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Platelet-leukocyte interactions in COVID-19: Contributions to hypercoagulability, inflammation, and disease severity

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Abstract
A State of the Art lecture titled “Platelet-leukocyte interactions in COVID-19: Contributions to hypercoagulability, inflammation and disease severity” was presented at the International Society for Thrombosis and Hemostasis (ISTH) congress in 2021. Severe coronavirus disease 2019 (COVID-19) has been associated with a high incidence of coagulopathy and thromboembolic events that contributes to disease severity and poor outcomes. Therefore, understanding the mechanisms of COVID-19-associated hypercoagulability and thromboinflammation has gained great interest. Here, we review the mechanisms involved in platelet activation and platelet interactions with leukocytes during COVID-19. We highlight recent evidence that platelet activation, platelet-monocyte, and platelet-neutrophil interactions in COVID-19 support pathological thromboinflammation, including in driving tissue factor expression and NETosis, which have been associated with thromboembolic complication and poor outcomes in critically ill patients. The contributions of platelet-leukocyte interactions to COVID-19 immunoregulation, inflammation, and hypercoagulability, as well as their potential implications in disease severity and therapeutic strategies, will be discussed. Finally, we summarize relevant new data on this topic presented during the 2021 ISTH Congress.

KEYWORDS
COVID-19, monocytes, neutrophils, platelets, thromboinflammation

Essentials
- Severe COVID-19 is associated with dysregulated inflammation and increased risk of blood clots.
- Platelet activation and platelet-leukocyte aggregates associate with severity in COVID-19.
- Platelet-leukocyte interactions amplify inflammatory and thrombotic responses in COVID-19.
- Investigation of platelet-leukocyte interactions in the long-term outcomes of COVID-19 is in need.

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INTRODUCTION

When the emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) completes its second anniversary, the coronavirus disease 2019 (COVID-19) pandemic will have already accumulated more than 300 million cases and 5 million deaths globally. A state of hypercoagulability is a major pathophysiological mechanism and the main cause of mortality in severe COVID-19. Individuals with severe COVID-19 syndrome frequently evolve with prothrombotic coagulation abnormalities, especially pulmonary embolism and deep vein thrombosis. Higher frequency of COVID-19-associated thromboembolic events, cardiovascular complications, and death during postdischarge is also observed. This hypercoagulable state is associated with respiratory distress syndrome in SARS-CoV-2-infected patients. Postmortem pathological findings show extensive areas of platelet-fibrin microvascular thrombosis containing neutrophil and macrophage infiltration, NETosis, and endothelial inflammation (Figure 1A). Platelet-neutrophil complexes and NET-containing pulmonary and extrapulmonary microvascular thromboses have been presented as a main mechanism of multiorgan impairment in autopsy studies from COVID-19 fatalities. Pulmonary histopathological studies revealed these thromboinflammatory vascular occlusions to be almost 10 times more frequent in COVID-19 than in influenza pneumonia fatalities. Aggravating an already complex situation, hypercoagulability and thromboinflammatory tissue damage have been reported despite prophylactic heparin anticoagulation, and antiplatelet therapy with P2Y12 inhibitors fails to improve hypercoagulability and organ impairment, highlighting the need of alternative antithrombotic strategies.

Platelet activation is a main feature of the COVID-19 hypercoagulable state. Platelet activation in severe COVID-19 patients is associated with markers of coagulation activation and inflammation and with increased incidence of thromboembolic complications. Platelets from severe COVID-19 patients are hyperresponsive to agonist stimulation and form aggregates with

**FIGURE 1** Platelet activation and hypercoagulability in COVID-19. (A) COVID-19-associated microvascular thrombosis: thromboinflammatory vascular occlusions presenting neutrophil and macrophage infiltration with NET-containing platelet-fibrin thrombi are observed in multiple organs during severe COVID-19. (B) Mechanisms of platelet activation and platelet-endothelial cell aggregation: soluble mediators in COVID-19 plasma including cytokines, procoagulant antibodies, coagulation, and complement factors, as well as SARS-CoV-2 itself, may participate in platelet and endothelial cell activation. Activated endothelial cells promote platelet aggregation through von Willebrand factor. (C) Platelet-monocyte and platelet-neutrophil aggregates formation in COVID-19: platelets induce monocyte TF expression through P-selectin and integrin αIIbβ3 signaling and neutrophil release of TF-containing NETs through mechanisms depending on Thrombin-PAR-1 and C5aR signaling.
monocytes, lymphocytes, and neutrophils.\textsuperscript{10,19,20} Through the use of different strategies to inhibit platelet activation and platelet-leukocyte aggregate formation, a role for platelets and platelet-leukocyte interactions in fueling coagulation and inflammation disturbances in COVID-19 has been examined. In this review, we highlight mechanisms and pathophysiological roles of platelet activation and platelet-leukocyte interactions in hypercoagulability, inflammation, and severity during COVID-19.

