediatric patients. In our experience as a COVID-19 epicenter, children and young adults do develop both the coagulopathy and the ALI of COVID-19. This review will discuss COVID-19 ALI from a hematological perspective with discussion of the distinct aspects of coagulation that are apparent in COVID-19. Current and potential interventions targeting the multiple thromboinflammatory mechanisms will be discussed.

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INTRODUCTION

Since the initial description in 2019, the novel coronavirus SARS-Cov-2 infection (COVID-19) pandemic has swept the globe, impacting almost every country with both human and economic loss. The most severe form of disease presents with fever and shortness of breath, which rapidly deteriorates to respiratory failure. At several COVID-19 hotspots around the world the healthcare delivery systems have been overwhelmed by the number of patients requiring ventilatory support for respiratory failure and acute lung injury (ALI). As more data is emerging it has become apparent that COVID-19 also presents with a severe coagulopathy [1]. This coagulopathy is distinct from disseminated intravascular coagulation. COVID-19 patients typically have high fibrinogen, nor-mal or modestly prolonged prothrombin time and activated partial thromboplastin time, platelet count $>100 \times 10^6$/mL, and do not typically bleed. In addition to the reported intravascular thrombosis, COVID-19 patients also frequently thrombose dialysis and ECMO circuits despite anticoagulation. While the majority of published data is on adult patients, there are parallels to pediatric patients. Pediatric patients have been reported to have fewer severe symptoms, fewer ICU admissions and fewer deaths from COVID-19 [2,3]. However, in our experience, children and young adults do develop the acute lung injury of COVID-19 and do manifest the severe coagulopathy with increased rate of thrombosis [4].

Based on the few published autopsy studies, which altogether include 16 adults, the primary cause of death in COVID-19 appears to be Acute Lung Injury [ALI] characterized by severe endothelial damage, inflammation and extensive thrombosis of the perialveolar capillaries [5–8]. This co-localization of thrombosis and inflammation is a typical feature of ALI from other causes and is an

E-mail address: wimitchell@montefiore.org

example of thromboinflammation, which is the convergence of thrombotic and inflammatory processes [9]. Although the hemostatic and immune functions are generally regarded separately, they are linked together by evolution from a single cell type in primitive organisms, the hemocyte, which performs all hemostatic, inflammatory and immune functions. This single-cell system has evolved into the highly complex coagulation cascade, the complement system, and the multicellular immune system. Although these systems are distinct in higher mammals, they remain interconnected and interdependent. Thrombosis evolved as part of the innate immune system as a means of isolating invading organisms. For example, platelets play multiple roles in innate immunity by such varied activities as binding to and removing pathogens directly from circulation [10,11], secreting serotonin to recruit immune cells to sites of infection, and activating neutrophils to produce neutrophil extracellular traps (NETs) [12].

This connection between thrombosis and inflammation is evident in the pathology of COVID-19 ALI. Available studies report mononuclear cell infiltrates around thrombosed small vessels and neutrophils within the fibrin thrombi. The capillary thrombotic component is rich in inflammatory cells including lymphocytes, macrophages and NETs. These findings are the signature of thromboinflammation. This review will discuss the COVID-19 ALI from a hematological perspective with discussion of the distinct aspects of coagulation that are apparent in both the clinical picture and the pulmonary pathology of COVID-19. Current and potential interventions targeting the multiple thromboinflammatory mechanisms will be discussed.

TRIGGERS OF THROMBOINFLAMMATION

Damage to the pulmonary vascular endothelium

Disruption of the pulmonary capillary endothelium is a consistent finding in the reported autopsy cases of COVID-19 ALI. The endothelial damage was reported to be more extensive in COVID-19 ALI than in H1N1-related ALI [7]. The endothelium showed loss of both intercellular junctions and contact with the basement membranes, thus exposing the highly thrombogenic extracellular matrix and basement membrane.

An intact vascular endothelium is a potent anti-thrombotic, and anti-inflammatory barrier [9]. This protective coating of the blood vessels prevents spontaneous activation of coagulation and protects against pathologic thromboses. There are multiple components that confer these protective properties to the endothelium (Table 1). Several components inhibit platelet activation, for example the surface receptor CD39, the secreted prostaglandin PGI2, and secreted nitric oxide (NO). PGI2 also inhibits leukocyte recruitment and activation, while NO also limits leukocyte adhesion to the endothelium. The endothelial surface is also normally decorated with tissue factor pathway inhibitor (TFPI), which inhibits initiation of coagulation, and glycosaminoglycans, which inhibit binding

Table 1
Protective mechanisms of the vascular endothelium.

