Received: 12 January 2022 | Revised: 29 March 2022 | Accepted: 31 March 2022

DOI: 10.1002/rth2.12709

rpth research & practice in thrombosis & haemostasis

STATE OF THE ART ISTH 2021

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

Eugenio D. Hottz PhD[1] | Patrícia T. Bozza MD, PhD[2]

[1]Laboratory of Immunothrombosis, Department of Biochemistry, Federal University of Juiz de Fora (UFJF), Juiz de Fora, MG, Brazil

[2]Laboratory of Immunopharmacology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, RJ, Brazil

Correspondence
Patrícia T. Bozza, Laboratory of Immunopharmacology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, RJ, Brazil.
Emails: pbozza@ioc.fiocruz.br; pbozza@gmail.com

Eugenio D. Hottz, Laboratory of Immunothrombosis, Department of Biochemistry, Federal University of Juiz de Fora (UFJF), Juiz de Fora, MG, Brazil.
Emails: eugenio.hottz@icb.ufjf.br; eugeniohottz@gmail.com

Funding information
Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; Fundação de Amparo à Pesquisa do Estado de Minas Gerais; Conselho Nacional de Desenvolvimento Científico e Tecnológico

Handling Editor: Prof. Yotis Senis

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.

1 | 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.[1] A state of hypercoagulability is a major pathophysiological mechanism and the main cause of mortality in severe COVID-19.[2-4] Individuals with severe COVID-19 syndrome frequently evolve with prothrombotic coagulation abnormalities, especially pulmonary embolism and deep vein thrombosis.[5,6] Higher frequency of COVID-19-associated thromboembolic events, cardiovascular complications, and death during postdischarge is also observed.[7,8] This hypercoagulable state is associated with respiratory distress syndrome in SARS-CoV-2-infected patients.[9,10] Postmortem pathological findings show extensive areas of platelet-fibrin microvascular thrombosis containing neutrophil and macrophage infiltration, NETosis, and endothelial inflammation (Figure 1A).[9-14] 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.[9,10,12,15] Pulmonary histopathological studies revealed these thromboinflammatory vascular occlusions to be almost 10 times more frequent in COVID-19 than in influenza pneumonia fatalities.[11,12,15] Aggravating an already complex situation, hypercoagulability and thromboinflammatory tissue damage have been reported despite prophylactic heparin anticoagulation,[10,16,17] and antiplatelet therapy with P2Y12 inhibitors fails to improve hypercoagulability and organ impairment,[18] highlighting the need of alternative antithrombotic strategies.

Platelet activation is a main feature of the COVID-19 hypercoagulable state.[19,20] Platelet activation in severe COVID-19 patients is associated with markers of coagulation activation and inflammation[10,19,21] and with increased incidence of thromboembolic complications.[22] Platelets from severe COVID-19 patients are hyperresponsive to agonist stimulation and form aggregates with