2 | PLATELET ACTIVATION AND HYPERRESPONSIVENESS IN COVID-19 HYPERCOAGULABILITY

Increased platelet activation in COVID-19 has been evidenced by alpha and dense granules release, integrin $\alpha_{IIb}/\beta_3$ activation, and platelet extracellular vesicle shedding.\textsuperscript{10,14,19,21-23,26} Also, platelets from COVID-19 patients were more adhesive to fibrinogen and collagen and more responsive to PAR-1 and P2Y$_{12}$ agonists, leading to TXA$_2$, synthesis, aggregation, and cytokine secretion.\textsuperscript{10,14,20,23,27} Platelet transcriptome analysis has revealed pathways involved in thromboinflammation, type I interferon-mediated antiviral response, and programmed cells death, which were associated with functional alterations in platelets from COVID-19 patients.\textsuperscript{14,20,27,28} These features of platelet activation and hyperresponsiveness were correlated to hypercoagulability, disease severity, respiratory distress syndrome, myocardial injury, and mortality in different cohorts of COVID-19 patients.\textsuperscript{10,19,21,29,30}

The mechanisms underlying platelet activation in severe COVID-19 are not yet completely understood. Our group and others have shown that circulating factors present in the plasma of severe COVID-19 patients activate platelets ex vivo.\textsuperscript{19,31,32} These circulating factors may include heightened thrombin generation, cytokine signaling, complement activation, and/or pro-coagulant antibodies (Figure 1B).\textsuperscript{31,33-38} Platelets from healthy volunteers become activated when exposed to plasma from COVID-19 patients,\textsuperscript{19,32,35,39} and whole blood reconstituted in COVID-19 plasma displays platelet activation, platelet-leukocyte aggregate formation, and tissue factor (TF) expression, which are all inhibited by the interleukin-6 receptor antibody tocilizumab.\textsuperscript{32} Anti-phospholipid antibodies are highly frequent in severe COVID-19 patients. Higher levels of anti-cardiolipin antibodies correlate with coagulation activation, NEtosis, and respiratory distress in infected patients.\textsuperscript{34} Immunoglobulins from patients have major thrombogenic effects, including triggering platelet activation, apoptosis, platelet-thrombi formation and NET extrusion in vitro,\textsuperscript{34,36,38} and driving intravascular NEtosis and accelerated thrombosis in mice.\textsuperscript{34} Aberrant glycosylation of anti-Spike antibodies and activation of Syk and PI3K/Akt signaling through FcRIlalpha and C5alpha are major signaling pathways in these processes.\textsuperscript{33,35,36,38,40} Downstream inhibition of FcRIlalpha/ITAM signaling through Syk inhibitor or the Bruton tyrosine kinase inhibitor ibrutinib completely abrogate the thrombogenic effect of anti-S antibodies, highlighting possible pathways for therapeutical intervention.\textsuperscript{35} These data highlight an interplay between innate and adaptive immune factors in triggering platelet activation and thromboinflammatory vascular occlusion in COVID-19.

Another thrombogenic mechanism of COVID-19 patients’ plasma involves endothelium activation through C5a-C5aR1 signaling, inducing Weibel-Palade bodies extrusion and von Willebrand factor-dependent platelet aggregation (Figure 1B).\textsuperscript{37} Similarly, the releasate from COVID-19 patients’ platelets also activates a thromboinflammatory program in endothelial cells.\textsuperscript{14} Combined analysis of platelets and endothelial cells transcriptomics, clinical data, and functional experiments revealed calprotectin (MRP8/14) as a major platelet-derived factor associated with endothelial inflammation, disease severity, the onset of thrombosis, and mortality in COVID-19.\textsuperscript{14} Also, the expansion of a myeloid suppressor cell subset capable of reducing L-arginine levels and activating platelets ex vivo has been reported in COVID-19 patients.\textsuperscript{26} Together, these data highlight complex interactions involving soluble factors, leukocyte subsets expansion, and vascular cells in activating platelets and generating the hypercoagulable state characteristic of severe COVID-19.