Endothelium feature	Anti-thrombotic Mechanism
CD39	Inhibits platelet activation
Prostaglandin PGI2	Inhibits platelet activation
	Inhibits leukocyte recruitment/ activation
Nitric Oxide	Inhibits platelet activation
Tissue Factor Pathway Inhibitor (TFPI)	Inhibits initiation of coagulation
Thrombomodulin	Activates protein C antithrombotic pathway

Table 2
Thromboinflammatory functions of thrombin.

Function	Mechanism	Comments
Drives coagulation	Proteolytic activation of coagulation factors	Primary driver of the coagulation cascade
Generates fibrin	Proteolysis of fibrinogen	Final step in the coagulation cascade
Activates platelets	Cleaves PAR1 and PAR4	Leads to complete granule release
Activates endothelial cells	Cleaves PAR1	Increases neutrophil recruitment Releases VWF into circulation
Activates leukocytes	Cleaves PAR1	Increased adhesion Release of cytokines
Activates endothelial smooth muscle cells	Cleaves PAR1	Release of cytokines

of prothrombotic factors. Lastly, endothelial thrombomodulin activates the potent antithrombotic protein C pathway. The severe damage to the endothelium seen in COVID-19 ALI would be expected to disrupt all of these protective mechanisms, leaving the capillaries vulnerable to thrombosis and inflammation.

Thrombin generation

The dense fibrin-rich microthrombi of the pulmonary capillaries reported in COVID-19 ALI indicate high activity of thrombin, which is the primary driver of coagulation and fibrin formation. The high thrombin activity is reflected by the high levels of thrombin-antithrombin (TAT) complexes seen in the most severely ill COVID-19 patients [13]. Thrombin is generated through the serial activation of proteases known as the coagulation cascade, or secondary hemostasis [14]. The pathway begins with the activation of tissue factor and the generation of a small amount of thrombin. This thrombin then activates the remainder of the cascade, leading to activation of factor X and amplification of thrombin formation. Thrombin finally generates fibrin from fibrinogen.

In general, thrombin plays multiple roles in thrombosis and inflammation (Table 2) [15]. In its thrombotic role [16], thrombin activates platelets, which serve as the phospholipid surface on which much of coagulation takes place. Thrombin also activates endothelial cells through PAR-1 leading to increased von Willebrand Factor (VWF) secretion from Weibel–Palade bodies. In its inflammatory role, thrombin increases the endothelial surface expression of P-selectin, thereby increasing neutrophil recruitment to the endothelium and subsequent activation. Thrombin also activates leukocytes and endothelial smooth muscle, leading to release of multiple cytokines and upregulation of surface adhesion markers.

Table 3
Thromboinflammatory functions of platelets.

Function	Mechanism	Comments
Primary hemostasis	Adheres to VWF and Collagen	Initiation of hemostasis
Granule content secretion	Platelet activation	Releases prothrombotic substances Releases inflammatory cytokines
PolyP release	Dense granule secretion	Initiation of contact pathway
Leukocyte binding	P-selectin	Increases leukocyte recruitment Activates leukocytes
Neutrophil binding	P-selectin	Increased neutrophil adhesion Release of cytokines Formation of NETs

An early clinical indication of the importance of thrombin in COVID-19 disease was the report from Wuhan showing a survival advantage of those patients who received heparin, which is a potent anti-thrombin agent [17]. In that retrospective study 99 out of 449 patients with severe COVID-19 received heparin for more than 7 days during their hospitalization. In the most severely ill patients (SIC score ≥4) 40% of those who received heparin succumbed, compared to 64% of those who did not ($p = 0.029$). This 20% survival advantage sparked the clinical use of heparin in severe COVID-19.

In addition to ALI, venous thromboembolism (VTE - deep vein thrombosis and/or pulmonary embolism) is widely reported in COVID-19. Since the Wuhan report heparin has been widely used in COVID-19 with partial success, but several centers have reported VTE rates of up to 30% in critically ill COVID-19 patients [18–22]. This included patients already on prophylactic anticoagulation with heparin. This high rate of failure of prophylactic heparin is in contrast to the expected 5–7% VTE breakthrough for critically ill patients while on prophylactic heparin [23]. Clearly, further anticoagulation is needed for these very ill patients. There are currently multiple clinical trials of anticoagulation in COVID-19 (Table 4).