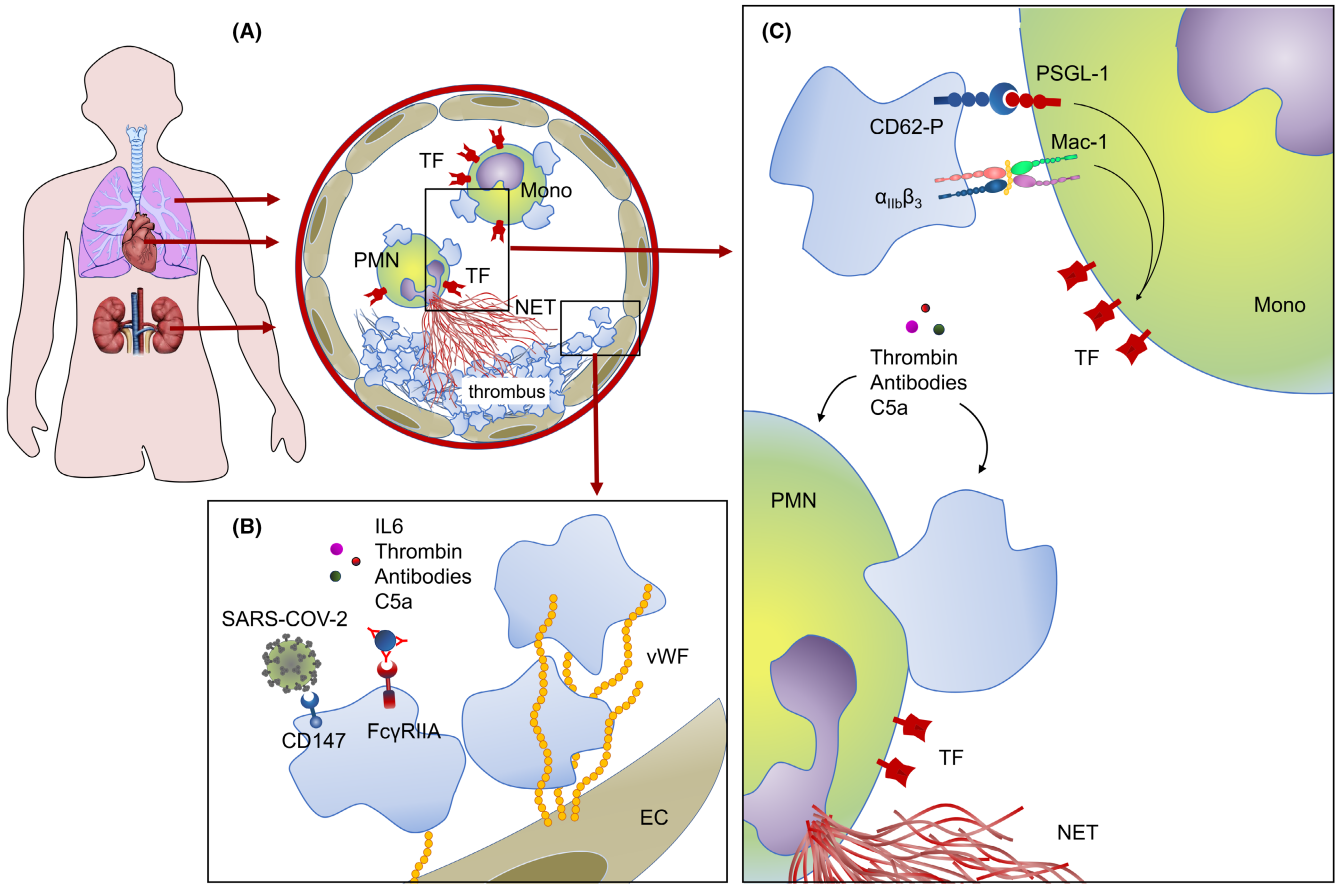

FIGURE 1 Platelet activation and hypercoagulability in COVID-19. (A) COVID-19-associated microvascular thrombosis: thromboinflammatory vascular occlusions presenting neutrophil and macrophage infiltration with NET-containing platelet-fibrin thrombi are observed in multiple organs during severe COVID-19. (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 α_{IIb}/β_3 signaling and neutrophil release of TF-containing NETs through mechanisms depending on Thrombin-PAR-1 and C5aR signaling