In vitro infection of platelets from healthy volunteers shows that platelets recognize and respond to SARS-CoV-2 becoming activated.\textsuperscript{21,28,41,42} Similar results have been observed in platelets exposed to recombinant Spike protein and Spike-containing pseudovirus.\textsuperscript{21,41,43} SARS-CoV-2-induced platelet activation in vitro is inhibited by competition with recombinant ACE-2, suggesting platelet activation through the Spike receptor-binding domain.\textsuperscript{41} However, whether platelets express or not ACE-2 has been a matter of controversy.\textsuperscript{20,21,23,27,28,41,42} Different studies using similar techniques to detect ACE-2 have shown that platelets express,\textsuperscript{28,41} and do not express ACE-2.\textsuperscript{20,21,23,27,42} Alternative receptors for SARS-CoV-2 attachment in platelets, including CD147 and CD42b, have been proposed.\textsuperscript{21,42,43} Neutralizing antibodies against CD147, but not ACE-2, prevent platelet activation after exposure to SARS-CoV-2, Spike-pseudovirus, or recombinant Spike protein.\textsuperscript{21} Similarly, Spike protein binds to CD42b and triggers Akt-protein kinase C pathway to induce platelet activation.\textsuperscript{42} Of note, SARS-CoV-2 RNA has been detected in platelets from infected patients,\textsuperscript{23,27,28} highlighting the feasibility of SARS-CoV-2-induced platelet activation in natural infections. Immunofluorescence staining, back-titration experiments, and ultrastructural studies have shown SARS-CoV-2 attachment and internalization in platelets and megakaryocytes from COVID-19 patients and from in vitro infection models.\textsuperscript{21,27,28,42} However, even though the subgenomic viral RNA is detected in infected platelets, no viral progeny is produced, indicating an abortive replication cycle.\textsuperscript{21}

3 | PLATELET-LEUKOCYTE INTERACTION IN COVID-19 HYPERCOAGULABILITY AND INFLAMMATION

Increased platelet-leukocyte aggregates formation in COVID-19 patients has been reported among the main leukocyte subsets,
including neutrophils, monocytes, and CD4+ and CD8+ T lymphocytes. Platelet-monocyte and platelet-neutrophil aggregate formation were especially higher in severe COVID-19 patients, and were associated with hyperinflammation and hypercoagulability. Importantly, circulating platelet-monocyte and platelet-neutrophil aggregates in severe COVID-19 patients expressed high levels of TF, the main trigger of intravascular coagulation and thrombosis (Figure 1C).

Increased platelet-monocyte aggregate formation has been shown in severe COVID-19 patients, but not in patients presenting mild COVID-19 syndrome or asymptomatic infections. Increased TF expression was observed on monocytes that were tethered with platelets compared with monocytes alone in the same sample, whereas isolated platelets from severe COVID-19 patients triggered TF expression in monocytes from healthy volunteers. Mechanistically, platelets from severe COVID-19 patients induced monocyte TF expression depending on P-selectin and integrin αIIb/β3 signaling (Figure 1C). Platelets activated by SARS-CoV-2 Spike protein also bind to monocytes through P-selectin and CD40-L, and signal proinflammatory monocyte activation with high IL-1β expression. Similarly, results from our group show that platelets from COVID-19 patients or in vitro-infected platelets also induce a proinflammatory program in monocytes, which is induced by P-selectin and integrin αIIb/β3 binding and is amplified by TF signaling. Platelet activation and monocyte TF expression positively correlate with plasma levels of D-dimers, supporting a role in hypercoagulability. In addition, increased platelet activation and monocyte TF expression at admission were predictive of patients’ poor outcomes, including the requirement of mechanical ventilation and mortality. These data highlight key roles of platelet-monocyte interaction in triggering pathological TF expression, contributing to hypercoagulability and inflammation in severe COVID-19 (Table 1).

Platelet-neutrophil aggregates and NETosis are evidenced alongside platelet-fibrin thrombi in lungs, kidneys, and heart histological preparations from COVID-19 autopsies (Figure 1A). Increased NETosis is associated with hypercoagulability, respiratory distress syndrome, and Sequential Organ Failure Assessment score values during COVID-19; and NETosis at admission is predictive of venous thromboembolism and mortality. Isolated neutrophils from COVID-19 patients show features of activation and spontaneously release NETs that are decorated with TF. Plasma from COVID-19 patients also activates control neutrophils and significantly induces NET extrusion ex vivo. Anti-phospholipid antibodies isolated from COVID-19 patients also induce NETs release in neutrophils from healthy volunteers.

