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Review Article

Rethinking the Immune Regulatory Role of Platelets during the COVID-19 Pandemic

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SUMMARY

Since the start of the COVID-19 pandemic, the recognition and management of thrombotic complications has become a clinical challenge, either in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) patients or in those receiving vector-based COVID-19 vaccination. In addition to blood clot formation, platelets can also respond to a variety of inflammatory cytokines and act in concert with circulating leukocytes to prevent pathogen infection. Herein, we review the basic biological roles of platelets in infection/inflammation, tools for assessment, and mechanisms of platelet activation to elucidate their immune regulatory roles.

1. Introduction

The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), rapidly spread across the globe since the first outbreak in Wuhan, China, at the end of 2019.

In addition to acute pulmonary deterioration, the disease also presents with severe coagulopathy with a high rate of venous and arterial thrombosis, as reported in several retrospective and prospective studies.[1–7] Earlier autopsy studies of COVID-19 cases reported co-localization of thrombosis and inflammation[8] within the pulmonary capillary vasculature.[9] Although COVID-19 vaccines are considered the most promising approach to controlling the pandemic,[10] vector-based vaccines have been associated with an unusual but not negligible amount of thrombotic events associated with thrombocytopenia (thrombosis with thrombocytopenia syndrome [TTS] or vaccine-induced immune thrombotic thrombocytopenia [VITT]).[11,12] The concerns around VITT unavoidably lead to misinformation and vaccine hesitancy.[13]

Platelets, in addition to hemostasis, also play an important role in the convergence of thrombosis and inflammation. For healthcare workers, early diagnosis, proper management, and prophylaxis of thromboembolic complications have been challenging throughout the course of SARS-CoV-2 infection and vaccination. In this article, we review the roles of platelets during the inflammatory response and COVID-19 pandemic from literature search in PubMed up to February 2022 in order to equip healthcare workers with a better understanding of the disease.

2. Platelet structure, platelet-associated granular constituents, and approaches to platelet function assessment

Platelets are short-lived (7–10 days), small (up to 2 μm in diameter), anucleate fragments.[14] According to transmission electron microscopy, the cytoplasm of a platelet is packed with complex membranous systems, such as surface-connected canalicular system (SCCS) and dense tubular system (DTS), and cytoplasmic organelles, including mitochondria, α-granules, dense granules (or dense bodies), peroxisomes, lysosomes, and glycogens.[14] α-Granules and dense granules are the major secretory granules for platelet activation. These platelet-associated secretory granules amplify the responses to stimuli and influence the surrounding environment. To date, more than 300 active substances have been identified.[15,16] The major constituents in platelet granules are summarized in Table 1. In addition to coagulant proteins, platelets secrete many cytokines, chemokines, and immunomodulators to promote platelet/endothelial and platelet/leukocyte interactions.[17]

Although the ultrastructure of platelets in patients with SARS-CoV-2 infection was found to be similar to those in healthy donors by transmission electron microscopy,[18] platelet hyperactivation as well as elevated plasma levels of coagulant proteins, such as fibrinogen, factor V, factor VIII, and von Willebrand factor (vWF),[19–22] have been reported. However, these factors can be released from other sources, such as damaged endothelium or liver tissue, and are not platelet-specific.

The identification and characterization of platelet dysfunction or hyperfunction have become an active area of research interest because of the COVID-19 pandemic. Using radioimmunoassays (RIAs) or enzyme-linked immunosorbent assays (ELISAs), the detection of soluble β-thromboglobulin (β-TG) or platelet factor 4 (PF4), which are released from α-granules of activated platelets, provides simple and useful measurements for the assessment of platelet activation *ex vivo*. Elevated plasma levels of β-TG and PF4 have been reported in several clinical studies of moderate COVID-19 patients,[23] suggesting platelet hyperactivation. However, these results may be artifactual due to venipuncture and blood handling.[24]

Alternatively, whole blood cytometric analysis of P-selectin or CD62p, also an alpha granular membrane protein that is expressed

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Table 1
Major platelet granules and their constituents (adapted from[16,17]).

I. α-Granule proteins	
(A) Coagulation factors	Fibrinogen (critical ligand for aggregation)
	α2-Antiplasmin
	α2-Antitrypsin
	α2-Macroglobulin
	Antithrombin
	Factor V (critical cofactor for coagulation)
	Factor VIII/XI/XIII
	PAI-1
	Plasmin
	Plasminogen
	Protease nexin-2
	Protein S
	Prothrombin
	TFPI
(B) Platelet-specific proteins	Platelet factor 4 (PF4) (also known as CXCL4) (marker for platelet activation)
	β-thromboglobulin (marker for platelet activation)
(C) Mitogenic and angiogenic factors	PDGF
	Transforming growth factor-β
	VEGF (relative high concentration in platelets)
	ADAM10
	ADAMTS13
	Angiostatin
	Angiopoietin-1
	BDNF
	bFGF
	BMP-2, BMP-4, BMP-6
	CTAP-III
	CTGF
	EGF
	Endostatin
	HGF
	IGF-1
	Kininogen
	MMP-1, MMP-2, MMP-9
	Thrombospondin
	TIMP-1, TIMP-4
(D) Adhesive glycoproteins and α-granule membrane-specific proteins	P-selectin (mediates platelet-leukocyte binding)
	vWF (mediates platelet adhesion)
	Multimerin
	Thrombospondin
	αIIβ3, αVβ3
	CD9
	Fibronectin
	GPIα
	Osteonectin
	PECAM
	Vitronectin
(E) Cytokines/chemokines	β-Thromboglobulin
	CCL4, CCL17
	ENA-78
	Gro-α
	IL-1, IL-7, IL-8
	MCP-1, MCP-3
	MIP-1α
	NAP-2
	PF4
	RANTES (also known as CCL5)
	sCD40L (major platelet-associated cytokines)
	SDF-1
(F) Immune mediators and others	C1 inhibitor
	Complement factors
	Factor D
	IgA, IgG, IgM
	Platelet factor H
	Thymosin-β4
	Albumin
	PDCI

Table 1 Continued.