monocytes, lymphocytes, and neutrophils.[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 α_{IIb}/β_3 activation, and platelet extracellular vesicle shedding.[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.[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.[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.[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.[19,31,32] These circulating factors may include heightened thrombin generation, cytokine signaling, complement activation, and/or pro-coagulant antibodies (Figure 1B).[31,33-38] Platelets from healthy volunteers become activated when exposed to plasma from COVID-19 patients,[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.[31] 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.[34] Immunoglobulins from patients have major thrombogenic effects, including triggering platelet activation, apoptosis, platelet-thrombi formation and NET extrusion in vitro,[34-36,38] and driving intravascular NETosis and accelerated thrombosis in mice.[34] Aberrant glycosylation of anti-Spike antibodies and activation of Syk and PI3K/Akt signaling through FcγRIIa and C5aR are major signaling pathways in these processes.[33,35,36,38-40] Downstream inhibition of FcγRIIa/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.[36] 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).[37] Similarly, the releasate from COVID-19 patients' platelets also activates a thromboinflammatory program in endothelial cells.[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.[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.[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.[21,28,41,42] Similar results have been observed in platelets exposed to recombinant Spike protein and Spike-containing pseudovirus.[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.[41] However, whether platelets express or not ACE-2 has been a matter of controversy.[20,21,23,27,28,41,42] Different studies using similar techniques to detect ACE-2 have shown that platelets express[28,41] and do not express ACE-2.[20,21,23,27,42] Alternative receptors for SARS-CoV-2 attachment in platelets, including CD147 and CD42b, have been proposed.[21,27,42,43] Neutralizing antibodies against CD147, but not ACE-2, prevent platelet activation after exposure to SARS-CoV-2, Spike-pseudovirus, or recombinant Spike protein.[21] Similarly, Spike protein binds to CD42b and triggers Akt-protein kinase C pathway to induce platelet activation.[43] Of note, SARS-CoV-2 RNA has been detected in platelets from infected patients,[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.[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.[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.[14,20] Platelet-monocyte and platelet-neutrophil aggregate formation were especially higher in severe COVID-19 patients, and were associated with hyperinflammation and hypercoagulability.[10,19,32,44] Importantly, circulating platelet-monocyte and platelet-neutrophil aggregates in severe COVID-19 patients express high levels of TF,[19,31,33] the main trigger of intravascular coagulation and thrombosis (Figure 1C).[45]

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.[19] 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.[19] Mechanistically, platelets from severe COVID-19 patients induced monocyte TF expression depending on P-selectin and integrin α_{IIb}/β_3 signaling (Figure 1C).[19] 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.[43] 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 α_{IIb}/β_3 binding and is amplified by TF signaling.[46] Platelet activation and monocyte TF expression positively correlate with plasma levels of D-dimers, supporting a role in hypercoagulability.[19] 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.[19] 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).[9,10] 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.[9,10,32,33] Isolated neutrophils from COVID-19 patients show features of activation and spontaneously release NETs[9,33] that are decorated with TF.[33] Plasma from COVID-19 patients also activates control neutrophils and significantly induces NET extrusion ex vivo.[9,32,47] Similarly, anti-phospholipid antibodies isolated from COVID-19 patients also induce NETs release in neutrophils from healthy volunteers.[34] 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.[33] Neutrophil extrusion of TF-containing NETs in response to PRP from COVID-19 patients depended on Thrombin-PAR-1 and C5aR signaling (Figure 1C).[33] 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.[48-51] Preliminary proteomic data on platelet and platelet releasate have revealed pathways associated with ribosomal protein synthesis, altered mitochondrial activity, and defective thrombopoietin signaling,[49] as well as platelet release of inflammation and vasoactive proteins, alongside proteins related to extracellular vesicles.[50] These reports are in line with evidence of mitochondrial dysfunction and platelet apoptosis presented by Althaus and colleagues[51] and of platelet exhaustion of alpha and dense granules content in severe COVID-19 patients presented by Manukjan and colleagues.[48]