Platelet activation

Platelet activation underlies thrombus formation. Autopsy analyses of COVID-19 ALI have reported that the alveolar capillaries were filled with fibrin-rich thrombi containing neutrophils, indicating extensive platelet activation. Microthrombi were found extensively in the pre- and post-capillary vessels throughout the lungs. Such extensive thrombosis would be expected to disrupt the normal physiology of oxygen exchange and ventilation, contributing to the COVID-19 ALI. It was also reported that the resident lung megakaryocytes were increased and actively producing platelets [5]. Young platelets have increased coagulation potential and the highest level of granule contents, making them more thrombogenic than older platelets. These resident megakaryocytes may be adding highly thrombogenic platelets to an already hypercoagulable environment.

Platelet activation is the first step in primary hemostasis and is initiated by binding of platelet surface GPIb/IX to von Willebrand factor (VWF) on damaged endothelium. These initial platelets then undergo activation largely by signaling through GP6 binding to collagen. Activated platelets then recruit more platelets and crosslink with them via fibrinogen (and other plasma proteins) binding to activated GPIIb/IIIa receptors. Activated platelets provide an important membrane surface for the cell-based coagulation cascade and production of thrombin and ultimately fibrin, as well as activation of the contact pathway. There are multiple approved antiplatelet therapies, some of which are in clinical trials against COVID-19 (Table 4).

Platelets also perform several functions beyond hemostasis that are likely contributing to the ALI of COVID-19 (Table 3). Upon activation, platelets secrete hundreds of substances. These include multiple proinflammatory cytokines such as IL-1beta [24] and pro angiogenic factors such as vascular endothelial growth factor (VEGF) [25]. Platelets also release polyphosphate (PolyP) multimers from their dense granules upon activation [26]. The largest of these multimers remain bound to the surface of the platelet and serve as a substrate for the activation of the contact pathway, which plays a role in pathologic thrombus formation. Activated platelets bind to leukocytes and promote leukocyte activation and extravasation [27]. Activated platelets also form aggregates with neutrophils and cause upregulation of MAC-1, which leads to stable neutrophil-endothelium biding [28]. Platelet neutrophil aggregates are frequently seen in thromboinflammation and are

reported to be elevated in COVID-19 patients. In transfusion-associated ALI, activated platelets induce neutrophils to generate NETs, which are both proinflammatory and procoagulant [29]. NETs were markedly increased in the blood of COVID-19 patients [30] and were noted in the pulmonary capillaries of COVID-19 ALI [5].

Infiltration of neutrophils and macrophages

Neutrophils and other inflammatory cells were observed within the capillary thrombi in COVID-19 ALI, and there was indication of NETs formation. Neutrophils are recruited to growing thrombi by activated platelets. Activated platelets also induce neutrophils to form NETs [28]. NETs are the organized extrusion of the chromatin of mature neutrophils [31]. NETs have many functions, including anti-bacterial and prothrombotic activity. NETs also damage the endothelium and inhibit fibrinolysis by trapping TFPI, which makes them highly thrombogenic. Cell free DNA is seen in the sera of COVID-19 patients, indicating NETs formation [30]. Macrophages are also recruited to fibrin thrombi and work to remodel the thrombus as part of the normal healing process [32]. Macrophages within the fibrin clot generate plasmin, which degrades fibrin to produce D-Dimers. Macrophages are also an alternate source of D-Dimer by uptake and degradation of fibrin via CD11b/CD18 (Mac-1) receptors [33]. These macrophage functions likely contribute to the unusually extreme elevation of D-dimers that is a unique feature of COVID-19.

Contact pathway activation

There is a second pathway of coagulation, known as the contact pathway, that is primarily initiated by factor XII binding to PolyP on the activated platelet surface. This pathway is expendable for secondary hemostasis but has been implicated in formation of pathological thrombi and thrombi formation on artificial surfaces [34]. One clue to the activation of the contact pathway in COVID-19 has been the repeated observation of low factor XII levels in very sick patients (personal communication). These low levels may indicate consumption of factor XII during activation of the contact pathway. There is growing interest in targeting the contact pathway for prevention of pathological thrombosis and inflammation, and there are novel anti-contact pathway therapies that could potentially target the thromboinflammation of COVID-19 [35].