II. Dense granule elements	
(A) Nucleotides	ADP (high concentrated, critical mediator for platelet aggregation)
	ATP
(B) Cations	Calcium
	Magnesium
(C) Bioactive amines	Serotonin
	Histamine
	GTPases
	Rab27a, rab27b
(D) Membrane proteins	αIIbβ3
	CD63
	GPIb
	LAMP-1, LAMP-2
	P-selectin
(E) Others	Polyphosphate, pyrophosphate
III. Lysosomes	
Enzymes	Several enzymes, including glycosidases and acid proteases (hold bactericidal activity)

Abbreviations: ADAM 10: a disintegrin and metalloproteinase domain-containing protein 10; ADAMTS: a disintegrin and metalloproteinase with thrombospondin motifs; ADP: adenosine diphosphate; ATP: adenosine triphosphate; BDNF: brain-derived neurotrophic factor; bFGF: basic fibroblast growth factor; BMP: bone morphogenetic protein; C1 inhibitor: platelet C1 esterase inhibitor; CCL: chemokine (C-C motif) ligand; CD: cluster of differentiation; CTAP-III: connective tissue activating peptide III; CTGF: connective tissue growth factor; CXCL4: chemokine (C-X-C motif) ligand 4; EGF: epidermal growth factor; ENA-78: endostatin epithelial neutrophil-activating peptide; GPI: glycoprotein I; GTP: Guanosine triphosphate; HGF: hepatocyte growth factor; Ig: Immunoglobin; IGF-1: insulin-like growth factor-1; IL: interleukin; LAMP: lysosomal-associated membrane protein; MCP: monocyte chemoattractant protein; MIP-1α: macrophage inflammatory protein-1α; MMP: matrix metalloproteinases; NAP-2: neutrophil activating peptide-2; PAI-1: plasminogen activator inhibitor 1; PDCI: platelet-derived collagenase inhibitor; PDGF: platelet-derived growth factor; PECAM: platelet endothelial cell adhesion molecule; PF4: platelet factor 4; RANTES: regulated upon activation normal T cell expressed and secreted; sCD40L, Soluble CD40-ligand; SDF-1: stromal cell derived factor 1; TFPI: tissue factor pathway inhibitor; TIMP: tissue inhibitors of matrix metalloproteinases; VEGF: vascular endothelial growth factor; vWF: von Willebrand factor.

only on the surface of activated platelets, can provide a powerful and less confounded assessment of platelet function. Manne BK et al., Hottz et al., and Taus et al. found increased P-selectin expression on platelets at either baseline or in a severe disease course, supporting platelet hyperactivation in SARS-CoV-2 infection.[18,25,26]

Using PF4 and serotonin as representative markers for platelet α-granules and dense granules, respectively, Zaid et al. found that both PF4 and serotonin were elevated in the plasma and reduced in the platelets of COVID-19 patients compared to those in healthy volunteers.[27] The observation further recapitulates platelet activation with degranulation during SARS-CoV-2 infection.

As a result of platelet activation, increased formation of platelet-leukocyte associations (PLAs), for example, platelet-neutrophil, platelet-T cell ($CD4^+$ and $CD8^+$), and platelet-monocyte aggregates, have been reported in COVID-19 patients.[18] PLA is a sensitive biomarker for *in vivo* platelet activation[28] and is used in the assessment of platelet activity.[29] Consistent with other approaches, PLA formation has been reported in severe COVID-19 patients[25] and linked to disease severity, mortality, respiratory condition, and vascular endothelial dysfunction.[30]

SARS-CoV-2 associated platelet hyperactivation has also been demonstrated in many laboratories with platelet aggregometry, a platelet functional assay measuring platelet aggregation,[18,27,31] or thromboelastography (TEG), a dynamic measure of clot formation in whole blood.[22,32]

In the absence of laboratory support, clinical assessment of platelet hyperfunction in COVID-19 patients can be challenging. Routine lab findings for COVID-19 include disproportionately increased D-dimer levels, elevated fibrinogen levels, high factor VIII activity, mild thrombocytopenia, minimal prolonged aPTT (activated partial thromboplastin time), and/or PT (prothrombin time).[33] This presentation is often referred to as a disseminated intravascular coagulopathy (DIC)-like status, since it does not fulfill the criteria of classic DIC as defined by the International Society of Thrombosis and Hemostasis (ISTH).[19] Clinicians should keep in mind that the major clinical feature in COVID-19 is thrombosis, while in acute decompensated DIC it is bleeding. Platelet hyperfunction, together with inflammation-associated endothelial injury and patient immobility, may contribute to hypercoagulable status/thrombosis formation and may worsen prognosis.

3. Platelet-derived inflammatory cytokines in COVID-19

As shown in Table 1, platelet α-granules serve as an important reservoir of many inflammatory cytokines. Zaid et al. studied platelets isolated from COVID-19 patients and found that several cytokines (Groα, interleukin-7 [IL-7], macrophage-derived chemokine [MDC], platelet-derived growth factor [PDGF-AB/BB], and regulated upon activation normal T cell expressed and secreted [RANTES]) were increased relative to a control. COVID-19 platelets were more prone to release IL-1β and soluble sCD40L upon stimulation relative to those from healthy individuals.[27] Similarly, Petrey et al. reported significantly increased vasculitis and vascular remodeling factors (PDGF-AA, PDGF-AB-BB, sCD40L, fibroblast growth factor [FGF], and IFN-γ-inducible protein 10 [IP10]) in COVID-19 patients compared to controls.[34] PDGF, FGF-2, and IP 10 were strongly associated with severe disease and intensive care unit (ICU) admission. Note that the major source of sCD40L is platelets and that PDGF and FGF-2 may partly be contributed by circulating platelets.[34,35] Platelet-secreted CD40L has been shown to interact with CD40 on endothelial cells and promote the expression of chemokines, adhesion molecules, E-selectin, P-selectin, and IL-6.[17] Thus, platelet-derived CD40L affects dendritic cells and lymphocytes.[17]

In addition to CD40L, other platelet α-granule-related substances, such as P-selectin, PF4, and RANTES (or chemokine ligand 5, CCL5), also play an important role in mediating platelet-leukocyte association. While P-selectin mediates platelet-leukocyte binding, PF4, also known as CXC chemokine ligand 4 (CXCL4), promotes neutrophil granule release/adhesion to endothelial cells and promotes monocytic differentiation into macrophages.[36] PF4 forms a

heterodimer with RANTES to promote monocyte recruitment to the endothelium.