A major mechanism of platelet activation in COVID-19 that was extensively explored in the 2021 ISTH congress is the presence of procoagulant antibodies.[51-55] Immunoglobulins isolated from COVID-19 patients were reported to induce platelet activation,[53] platelet apoptosis,[51] procoagulant activity in monocytes, endothelial cells in culture,[52] and accelerated thrombosis in mice.[55] The presented abstracts have shed new light on the signaling mechanisms of antibody-mediated platelet activation that involved FcγRIIA and[51,54] PI3K/Akt signaling pathway[53] and was preventable by Iloprost-induced cAMP increase.[54] 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,[55] 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 α_{IIb}/β_3 amplification.[56] Regarding platelet-mediated protective responses in COVID-19, Ishizuka et al.[57] 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.[58,59] Recently, platelet antigen presentation was associated with CD8+ T-cell suppression in sepsis.[58] 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/PLA_2 leading to TXA_2 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 TXB_2, 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 (PaO_2/FiO_2), 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 α_{IIb}/β_3 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; PLA_2, phospholipase A_2; 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; TXA_2, thromboxane A_2; VTE, venous thromboembolism

in malaria.[59] 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.[60,61] 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.[7,8,62-64] Postdischarge thromboprophylaxis may be beneficial.[7] However, new studies are still necessary to investigate the participation of platelets and platelet-leukocyte aggregate formation in the persisting inflammation and thromboembolic risk of COVID-19 survivors.

ACKNOWLEDGMENTS

The work of the authors is supported by CNPq, CAPES, FAPERJ, FAPEMIG, and INOVA/FIOCRUZ.

RELATIONSHIP DISCLOSURE

The authors declare that they have no conflicts of interest relevant to the content of this manuscript. P.T.B. has received fees as lecturer for GSK.

AUTHOR CONTRIBUTIONS

Both authors wrote and edited the manuscript.

ORCID

Eugenio D. Hottz https://orcid.org/0000-0002-2201-1742
Patrícia T. Bozza https://orcid.org/0000-0001-8349-9529