In another study, however, plasma from COVID-19 patients induced neutrophil TF expression without inducing NETosis, while platelet-rich plasma (PRP) was required to induce the release of TF-containing NETs. Neutrophil extrusion of TF-containing NETs in response to PRP from COVID-19 patients depended on Thrombin-PAR-1 and C5aR signaling (Figure 1C). Collectively, these data highlight complex platelet-leukocyte interactions as key events for monocyte and neutrophil thromboinflammatory responses in severe COVID-19, including in driving TF expression and hypercoagulability (Table 1).

4 | ISTH 2021 CONGRESS REPORT

Many abstracts presented in the XXIX Congress of the International Society for Thrombosis and Hemostasis (ISTH, Philadelphia, PA, USA, 2021) reported increased platelet activation and platelet-leukocyte aggregates formation in patients with COVID-19. Preliminary proteomic data on platelet and platelet releasate have revealed pathways associated with ribosomal protein synthesis, altered mitochondrial activity, and defective thrombopoietin signaling, as well as platelet release of inflammation and vasoactive proteins, alongside proteins related to extracellular vesicles. These reports are in line with evidence of mitochondrial dysfunction and platelet apoptosis presented by Althaus and colleagues and of platelet exhaustion of alpha and dense granules content in severe COVID-19 patients presented by Manukjan and colleagues.

A major mechanism of platelet activation in COVID-19 that was extensively explored in the 2021 ISTH congress is the presence of procoagulant antibodies. Immunoglobulins isolated from COVID-19 patients were reported to induce platelet activation, platelet apoptosis, procoagulant activity in monocytes, endothelial cells in culture, and accelerated thrombosis in mice. The presented abstracts have shed new light on the signaling mechanisms of antibody-mediated platelet activation that involved FcγRIIA and PI3K/Akt signaling pathway and was preventable by iloprost-induced cAMP increase. Moreover, accelerated thrombosis induced by the injection of antiphospholipid antibodies from COVID-19 patients in mice was prevented by a specific inhibitor of the TF initiation complex, highlighting the importance of the TF pathway and signaling in COVID-19 hypercoagulability state.

Another mechanism of platelet activation was direct stimulation with Spike protein and its receptor binding domain, which activated platelets requiring integrin αIIb/β3 amplification. Regarding platelet-mediated protective responses in COVID-19, Ishizuka et al. has shown that PF4 enhances SARS-CoV-2 sequestration in NETs. Collectively, these reports highlight mechanisms of platelet activation and hypercoagulability in COVID-19 and platelet involvement in inflammatory response to SARS-CoV-2 infection.

5 | FUTURE DIRECTIONS

Although platelet-neutrophil and platelet-monocyte aggregate formation are well documented, platelet-lymphocyte interactions have been far less explored. The ability of platelets to present antigens through HLA class I has been previously shown. Recently, platelet antigen presentation was associated with CD8+ T-cell suppression in sepsis. On the other hand, platelet antigen presentation has been previously shown to activate CD8+ T-cell effector responses
### TABLE 1 Platelet phenotypes and clinical correlates in COVID-19