These findings imply that cytokines released from activated platelets enhance the immune response to stimuli and communication between thrombosis and immunity to promote restoration of normal tissue function following injury.[15,28]

Although it remains to be elucidated, the above reports also suggest that platelets may play a critical role in contributing to the hyperinflammatory response in severe SARS-CoV-2 infection.

4. Interaction between platelets and pathogens

Platelets are abundant in circulation and therefore have a higher chance of encountering pathogens than any circulating leukocytes.[37] Through platelet surface glycoprotein IIb/IIIa, platelets can bind to several bacteria either directly[38] or indirectly via interaction between fibrinogen and the fibronectin-binding proteins on the bacteria.[39] The bacteria-platelet interaction can lead to platelet activation/aggregation and thromboinflammation.[40]

A variety of viral particles, such as HIV,[41] dengue,[42,43] influenza,[44,45] and SARS-CoV-2,[27] can be found inside platelets. In dengue fever, this interaction is followed by activation in CD61-positive and other hematopoietic progenitor cells as well as a release of a number of cytokines, which is associated with severe bone pain.[46] As dengue virus particles are engulfed by platelets, a decline in RNA viremia is often observed after 3–5 d of infection.[46]

The importance of platelets in viral infection has been demonstrated *in vivo* using mice with or without platelet depletion. Negrotto et al. inoculated mice with coxsackievirus B (CVB) and found that CVB was engulfed by platelets but did not replicate.[47] CVB-infected mice showed rapid thrombocytopenia and increased numbers of platelet-leukocyte aggregates. Platelet-depleted mice had significantly higher viremia, more myocarditis, and worse survival outcomes than the normal controls did. This further supports the critical role of platelet-neutrophil interactions in viral infections.

In a small proportion of COVID-19 patients (2 out of 25, 8%), transcriptional expression of the N1 SARS-CoV-2 gene was found in isolated platelets.[18] Another study found SARS-CoV-2 RNA in platelets from either non-severe (9 out of 38, 23.7%) or severe (2 out of 11, 18.2%) COVID-19 patients.[27] SARS-CoV-2 in megakaryocytes, the precursors of platelets, from bone marrow or lung tissue was also reported in autopsy results.[31] However, the mechanisms by which SARS-CoV-2 enters platelets or megakaryocytes remain to be elucidated.[48] It is well known that the host receptor for SARS-CoV-2 cell entry is angiotensin-converting enzyme 2 (ACE2). As several studies failed to identify ACE2 expression in platelets, the inclusion of SARS-CoV-2 viral particles may be mediated through an ACE2-independent mechanism.[48]

5. Molecular mechanism of platelet-involving immune activation

The molecular mechanism of platelet-involving immunity can be divided into two types: pattern recognition receptors (PRR) and cell-cell receptors (CCR).[49] PRR mainly reacts or co-reacts with pathogens, such as viral genome, bacterial protein A, and exogenous antigen, through receptors including several kinds of C-type lectin receptors (CLRs) and toll-like receptor (TLR).[50] Whereas CCR reacts to endogenous signals, such as P-selectin glycoprotein ligand 1 (PSGL-1) from neutrophils, or detection of a damaged endothelial cell's collagen by platelet surface integrin.[51] Upon activation, PRR and CCR can induce cascades of immune-related signal substance release and activate innate or adapted immune responses, such as complement activation, aggregation with activated T-cytolytic and T-helper cells, and formation of neutrophil extracellular traps (NETs), which consist of modified condensed chromatin and bactericidal proteins from granules and cytoplasm.[52] Activated PRR and CCR are also associated with platelet aggregation, which contributes to the pathology of thrombosis and thrombocytopenia.

Alterations in the number and function of platelets have also been reported in COVID-19 adenoviral vector-based vaccination,[53] though through different molecular mechanisms from those in SARS-CoV-2 infection. In SARS-CoV-2 infection, thrombi are likely induced following viral RNAemia and host cytokine storm. Through the interaction between SARS-CoV-2's RNA and platelets by TLR-7 and TLR-9,[54] NET formation accompanied by the cell death (NETosis) and thrombosis were induced (Figure 1).[52] On the other hand, VITT is likely induced by extravascular vector DNA-PF4 interaction.[55] PF4 is a chemokine stored in platelet α-granules and participates in immune cell regulation and pathogen opsonization and killing.[52] In COVID-19 VITT, the vector viral DNA-PF4 complexes form a kind of antibody that target PF4 produced by splenic B cells,[56,57] which, in turn, attack the DNA-PF4 complexes and cause thrombus formation and further thrombocytopenia. PF4-induced thrombotic thrombocytopenia also occurs in heparin-induced thrombocytopenia (HIT), in which heparin forms a large complex with PF4, thereby inducing an autoimmune attack. Thrombus formation in VITT takes two weeks to occur, suggesting that VITT may delay adaptive immunity activation, unlike HIT in which presensitizing anti-PF4 antibodies are present. The mechanism of autoimmune attack in COVID-19 VITT may explain the higher risk of VITT in younger women. Since adenoviral vector-based vaccines are injected intramuscularly, endothelial cell injuries from needle pricks may contribute to the release of PF4 from platelets and the induction of DNA-PF4 complexes (Figure 2).[55,58]