THERAPIES FOR THROMBOINFLAMMATION

The pathological findings in COVID-19 ALI paint a highly thrombogenic and inflammatory picture. What can be done to ameliorate or prevent this from occurring? Since the mechanism underlying ALI thromboinflammation is multifactorial there are multiple potential targets for therapy. Given the prominence of the thrombotic microangiopathy in the COVID-19 ALI pathology, those therapies directed at preventing or reversing the coagulopathy of ALI may be efficacious for survival. Due to the multifactorial nature of thromboinflammation multiple complementary anticoagulant/antithrombotic modalities may be needed.

Heparin

Heparin derails the coagulation cascade by deactivating activated factor X, which is the primary producer of thrombin, thereby slowing secondary hemostasis. Since the report from Wuhan suggesting that heparin use was associated with improved survival, most large centers have adopted a prophylactic heparin protocol for COVID-19 patients, as well as criteria for escalation to

Table 4
Active clinical trials targeting thromboinflammation.

Target	NCT number	Title
Endothelium	NCT04398290	iNOPulse for COVID-19
	NCT04397692	Inhaled NO for the Treatment of COVID-19 Caused by SARS-CoV-2 (US Trial)
	NCT04388683	Inhaled Nitric Oxide for Preventing Progression in COVID-19
	NCT04383002	High Dose Inhaled Nitric Oxide for COVID-19 (ICU Patients)
	NCT04358588	Pulsed Inhaled Nitric Oxide for the Treatment of Patients With Mild or Moderate COVID-19
	NCT04338828	Nitric Oxide Inhalation Therapy for COVID-19 Infections in the ED
Thrombin	NCT04408235	High Versus Low LMWH Dosages in Hospitalized Patients With Severe COVID-19 Pneumonia and Coagulopathy
	NCT04406389	Anticoagulation in Critically Ill Patients With COVID-19 (The IMPACT Trial)
	NCT04401293	Full Dose Heparin Vs. Prophylactic Or Intermediate Dose Heparin in High Risk COVID-19 Patients
	NCT04397510	Nebulized Heparin for the Treatment of COVID-19 Induced Lung Injury
	NCT04394377	Full Anticoagulation Versus Prophylaxis in COVID-19: COALIZAO ACTION Trial
	NCT04393805	Heparins for Thromboprophylaxis in COVID-19 Patients: HETHICO Study in Veneto
	NCT04377997	Safety and Efficacy of Therapeutic Anticoagulation on Clinical Outcomes in Hospitalized Patients With COVID-19
	NCT04373707	Weight-Adjusted vs Fixed Low Doses of Low Molecular Weight Heparin For Venous Thromboembolism Prevention in COVID-19
	NCT04372589	Antithrombotic Therapy to Ameliorate Complications of COVID-19 (ATTACC)
	NCT04367831	Intermediate or Prophylactic-Dose Anticoagulation for Venous or Arterial Thromboembolism in Severe COVID-19
	NCT04366960	Comparison of Two Doses of Enoxaparin for Thromboprophylaxis in Hospitalized COVID-19 Patients
	NCT04362085	Coagulopathy of COVID-19: A Pragmatic Randomized Controlled Trial of Therapeutic Anticoagulation Versus Standard Care
	NCT04360824	Covid-19 Associated Coagulopathy
	NCT04359277	A Randomized Trial of Anticoagulation Strategies in COVID-19
	NCT04359212	Increased Risk of VTE and Higher Hypercoagulability in Patients Recovered in ICU and in Medical Ward for COVID-19
	NCT04345848	Preventing COVID-19 Complications With Low- and High- dose Anticoagulation
	NCT04344756	Trial Evaluating Efficacy and Safety of Anticoagulation in Patients With COVID-19 Infection, Nested in the Corimmuno-19 Cohort
	NCT04343001	Coronavirus Response - Active Support for Hospitalised Covid-19 Patients
	NCT04333407	Preventing Cardiac Complication of COVID-19 Disease With Early Acute Coronary Syndrome Therapy: A Randomised Controlled Trial.
Platelets	NCT04391179	Dipyridamole to Prevent Coronavirus Exacerbation of Respiratory Status (DICER) in COVID-19
	NCT04363840	The LEAD COVID-19 Trial: Low-risk, Early Aspirin and Vitamin D to Reduce COVID-19 Hospitalizations
	NCT04368377	Enhanced Platelet Inhibition in Critically Ill Patients With COVID-19
	NCT04365309	Protective Effect of Aspirin on COVID-19 Patients

treatment dosing. Given the high rate of prophylaxis failure, better prophylaxis appears to be needed. In pediatrics this may be provided by using anti-Xa level-based dosing of low molecular weight heparin. Some primarily adult centers are starting to use anti-Xa based dosing to try and decrease their rates of breakthrough VTE, including at our center.