TWITTER

Eugenio D. Hottz @EugenioHottz

REFERENCES

1. World Health Organization. WHO coronavirus (COVID-19) dashboard | WHO coronavirus (COVID-19) dashboard with vaccination data [Internet] [cited 2022 Jan 7]. Available from: https://covid19.who.int/
2. 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(4):844-847.
3. Klok FA, Kruip MJHA, van der Meer NJM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res*. 2020;191:145-147.
4. Middeldorp S, Coppens M, van Haaps TF, et al. Incidence of venous thromboembolism in hospitalized patients with COVID-19. *J Thromb Haemost*. 2020;18(8):1995-2002. doi:10.1111/jth.14888
5. Bikdeli B, Madhavan MV, Jimenez D, et al. COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. *J Am Coll Cardiol*. 2020;75(23):2950-2973. doi:10.1016/j.jacc.2020.04.031
6. Asakura H, Ogawa H. COVID-19-associated coagulopathy and disseminated intravascular coagulation. *Int J Hematol*. 2021;113(1):45-57. doi:10.1007/s12185-020-03029-y
7. Giannis D, Allen SL, Tsang J, et al. Postdischarge thromboembolic outcomes and mortality of hospitalized patients with COVID-19: the CORE-19 registry. *Blood*. 2021;137(20):2838-2847. doi:10.1182/blood.2020010529
8. Xie Y, Xu E, Bowe B, Al-Aly Z. Long-term cardiovascular outcomes of COVID-19. *Nat Med*. 2022;28(3):583-590. doi:10.1038/s41591-022-01689-3
9. Middleton EA, He XY, Denorme F, et al. Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. *Blood*. 2020;136(10):1169-1179. doi:10.1182/blood.2020007008
10. Nicolai L, Leunig A, Brambs S, et al. Immunothrombotic dysregulation in COVID-19 pneumonia is associated with respiratory failure and coagulopathy. *Circulation*. 2020;142(12):1176-1189. doi:10.1161/CIRCULATIONAHA.120.048488
11. McMullen PD, Cho JH, Miller JL, Husain AN, Pytel P, Krausz T. A Descriptive and quantitative immunohistochemical study demonstrating a spectrum of platelet recruitment patterns across pulmonary infections including COVID-19. *Am J Clin Pathol*. 2021;155(3):354-363. doi:10.1093/ajcp/aqaa230
12. Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in covid-19. *N Engl J Med*. 2020;383(2):120-128. doi:10.1056/NEJMoa2015432
13. Aid M, Busman-Sahay K, Vidal SJ, et al. Vascular disease and thrombosis in SARS-CoV-2-infected rhesus macaques. *Cell*. 2020;183(5):1354-1366.e13. doi:10.1016/j.cell.2020.10.005
14. Barrett TJ, Cornwell M, Myndzar K, et al. Platelets amplify endotheliopathy in COVID-19. *Sci Adv*. 2021;7(37):eabh2434. doi:10.1126/sciadv.abh2434
15. Nicolai L, Leunig A, Brambs S, et al. Vascular neutrophilic inflammation and immunothrombosis distinguish severe COVID-19 from influenza pneumonia. *J Thromb Haemost*. 2021;19(2):574-581. doi:10.1111/jth.15179
16. Blasi A, von Meijenfeldt FA, Adelmeijer J, et al. In vitro hypercoagulability and ongoing in vivo activation of coagulation and fibrinolysis in COVID-19 patients on anticoagulation. *J Thromb Haemost*. 2020;18(10):2646-2653. doi:10.1111/jth.15043
17. Sadeghipour P, Talasaz AH, Rashidi F, et al. Effect of intermediate-dose vs standard-dose prophylactic anticoagulation on thrombotic events, extracorporeal membrane oxygenation treatment, or mortality among patients with COVID-19 admitted to the intensive care unit: the INSPIRATION randomized clinical trial. *JAMA*. 2021;325(16):1620-1630. doi:10.1001/jama.2021.4152
18. Berger JS, Kornblith LZ, Gong MN, et al. Effect of P2Y12 inhibitors on survival free of organ support among non-critically Ill hospitalized patients with COVID-19: a randomized clinical trial. *JAMA*. 2022;327(3):227-236. doi:10.1001/jama.2021.23605
19. Hottz ED, Azevedo-Quintanilha IG, Palhinha L, et al. Platelet activation and platelet-monocyte aggregate formation trigger tissue factor expression in patients with severe COVID-19. *Blood*. 2020;136(11):1330-1341. doi:10.1182/blood.2020007252
20. Kanth Manne B, Denorme F, Middleton EA, et al. Platelet gene expression and function in patients with COVID-19. *Blood*. 2020;136(11):1317-1329. doi:10.1182/blood.2020007214
21. Maugeri N, De Lorenzo R, Clementi N, et al. Unconventional CD147-dependent platelet activation elicited by SARS-CoV-2 in COVID-19. *J Thromb Haemost*. 2022;20(2):434-448. doi:10.1111/jth.15575
22. Barrett TJ, Lee AH, Xia Y, et al. Platelet and vascular biomarkers associate with thrombosis and death in coronavirus disease. *Circ Res*. 2020;127(7):945-947. doi:10.1161/CIRCRESAHA.120.317803