6. Platelet derangement in elderly patients with COVID-19

Comparing patients less and more than 60 years old, the incidence rate of idiopathic thrombocytopenic purpura (ITP) more than doubled in the older age group.[59] Thrombocytopenia is a risk factor for increased morbidity and mortality in patients with SARS-CoV-2 infection. In addition to viral infection, thrombocytopenia in COVID-19 patients may be the result of DIC, sepsis or drug-induced. Early reports show no statistically significant difference in the incidence of thrombocytopenia (platelet counts less than 100×10^9/L) among older adults (5.15% for patients > 60 years versus 3.07% for patients < 60 years, $p = 0.295$).[60] Using a different cut-off point (platelet counts less than 150×10^9/L), other study reported upto 36.2% of COVID-19 patients presents with thrombocytopenia, which is more prominent among severe cases.[61] With careful evaluation, immune complications associated with thrombocytopenia such as ITP[62–69] and autoimmune thrombotic thrombocytopenic purpura (TTP)[70–73] in elderly patients with COVID-19 have been reported. The clinical characteristic and treatment outcomes from case reports or case series that investigate the association between COVID-19 infection and ITP in the Science Citation Index (SCI)-listed journals are reviewed and summarized in Table 2. ITP is an autoimmune disease, which can be triggered by or associated with many viruses, including hepatitis C, cytomegalovirus, Epstein-Barr virus and others like SARS-CoV-1 and SARS-CoV-2.[65] As shown in Table 2, most elderly patients developed ITP within 2 weeks after COVID-19 infection and recovered in 1–4 week from ITP with steroids and intravenous immunoglobulin (IVIG) treatment and one had ITP relapsed 1 month later. Among three fatal cases, one died of intracerebral hemorrhage and the other two patients died of non-hematologic complica-

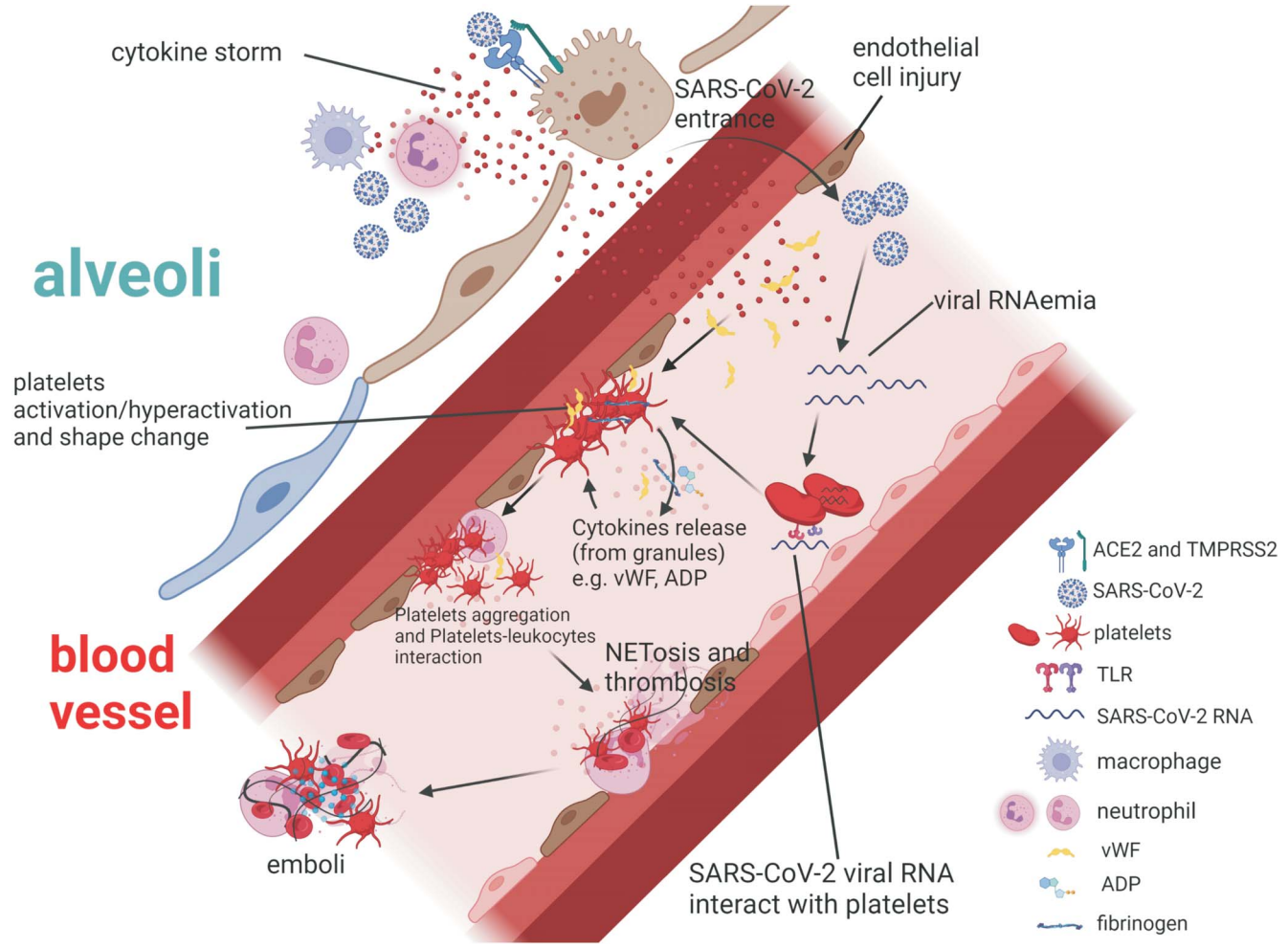

Figure 1. Proposed mechanism of platelet activation and immune consequences following SARS-CoV-2 infection (adapted from[48,52,55]). After SARS-CoV-2 infection, the virus enters the host's circulatory system and proceeds to spread viral RNA. Platelets are activated either by endothelial injury or the entry of viral RNA particles likely through the interaction with TLR-7 and TLR-9. Following platelet activation, more cytokines are released from granules which reinforces platelet aggregation, promotes PLA, induces the formation of NETosis with neutrophils and thrombosis. Created with BioRender.com. Abbreviations: ACE2, angiotensin-converting enzyme 2; ADP, adenosine diphosphate; NETosis, neutrophil extracellular traps activation and release; PLA: platelet-leukocyte associations; TLR, toll-like receptor; TMPRSS2, transmembrane protease, serine 2; vWF, von Willebrand factor.

tions.[63,68–69] Treatment for elderly COVID-19 patients with ITP should not be different from younger patients. For individuals at risk of serious bleeding, IVIG is recommended as it can achieve platelet count increments in 12–48 h, whereas it generally takes 2–5 days to get treatment response with steroids.[74] Thrombopoietin receptor agonists such as eltromopag or romiplostim, are usually recommended as second-line treatment with the concerns of increased thrombotic complications and hepatotoxicity.