| Platelet phenotype | Outcomes and thromboinflammatory implications | Ref |
|--------------------|-----------------------------------------------|-----|
| Increased platelet activation and hyperresponsiveness | Associated with hypercoagulability (D-dimer and fibrinogen), requirement of mechanical ventilation and mortality | (19) |
| | Activation of ERK/p38/PLA₂ leading to TXA₂ synthesis and platelet hyperaggregability | (20) |
| | Associated with inflammation, hypercoagulability, and disease severity | (10) |
| | Associated with critical illness and viremia | (41) |
| | Activated platelets from COVID-19 patients increase factor XII formation in control plasma | (65) |
| | Associated with ICU admission myocardial injury and mortality | (29) |
| | Platelet hyperactivity is observed regardless of presenting ARDS | (24) |
| Increased shedding of platelet-derived factors and EVs | High levels of TXB₂, sCD40L, and sCD62-P in plasma associated with increased risk of mortality and thrombosis | (22) |
| | Platelet shedding of sCD62-P and HMGB-1-containing EVs associated with inflammation (CRP), hypercoagulability (D-dimer), respiratory distress (PaO₂/FiO₂), and disease severity | (21) |
| | High levels of sCD62-P in plasma predictive of mechanical ventilation and mortality | (30) |
| | Platelets from patients secrete higher levels of IL-1β and sCD40-L. PF4 and serotonin are reduced in platelets and augmented in plasma from patients | (23) |
| | High levels of sCD62-P, sGPVI, and PF4 in plasma | (42) |
| Increased platelet apoptosis | Increased in ICU-admitted patients and associated with thrombocytopenia, hypercoagulability, SOFA score, thrombosis, and mortality. IgG fraction from COVID-19 serum activates platelets through FcγRIIA | (38) |
| | Morphological features of platelet activation, cell shrinking, and cell death. MLKL phosphorylation and caspase-3 cleavage were observed in platelets that were positive to Spike protein both in patients and after SARS-CoV-2 infection in vitro | (28) |
| Increased platelet-neutrophil aggregates and NETosis | NETosis in blood, airways, and postmortem lung histopathological analysis were associated with respiratory distress (PaO₂/FiO₂), requirement of mechanical ventilation, SOFA score, and mortality. | (9) |
| | Neutrophil activation and NETosis are observed in blood and in lung, kidneys, and heart histopathological analysis. Associated with hypercoagulability and respiratory distress. PRP from COVID-19 patients induce NETosis in control neutrophils | (10) |
| | Platelet-neutrophil aggregate formation was associated with disease severity and with the inflammatory markers (CRP and IL-6) | (44) |
| | Release of NETs decorated with TF. PRP from COVID-19 patients induced TF-positive NETs in control neutrophils through PAR1 and C5AR signaling | (33) |
| | Increased TF expression in platelets and platelet-neutrophil aggregates. Plasma from COVID-19 patients induces a similar phenotype in control blood, which is prevented by aspirin, P2Y12 inhibitors, or IL-6R blocking | (31) |
| | NETosis but not platelet activation was associated with ICU admission, requirement of mechanical ventilation, and VTE | (32) |
| Increased platelet-monocyte aggregates | Platelets induce TF expression in monocytes through CD62P and integrin α₁b/β₃ signaling. Monocyte TF expression was associated with hypercoagulability (D-dimer and fibrinogen), requirement of mechanical ventilation, and mortality. | (19) |
| | Associated with disease severity and with inflammatory markers (CRP and IL-6) | (44) |
| | Increased TF expression in platelets and platelet-leukocyte aggregates. Plasma from COVID-19 patients induces a similar phenotype in control blood, which is prevented by aspirin, P2Y12 inhibitors, or IL-6R blocking | (31) |
| | Platelets form aggregates especially with CD16+ inflammatory monocytes. Platelet-monocyte interactions reciprocally activate monocytes and platelets, inducing the secretion of inflammatory mediators. Platelet adhesion is a primary signaling mechanism inducing mediator secretion and TF expression, whereas TF activity amplifies inflammation by inducing TNF-α and IL-1β through PAR1 and 2 signaling | (46) |

Abbreviations: ADP, adenosine diphosphate; ARDS, acute respiratory distress syndrome; C5aR, complement factor 5a receptor; CRP, C-reactive protein; EVs, extracellular vesicles; HMGB-1, high mobility group box 1; ICU, intensive care unit; NET, neutrophil extracellular traps; PAR, protease-activated receptor; PAR1, protease-activated receptor 1; PF-4, platelet factor 4; PLA₂, phospholipase A₂; PRP, platelet-rich plasma; sCD40L, soluble CD40 ligand; sCD62P, soluble P-selectin; sGPVI, soluble glycoprotein VI; SOFA, Sequential Organ Failure Assessment; TF, tissue factor; TRAP, thrombin receptor-activating peptide; TXA₂, thromboxane A₂; VTE, venous thromboembolism
in malaria. Therefore, platelet-lymphocyte interaction is potentially involved in T-cell features with major implications to COVID-19 pathophysiology, including immunosuppression through T-cell exhaustion or inflammatory amplification and tissue damage through cytotoxic activity.

As the knowledge on COVID-19 pathophysiology advances, thromboinflammatory vascular occlusions emerge as central pathological features driving clinical complications. Despite the progressive increase in survival, many postdischarge patients still present a post-COVID-19 syndrome with pulmonary and extrapulmonary features. Among many persisting symptoms in COVID-19-recovered patients, thrombotic complications and long-term cardiovascular outcomes have been observed for months after the acute infection. Postdischarge thromboprophylaxis may be beneficial. However, new studies are still necessary to investigate the participation of platelets and platelet-leukocyte aggregate formation in the persisting inflammatory and thromboembolic risk of COVID-19 survivors.

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AUTHOR CONTRIBUTIONS

Both authors wrote and edited the manuscript.

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