23. Zaid Y, Puhm F, Allaeys I, et al. Platelets can associate with SARS-CoV-2 RNA and are hyperactivated in COVID-19. *Circ Res*. 2020;127(11):1404-1418. doi:10.1161/CIRCRESAHA.120.317703
24. Zaid Y, Guessous F, Puhm F, et al. Platelet reactivity to thrombin differs between patients with COVID-19 and those with ARDS unrelated to COVID-19. *Blood Adv*. 2021;5(3):635-639. doi:10.1182/bloodadvances.2020003513
25. Accioly MT, Pacheco P, Maya-Monteiro CM, et al. Lipid bodies are reservoirs of cyclooxygenase-2 and sites of prostaglandin-E2 synthesis in colon cancer cells. *Cancer Res*. 2008;68(6):1732-1740.
26. Sacchi A, Grassi G, Notari S, et al. Expansion of myeloid derived suppressor cells contributes to platelet activation by L-arginine deprivation during SARS-CoV-2 infection. *Cells*. 2021;10(8):2111. doi:10.3390/cells10082111
27. Barrett TJ, Bilaloglu S, Cornwell M, et al. Platelets contribute to disease severity in COVID-19. *J Thromb Haemost*. 2021;19(12):3139-3153. doi:10.1111/jth.15534
28. Koupenova M, Corkrey HA, Vitseva O, et al. SARS-CoV-2 initiates programmed cell death in platelets. *Circ Res*. 2021;129(6):631-646. doi:10.1161/CIRCRESAHA.121.319117
29. Campo G, Contoli M, Fogagnolo A, et al. Over time relationship between platelet reactivity, myocardial injury and mortality in patients with SARS-CoV-2-associated respiratory failure. *Platelets*. 2021;32(4):560-567. doi:10.1080/09537104.2020.1852543
30. Yatim N, Boussier J, Chocron R, et al. Platelet activation in critically ill COVID-19 patients. *Ann Intensive Care*. 2021;11(1):113. doi:10.1186/s13613-021-00899-1
31. Canzano P, Brambilla M, Porro B, et al. Platelet and endothelial activation as potential mechanisms behind the thrombotic complications of COVID-19 patients. *JACC Basic to Transl Sci*. 2021;6(3):202-218. doi:10.1016/j.jacbts.2020.12.009
32. Petito E, Falcinelli E, Paliani U, et al. Association of neutrophil activation, more than platelet activation, with thrombotic complications in coronavirus disease 2019. *J Infect Dis*. 2021;223(6):933-944. doi:10.1093/infdis/jiaa756
33. Skendros P, Mitsios A, Chrysanthopoulou A, et al. Complement and tissue factor–enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis. *J Clin Invest*. 2020;130(11):6151-6157. doi:10.1172/JCI141374
34. Zuo Y, Estes SK, Ali RA, et al. Prothrombotic autoantibodies in serum from patients hospitalized with COVID-19. *Sci Transl Med*. 2020;12(570):eabd3876. doi:10.1126/scitranslmed.abd3876
35. Nazy I, Jevtic SD, Moore JC, et al. Platelet-activating immune complexes identified in critically ill COVID-19 patients suspected of heparin-induced thrombocytopenia. *J Thromb Haemost*. 2021;19(5):1342-1347. doi:10.1111/jth.15283
36. Bye AP, Hoepel W, Mitchell JL, et al. Aberrant glycosylation of anti-SARS-CoV-2 spike IgG is a prothrombotic stimulus for platelets. *Blood*. 2021;138(16):1481-1489. doi:10.1182/blood.2021011871
37. Aiello S, Gastoldi S, Galbusera M, et al. C5a and C5aR1 are key drivers of microvascular platelet aggregation in clinical entities spanning from aHUS to COVID-19. *Blood Adv*. 2022;6(3):866-881. doi:10.1182/bloodadvances.2021005246
38. Althaus K, Marini I, Zlamal J, et al. Antibody-induced procoagulant platelets in severe COVID-19 infection. *Blood*. 2021;137(8):1061-1071. doi:10.1182/blood.2020008762
39. Apostolidis SA, Sarkar A, Giannini HM, et al. Signaling through FcγRIIA and the C5a–C5aR pathway mediates platelet hyperactivation in COVID-19. *bioRxiv Prepr Serv Biol*. 2021. doi:10.1101/2021.05.01.442279
40. Pelzl L, Singh A, Funk J, et al. Antibody-mediated procoagulant platelet formation in COVID-19 is AKT dependent. *J Thromb Haemost*. 2022;20(2):387-398. doi:10.1111/jth.15587
41. Zhang S, Liu Y, Wang X, et al. SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19. *J Hematol Oncol*. 2020;13(1):120. doi:10.1186/s13045-020-00954-7