7. Conclusions

The clinical presentation of COVID-19 ranges from asymptomatic infection to mild to critically fatal illness. The COVID-associated cytokine storm and microvascular thrombosis are among the main factors affecting disease severity and patient mortality. Moreover, post-COVID-19 syndrome, manifested by persistent and prolonged aftereffects, has been linked to COVID-19-associated microvascular thrombosis.[75] Although vaccination is considered the most promising approach in controlling the COVID-19 pandemic,[10] its success is challenged by the rare cases of thrombosis associated with thrombocytopenia following vector-based vaccination. This has led to mass distrust and vaccination hesitancy, which has become a major challenge for public health officials.

In addition to homeostasis, platelets are actively involved in disease development. Several studies have shown the internalization of viral particles in platelets, release of platelet-associated granular substances, platelet hyperactivation, and increased formation of platelet-leukocyte aggregates (PLA) in patients with moderate-to-severe SARS-CoV-2 infection. A limitation of the review is that COVID-19 is a newly described disease and our understanding of SARS-CoV-2-platelet interaction is still evolving, therefore, future studies are needed to explore the role of platelets during viral inflammation and develop therapeutic strategies that incorporate platelet function in the treatment of the disease.

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Conflicts of interest

The authors declare no conflict of interest.

Author statements

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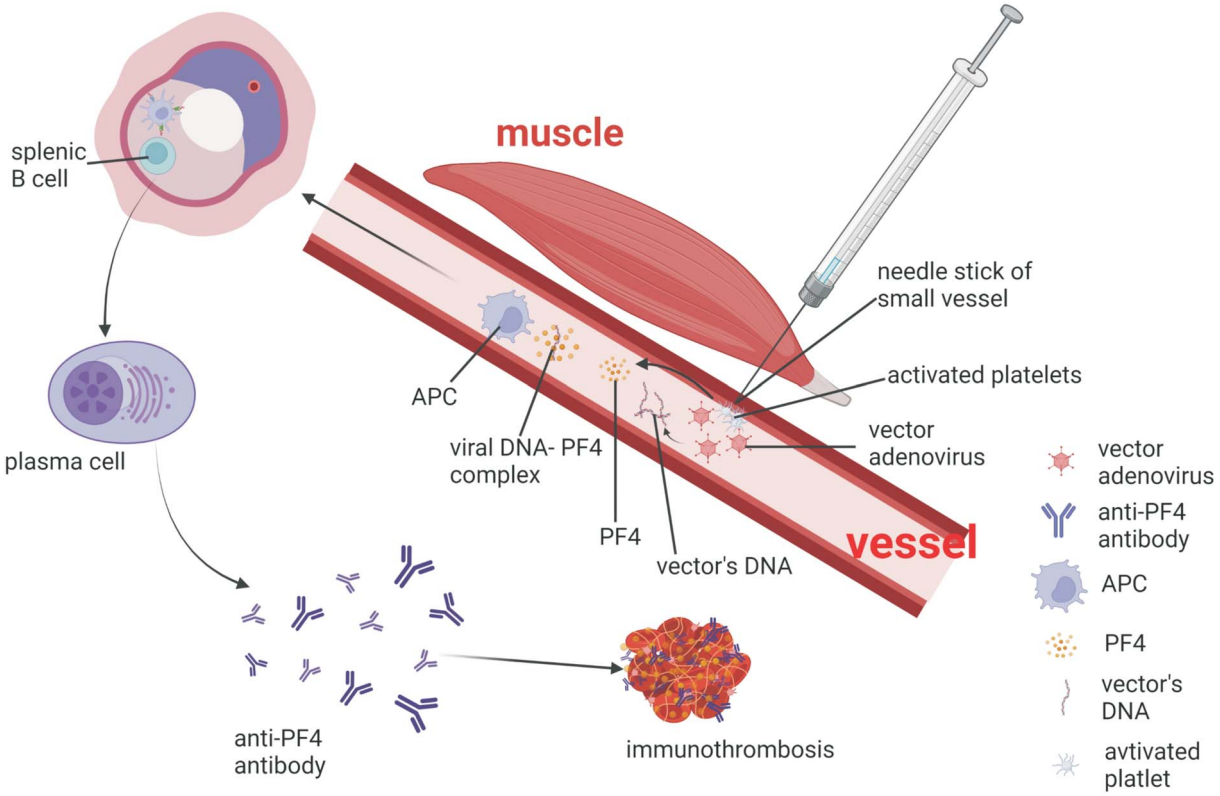

Figure 2. The development of vaccine-induced thrombosis (adapted from[54,55]). Following vaccine injection, the needle prick causes vessel injury and induces platelet activation, aggregation, and release of platelet-associated α-granular substances, such as platelet factor 4 (PF4). PF4 subsequently interacts with COVID-19 vector adenovirus DNA and forms the DNA-PF4 complex. This complex, which is recognized by antigen-presenting cells, triggers anti-PF4 antibody production in B cellsas part of an autoimmune attack that causes thrombosis and thrombocytopenia. Created with BioRender.com. Abbreviations: APC, antigen-presenting cells; PF4, platelet factor 4.

Table 2
Summary of immune thrombocytopenic purpura (ITP) case reports in elderly patients (age ≥ 65) with COVID-19.