Heparin is a potent anticoagulant, but it also has other potential roles in thromboinflammation and ALI. In an animal model of ALI, local heparin attenuated acute lung injury (ALI) in lung tissue by decreasing multiple proinflammatory pathways, including decreasing production of interleukin 6 and tumor necrosis factor alpha [36]. These cytokines are markedly elevated in critically ill COVID-19 patients. In alveolar macrophages heparin reduced expression of procoagulant genes for transforming growth factor beta and nuclear factor kappa B. Heparin also protects the endothelium from NETs and histones, sequesters cytokines and complement, and protects against recruitment of inflammatory cells [37,38]. There is also data that heparin interacts with spike proteins of several viruses, including the SARS-CoV-2 spike protein receptor binding domain, suggesting that it may be able to modulate that protein's interactions with the endothelium [39]. Of note, aerosolized heparin has been explored in human ALI with results indicating decreased inflammation and thrombosis [40,41]. As more data is reported a clearer picture of the effect of heparin on COVID-19 ALI, coagulopathy, and survival will emerge. At the time of writing there are 19 clinical trials of heparin in COVID-19 listed on clinicaltrials.gov (Table 4).

Direct anti-thrombin and anti-Xa inhibitors

These agents bind directly to and inhibit thrombin or factor Xa. They are attractive because of the ease of administration and no requirement for monitoring. However, there is limited data for their use in children who way less than 40 kg. There is no large population study yet using these agents in COVID-19, but theoretically they would have both anticoagulation and anti-inflammatory activity.

Anti-platelet agents

Given the microangiopathy of COVID-19 ALI, platelets are an attractive drug target. There are already several types of anti-platelet agents available. To be most effective however, the agent of choice may need to suppress platelet granule release in order to decrease the secondary effects of platelet activation. Aspirin will not do this, but P2y12 inhibitors and GPIIb/IIIa inhibitors could in high enough doses. At time of writing there are four clinical trials of antiplatelet agents in COVID-19 listed on clinicaltrials.gov (Table 4).

Contact pathway inhibitors

The contact pathway is an attractive anticoagulation target since loss of contact pathway function does not lead to bleeding in animals or humans. However, initiation of the contact pathway plays a role in pathological thrombosis in mouse models. Thus, the contact pathway is dispensable for hemostasis but essential for certain types of thrombosis. The contact factor pathway also has an important inflammatory role as it produces bradykinin, a potent inflammatory protein. It is not known at this time whether the contact pathway plays a role in COVID-19 ALI. Multiple factor XI and XII inhibitors are under development and a few are approved for limited indications. At the time of writing there are no contact pathway inhibitor trials listed in clinicaltrials.gov.

SUMMARY AND CONCLUSIONS

Severe COVID-19 is characterized by ALI and respiratory failure. While current pathology reports are all of adult cases, in our experience pediatric patients also acquire severe COVID-19 and require mechanical ventilation. It seems likely that children have a similar ALI pathology, although their endothelium may be healthier to begin with. The ALI of COVID-19 has a severe thromboinflammatory component characterized by neutrophil and lymphocyte infiltrates into fibrous thrombi with evidence of NETs formation. Multiple thrombotic pathways likely converge to create the thromboinflammation of COVID-19 ALI, including endothelial damage, thrombin activation, platelet activation, NETs formation, and contact pathway activation. These pathways offer multiple targets for mitigation of COVID-19 coagulopathy and may potentially ameliorate the ALI contribution to morbidity and mortality in COVID-19.

DIRECTIONS FOR FUTURE RESEARCH

- Studies on the coagulopathy of COVID-19 in children are needed.
- Studies to determine optimal prophylactic heparin anticoagulation regimen to prevent DVT/PE are needed.
- Studies of antiplatelet agents are needed
- Studies of other antithrombotic therapies, including direct thrombin inhibitors, direct factor Xa inhibitors and contact pathway inhibitors should be considered.

Acknowledgement

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