42. Shen S, Zhang J, Fang Y, et al. SARS-CoV-2 interacts with platelets and megakaryocytes via ACE2-independent mechanism. *J Hematol Oncol*. 2021;14(1):1-5. doi:10.1186/s13045-021-01082-6
43. Li T, Yang Y, Li Y, et al. Platelets mediate inflammatory monocyte activation by SARS-CoV-2 spike protein. *J Clin Invest*. 2022;132(4):e150101. doi:10.1172/JCI150101
44. Le Joncour A, Biard L, Vautier M, et al. Neutrophil-platelet and monocyte-platelet aggregates in COVID-19 patients. *Thromb Haemost*. 2020;120(12):1733-1735. doi:10.1055/s-0040-1718732
45. Grover SP, Mackman N. Tissue factor: an essential mediator of hemostasis and trigger of thrombosis. *Arterioscler Thromb Vasc Biol*. 2018;38(4):709-725. doi:10.1161/ATVBAHA.117.309846
46. Hottz ED, Martins-Gonçalves R, Palhinha L, et al. Platelet-monocyte interaction amplifies thromboinflammation through tissue factor signaling in COVID-19. *Blood Adv*. 2022; ahead of print. https://doi.org/10.1182/bloodadvances.2021006680
47. Zuo Y, Yalavarthi S, Shi H, et al. Neutrophil extracellular traps in COVID-19. *JCI Insight*. 2020;5(11):e138999. doi:10.1172/jci.insight.138999
48. Manukjan G, Herrmann J, Kleiß J, et al. Systemic platelet exhaustion in critically-ill covid-19 patients - ISTH Congress abstracts. *Res Pract Thromb Haemost*. 2021;5(suppl 2):PB0218. https://abstracts.isth.org/abstract/systemic-platelet-exhaustion-in-critically-ill-covid-19-patients/
49. Goudswaard L, Williams C, Hamilton F, Arnold D, Davidson A, Poole AHI. Altered platelet proteome and platelet-leukocyte interactions in patients with COVID-19 - ISTH Congress abstracts. *Res Pract Thromb Haemost*. 2021;5(suppl 2):OC 15.2. https://abstracts.isth.org/abstract/altered-platelet-proteome-and-platelet-leukocyte-interactions-in-patients-with-covid-19/
50. Comer S, Cullivan S, Szklanna P, et al. Impact of COVID-19 on platelet activity and the platelet releasate - ISTH Congress abstracts. *Res Pract Thromb Haemost*. 2021;5(suppl 2):PB0137. https://abstracts.isth.org/abstract/impact-of-covid-19-on-platelet-activity-and-the-platelet-releasate/
51. Althaus K, Marini I, Zlamal J, et al. COVID-19 infection is associated with antibody-mediated procoagulant platelets: data from an observational study - ISTH Congress abstracts. *Res Pract Thromb Haemost*. 2021;5(suppl 2):PB0179. https://abstracts.isth.org/abstract/covid-19-infection-is-associated-with-antibody-mediated-procoagulant-platelets-data-from-an-observational-study/
52. Hollerbach A, Müller-Calleja N, Pedrosa D, et al. Lipid-binding antiphospholipid antibodies trigger autoimmune signaling in severe COVID-19 - ISTH Congress abstracts. *Res Pract Thromb Haemost*. 2021;5(suppl 2):OC 03.4. https://abstracts.isth.org/abstract/lipid-binding-antiphospholipid-antibodies-trigger-autoimmune-signaling-in-severe-covid-19/
53. Pelzl L, Singh A, Funk J, et al. Platelet activation via PI3K/AKT signaling pathway in COVID-19 - ISTH Congress abstracts. *Res Pract Thromb Haemost*. 2021;5(suppl 2):OC 70.4. https://abstracts.isth.org/abstract/platelet-activation-via-pi3k-akt-signaling-pathway-in-covid-19/
54. Zlamal J, Althaus K, Jaffal H, et al. cAMP prevents antibody induced thrombus formation in COVID-19 - ISTH Congress abstracts. *Res Pract Thromb Haemost*. 2021;5(suppl 2):OC 70.1. https://abstracts.isth.org/abstract/camp-prevents-antibody-induced-thrombus-formation-in-covid-19/
55. Pedrosa D, Müller-Calleja N, Hollerbach A, Lackner KRW. Blockade of COVID-19 antiphospholipid antibody-induced thrombosis by a specific inhibitor of the TF initiation complex - ISTH Congress abstracts. *Res Pract Thromb Haemost*. 2021;5(suppl 2):LPB0102. https://abstracts.isth.org/abstract/blockade-of-covid-19-antiphospholipid-antibody-induced-thrombosis-by-a-specific-inhibitor-of-the-tf-initiation-complex/
56. Williams C, Khalil J, Walsh T, et al. SARS-CoV2 spike protein can activate platelets through integrin alpha IIb beta 3 - ISTH Congress