No	Age	G.	Comorbidities	Platelet counts at presentation or lowers counts during COVID-19	Treatment	Time of platelet counts ≥ 10,000/μL	ITP outcomes	Ref.
1	65	F	Hypertension, Autoimmune hypothyroidism	D4: 66,000/μL D9: 2000/μL	IVIG, Prednisolone, Eltromopag	D10: 10,000/μL	Recovery	[62]
2	66	F	Hypertension	D1: 2,000/μL	Dexamethasone, IVIG	D22: 320,000/μL	Recovery	[63]
3	86	M	Hypertension, DM	D1: 10,000/μL	IVIG, Prednisolone	D10: 100,000/μL	Recovery	[64]
4	66	M	N/A	Lowest: 1,000/μL	IVIG, Eltrombopag	N/A	Recovery	[65]
5	74	M	N/A	Lowest: < 1,000/μL	Prednisone	N/A	Recovery	[65]
6	65	M	N/A	Lowest: 17,000/μL	Dexamethasone	N/A	Recovered initially relapsed later (D30)	[65]
7	66	F	N/A	Lowest: 8,000/μL	Methylprednisolone, IVIG, Eltrombopag	N/A	Recovery	[65]
8	79	F	N/A	Lowest: 9,000/μL	IVIG	N/A	Recovery	[65]
9	69	F	N/A	Lowest: < 10,000/μL	IVIG, Romiplostim	N/A	Recovery	[65]
10	72	M	N/A	Lowest: 8,000/μL	IVIG	N/A	Recovery	[65]
11	70	M	Hypertension, COPD, IHD	Initial: 150,000/μL 10 days later (D1): 10,000/μL	Hydrocortisone, Dexamethasone, IVIG	D8: 140,000/μL	Recovery	[66]
12	83	F	N/A	D1: 2,000/μL	Prednisone, IVIG, Eltrombopag	D24: > 100,000/μL	Recovery	[67]
13	67	M	Hypertension, DM	Initial: 338,000/μL, D12: 3000/μL	Anticoagulant, intubated	N/A	Expired due to ICH	[63]
14	89	M	CHF, Hypertension, DM, CKD, Af	D1: 175000/μL D13: 2000/μL (DVT on D13)	Warfarin Prophylactic Enoxaparin, Argatroban, Dexamethasone, IVIG	N/A (Short-term response achieved at D17, 79 000/μL)	Expired on D20 (cause of death: not stated)	[68]
15	96	F	Af, IHD, CKD	D1: 109,000/μL D5: 3000/μL	IVIG	N/A (Short-term response achieved on D10, 16,000/μL)	Expired (due to respiratory failure)	[69]

Abbreviation: Af: atrial fibrillation; CHF: congestive heart failure; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; CVA: cerebrovascular accident; DM: type 2 diabetes mellitus; G.: gender; ICH: intracerebral hemorrhage; ITP: immune thrombocytopenic purpura; IHD: ischaemic heart disease; IVIG: Intravenous immunoglobulin; N/A: not available; No.: case number; Ref.: references.

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References

1. Rashidi F, Barco S, Kamangar F, et al. Incidence of symptomatic venous thromboembolism following hospitalization for coronavirus disease 2019: Prospective results from a multi-center study. *Thromb Res.* 2021;198: 135–138. doi:10.1016/j.thromres.2020.12.001
2. Smit JM, Lopez Matta JE, Vink R, et al. Coronavirus disease 2019 is associated with catheter-related thrombosis in critically ill patients: A multicenter case-control study. *Thromb Res.* 2021;200:87–90. doi: 10.1016/j.thromres.2021.01.013
3. Planquette B, Le Berre A, Khider L, et al. Prevalence and characteristics of pulmonary embolism in 1042 COVID-19 patients with respiratory symptoms: A nested case-control study. *Thromb Res.* 2021;197:94–99. doi: 10.1016/j.thromres.2020.11.001
4. Sindet-Pedersen C, Olesen JB, Blanche P, et al. Effect of government interventions to contain the COVID-19 pandemic on incidence of pulmonary embolism—A Danish nationwide register-based cohort study. *Thromb Res.* 2021;199:97–100. doi:10.1016/j.thromres.2020.12.025
5. Esenwa C, Unda SR, Altschul DJ, et al. The effect of race on composite thrombotic events in patients with COVID-19. *Thromb Res.* 2021;199: 10–13. doi:10.1016/j.thromres.2020.12.015
6. Miró Ò, Llorens P, Aguirre A, et al. Association between Covid-19 and Pulmonary Embolism (AC-19-PE study). *Thromb Res.* 2020;196:322–324. doi:10.1016/j.thromres.2020.09.010
7. Bourguignon A, Beaulieu C, Belkaid W, et al. Incidence of thrombotic outcomes for patients hospitalized and discharged after COVID-19 infection. *Thromb Res.* 2020;196:491–493. doi:10.1016/j.thromres.2020.10.017
8. 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
9. Ekdahl KN, Teramura Y, Asif S, et al. Thromboinflammation in therapeutic medicine. *Adv Exp Med Biol.* 2015;865:3–17. doi:10.1007/978-3-319-18603-0_1
10. Cohn AC, Mahon BE, Walensky RP. One year of COVID-19 vaccines: A shot of hope, a dose of reality. *JAMA.* 2022;327(2):119–120. doi:10.1001/jama.2021.23962
11. Schultz NH, Sørvoll IH, Michelsen AE, et al. Thrombosis and thrombocytopenia after ChAdOx1 nCoV-19 vaccination. *N Engl J Med.* 2021; 384(22):2124–2130. doi:10.1056/NEJMoa2104882
12. Greinacher A, Thiele T, Warkentin TE, et al. Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination. *N Engl J Med.* 2021;384(22): 2092–2101. doi:10.1056/NEJMoa2104840
13. Paris C, Bénézit F, Geslin M, et al. COVID-19 vaccine hesitancy among healthcare workers. *Infect Dis Now.* 2021;51(5):484–487. doi:10.1016/j.idnow.2021.04.001
14. Smyth SS. Platelet Structure and Function in Hemostasis and Thrombosis. In: Greer JP, Arber DA, Glader B, et al., eds. *Wintrobe's Clinical Hematology.* 13th ed. Philadelphia, USA: Lippincott Williams & Wilkins, 2014: 389–410.
15. Golebiewska EM, Poole AW. Platelet secretion: From haemostasis to wound healing and beyond. *Blood Rev.* 2015;29(3):153–162. doi:10.1016/j.blre.2014.10.003
16. Sut C, Tariket S, Aubron C, et al. The non-hemostatic aspects of transfused platelets. *Front Med (Lausanne).* 2018;5:42. doi:10.3389/fmed.2018.00042
17. Yun SH, Sim EH, Goh RY, et al. Platelet activation: The mechanisms and potential biomarkers. *Biomed Res Int.* 2016;2016:9060143. doi:10.1155/2016/9060143
18. Manne BK, 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
19. Al-Samkari H, Karp Leaf RS, Dzik WH, et al. COVID-19 and coagulation: Bleeding and thrombotic manifestations of SARS-CoV-2 infection. *Blood.* 2020;136(4):489–500. doi:10.1182/blood.2020006520
20. Tang N, Li D, Wang X, et al. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost.* 2020;18(4):844–847. doi:10.1111/jth.14768
21. Spiezia L, Boscolo A, Poletto F, et al. COVID-19-related severe hypercoagulability in patients admitted to intensive care unit for acute respiratory failure. *Thromb Haemost.* 2020;120(6):998–1000. doi:10.1055/s-0040-1710018
22. Panigada M, Bottino N, Tagliabue P, et al. Hypercoagulability of COVID-19 patients in intensive care unit: A report of thromboelastography findings and other parameters of hemostasis. *J Thromb Haemost.* 2020;18(7): 1738–1742. doi:10.1111/jth.14850
23. Cacciola R, Gentilini Cacciola E, Vecchio V, et al. Cellular and molecular mechanisms in COVID-19 coagulopathy: Role of inflammation and endotheliopathy. *J Thromb Thrombolysis.* 2022;53(2):282–290. doi:10.1007/s11239-021-02583-4
24. Harrison P. Progress in the assessment of platelet function. *Br J Haematol.* 2000;111(3):733–744. doi:10.1111/j.1365-2141.2000.02269.x
25. 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
26. 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
27. 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
28. Guo L, Rondina MT. The era of thromboinflammation: Platelets are dynamic sensors and effector cells during infectious diseases. *Front Immunol.* 2019;10:2204. doi:10.3389/fimmu.2019.02204
29. Finsterbusch M, Schrottmaier WC, Kral-Pointner JB, et al. Measuring and interpreting platelet-leukocyte aggregates. *Platelets.* 2018;29(7):677–685. doi:10.1080/09537104.2018.1430358
30. Nishikawa M, Kanno H, Zhou Y, et al. Massive image-based single-cell profiling reveals high levels of circulating platelet aggregates in patients with COVID-19. *Nat Commun.* 2021;12(1):7135. doi:10.1038/s41467-021-27378-2
31. 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
32. Hartmann J, Ergang A, Mason D, et al. The role of TEG analysis in patients with COVID-19-associated coagulopathy: A systematic review. *Diagnostics (Basel).* 2021;11(2):172. doi:10.3390/diagnostics11020172
33. Wool GD, Miller JL. The impact of COVID-19 disease on platelets and coagulation. *Pathobiology.* 2021;88(1):15–27. doi:10.1159/000512007
34. Petrey AC, Qeadan F, Middleton EA, et al. Cytokine release syndrome in COVID-19: Innate immune, vascular, and platelet pathogenic factors differ in severity of disease and sex. *J Leukoc Biol.* 2021;109(1):55–66. doi: 10.1002/JLB.3COVA0820-410RRR
35. Hamzeh-Cognasse H, Mansour A, Reizine F, et al. Platelet-derived sCD40L: Specific inflammatory marker for early-stage severe acute respiratory syndrome coronavirus 2 infection. *Virol J.* 2021;18(1):211. doi:10.1186/s12985-021-01680-3
36. Thomas MR, Storey RF. The role of platelets in inflammation. *Thromb Haemost.* 2015;114(3):449–458. doi:10.1160/TH14-12-1067
37. Koupenova M, Freedman JE. Platelets and immunity: Going viral. *Arterioscler Thromb Vasc Biol.* 2020;40(7):1605–1607. doi:10.1161/ATVBAHA.120.314620
38. Petersen HJ, Keane C, Jenkinson HF, et al. Human platelets recognize a novel surface protein, PadA, on Streptococcus gordonii through a unique interaction involving fibrinogen receptor GPIIbIIIa. *Infect Immun.* 2010; 78(1):413–422. doi:10.1128/IAI.00664-09
39. Liesenborghs L, Verhamme P, Vanassche T. Staphylococcus aureus, master manipulator of the human hemostatic system. *J Thromb Haemost.* 2018;16(3):441–454. doi:10.1111/jth.13928
40. Palankar R, Kohler TP, Krauel K, et al. Platelets kill bacteria by bridging innate and adaptive immunity via platelet factor 4 and FcγRIIA. *J Thromb Haemost.* 2018;16(6):1187–1197. doi:10.1111/jth.13955
41. Youssefian T, Drouin A, Massé JM, et al. Host defense role of platelets: Engulfment of HIV and Staphylococcus aureus occurs in a specific subcellular compartment and is enhanced by platelet activation. *Blood.* 2002;99(11):4021–4029. doi:10.1182/blood-2001-12-0191
42. Noisakran S, Gibbons RV, Songprakhon P, et al. Detection of dengue virus in platelets isolated from dengue patients. *Southeast Asian J Trop Med Public Health.* 2009;40(2):253–262.
43. Simon AY, Sutherland MR, Pryzdial EL. Dengue virus binding and replication by platelets. *Blood.* 2015;126(3):378–385. doi:10.1182/blood-