abstracts. *Res Pract Thromb Haemost*. 2021;5(suppl 2):PB0157. https://abstracts.isth.org/abstract/sars-cov2-spike-protein-can-activate-platelets-through-integrin-alpha-iib-beta-3/

57. Ishizuka M, Estevez B, Sarkar A, et al. Platelet factor 4 (PF4) enhances in vitro neutrophil extracellular traps (NET) capture of coronaviruses: clinical and therapeutic implications - ISTH Congress abstracts. *Res Pract Thromb Haemost*. 2021;5 (suppl 2):OC 15.1. https://abstracts.isth.org/abstract/platelet-factor-4-pf4-enhances-in-vitro-neutrophil-extracellular-traps-net-capture-of-coronaviruses-clinical-and-therapeutic-implications/
58. Guo L, Shen S, Rowley JW, et al. Platelet MHC class I mediates CD8+ T cell suppression during sepsis. *Blood*. 2021;138(5):401-416. doi:10.1182/blood.2020008958
59. Chapman LM, Aggrey AA, Field DJ, et al. Platelets present antigen in the context of MHC class I. *J Immunol*. 2012;189(2):916-923. doi:10.4049/jimmunol.1200580
60. Huang L, Yao Q, Gu X, et al. 1-year outcomes in hospital survivors with COVID-19: a longitudinal cohort study. *Lancet (London, England)*. 2021;398(10302):747-758. doi:10.1016/S0140-6736(21)01755-4
61. Mandal S, Barnett J, Brill SE, et al. "Long-COVID": a cross-sectional study of persisting symptoms, biomarker and imaging abnormalities following hospitalisation for COVID-19. *Thorax*. 2021;76(4):396-398. doi:10.1136/thoraxjnl-2020-215818
62. Patell R, Bogue T, Koshy A, et al. Postdischarge thrombosis and hemorrhage in patients with COVID-19. *Blood*. 2020;136(11):1342-1346. doi:10.1182/blood.2020007938
63. Townsend L, Fogarty H, Dyer A, et al. Prolonged elevation of D-dimer levels in convalescent COVID-19 patients is independent of the acute phase response. *J Thromb Haemost*. 2021;19(4):1064-1070. doi:10.1111/jth.15267
64. Fogarty H, Townsend L, Morrin H, et al. Persistent endotheliopathy in the pathogenesis of long COVID syndrome. *J Thromb Haemost*. 2021;19(10):2546-2553. doi:10.1111/jth.15490
65. Taus F, Salvagno G, Canè S, et al. Platelets promote thromboinflammation in SARS-CoV-2 pneumonia. *Arterioscler Thromb Vasc Biol*. 2020;40(12):2975-2989. doi:10.1161/ATVBAHA.120.315175

How to cite this article: Hottz ED, Bozza PT. Platelet-leukocyte interactions in COVID-19: Contributions to hypercoagulability, inflammation, and disease severity. *Res Pract Thromb Haemost*. 2022;6:e12709. doi:10.1002/rth2.12709