2014-09-598029
44. Koupenova M, Corkrey HA, Vitseva O, et al. The role of platelets in mediating a response to human influenza infection. *Nat Commun.* 2019; 10(1):1780. doi:10.1038/s41467-019-09607-x
45. Jansen AJG, Spaan T, Low HZ, et al. Influenza-induced thrombocytopenia is dependent on the subtype and sialoglycan receptor and increases with virus pathogenicity. *Blood Adv.* 2020;4(13):2967–2978. doi:10.1182/bloodadvances.2020001640
46. Noisakran S, Onlamoon N, Pattanapanyasat K, et al. Role of CD61+ cells in thrombocytopenia of dengue patients. *Int J Hematol.* 2012;96(5):600–610. doi:10.1007/s12185-012-1175-x
47. Negrotto S, Jaquenod de Giusti C, Rivadeneyra L, et al. Platelets interact with Coxsackieviruses B and have a critical role in the pathogenesis of virus-induced myocarditis. *J Thromb Haemost.* 2015;13(2):271–282. doi:10.1111/jth.12782
48. Zaid Y, Guessous F. The ongoing enigma of SARS-CoV-2 and platelet interaction. *Res Pract Thromb Haemost.* 2022;6(1):e12642. doi:10.1002/rth2.12642
49. O'Reilly D, Murphy CA, Drew R, et al. Platelets in pediatric and neonatal sepsis: Novel mediators of the inflammatory cascade. *Pediatr Res.* 2022; 91(2):359–367. doi:10.1038/s41390-021-01715-z
50. Sung PS, Hsieh SL. CLEC2 and CLEC5A: Pathogenic host factors in acute viral infections. *Front Immunol.* 2019;10:2867. doi:10.3389/fimmu.2019.02867
51. Duerschmied D, Bode C, Ahrens I. Immune functions of platelets. *Thromb Haemost.* 2014;112(4):678–691. doi:10.1160/TH14-02-0146
52. Koupenova M, Clancy L, Corkrey HA, et al. Circulating platelets as mediators of immunity, inflammation, and thrombosis. *Circ Res.* 2018;122(2): 337–351. doi:10.1161/CIRCRESAHA.117.310795
53. Goldman M, Hermans C. Thrombotic thrombocytopenia associated with COVID-19 infection or vaccination: Possible paths to platelet factor 4 autoimmunity. *PLoS Med.* 2021;18(5):e1003648. doi:10.1371/journal.pmed.1003648
54. Page EM, Ariëns RAS. Mechanisms of thrombosis and cardiovascular complications in COVID-19. *Thromb Res.* 2021;200:1–8. doi:10.1016/j.thromres.2021.01.005
55. McGonagle D, De Marco G, Bridgewood C. Mechanisms of immunothrombosis in vaccine-induced thrombotic thrombocytopenia (VITT) compared to natural SARS-CoV-2 infection. *J Autoimmun.* 2021;121:102662. doi:10.1016/j.jaut.2021.102662
56. Staibano P, Arnold DM, Bowdish DM, et al. The unique immunological features of heparin-induced thrombocytopenia. *Br J Haematol.* 2017; 177(2):198–207. doi:10.1111/bjh.14603
57. Kelton JG, Arnold DM, Nazy I. Lessons from vaccine-induced immune thrombotic thrombocytopenia. *Nat Rev Immunol.* 2021;21(12):753–755. doi:10.1038/s41577-021-00642-8
58. Arepally GM, Cines DB. Pathogenesis of heparin-induced thrombocytopenia. *Transl Res.* 2020;225:131–140. doi:10.1016/j.trsl.2020.04.014
59. Frederiksen H, Schmidt K. The Incidence of idiopathic thrombocytopenic purpura in adults increases with age. *Blood*. 1999;94(3):909–913. doi:10.1182/blood.V94.3.909.415k02_909_913
60. Lian J, Jin X, Hao S, et al. Analysis of epidemiological and clinical features in older patients with coronavirus disease 2019 (COVID-19) outside Wuhan. *Clin Infect Dis*. 2020;71(15):740–747. doi:10.1093/cid/ciaa242
61. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, et al. Hematological findings and complications of COVID-19. *Am J Hematol.* 2020;95:834–847. doi:10.1002/ajh.25829
62. Zulfiqar AA, Lorenzo-Villalba N, Hassler P, et al. Immune thrombocytopenic purpura in a patient with Covid-19. *N Engl J Med.* 2020;382:e43. doi:10.1056/NEJMc2010472
63. Bomhof G, Mutsaers PGNJ, Leebeek FWG, et al. COVID-19-associated immune thrombocytopenia. *Br J Haematol.* 2020;190(2):e61–e64. doi:10.1111/bjh.16850
64. Hindilerden F, Yonal-Hindilerden I, Sevtap S, et al. Immune thrombocytopenia in a very elderly patient with Covid-19. *Front Med (Lausanne).* 2020;7:404. doi:10.3389/fmed.2020.00404
65. Mahévas M, Moulis G, Andres E, et al. Clinical characteristics, management and outcome of COVID-19-associated immune thrombocytopenia: a French multicentre series. *Br J Haematol.* 2020;190:e224–e229. doi:10.1111/bjh.17024
66. Kupietzky A, Parnasa E, Fischer M, et al. Immune thrombocytopenia secondary to COVID-19 infection. *Isr Med Assoc J.* 2021;23:342–343.
67. Gruden G, Beggiato E, Camerino E, et al. Treatment with eltrombopag of severe immune thrombocytopenia and hemolytic anemia associated with COVID-19 pneumonia: a case report. *Ther Adv Hematol.* 2021;12: 20406207211011353. doi:10.1177/20406207211011353
68. Kewan T, Almhana F, Schwartzman L, et al. COVID-19 patient with immune thrombocytopenic purpura. *Int J Lab Hem.* 2020;42:e260–e262. doi:10.1111/ijlh.13303
69. Ahmed MZ, Khakwani M, Venkatadasari I, et al. Thrombocytopenia as an initial manifestation of COVID-19; case series and literature review. *Br J Haematol.* 2020;189:1057–1058. doi:10.1111/bjh.16769
70. Hindilerden F, Yonal-Hindilerden I, Akar E, et al. Covid-19 associated autoimmune thrombotic thrombocytopenic purpura: Report of a case. *Thromb Res*. 2020;195:136–138. doi:10.1016/j.thromres.2020.07.005
71. El Sissy C, Saldman A, Zanetta G, et al. COVID-19 as a potential trigger of complement-mediated atypical HUS. *Blood*. 2021;138(18):1777–1782. doi:10.1182/blood.2021012752
72. Leone VF, Imeraj A, Gastoldi S, et al. Case report: Tackling complement hyperactivation with eculizumab in atypical hemolytic uremic syndrome triggered by COVID-19. *Front Pharmacol*. 2022;13:842473. doi:10.3389/fphar.2022.842473
73. Cognard N, Gautier-Vargas G, Perrin P, et al. COVID-19 in a kidney transplant recipient treated with eculizumab for atypical hemolytic uremic syndrome: a case report. *J Nephrol*. 2021;34(4):1045–1048. doi:10.1007/s40620-021-01057-3
74. Alharbi MG, Alanazi N, Yousef A, et al. COVID-19 associated with immune thrombocytopenia: a systematic review and meta-analysis. *Expert Rev Hematol*. 2022;15(2):157–166. doi:10.1080/17474086.2022.2029699
75. Nalbandian A, Sehgal K, Gupta A, et al. Post-acute COVID-19 syndrome. *Nat Med.* 2021;27(4):601–615. doi:10.1038/s41591-021-01283-z