Platelet Activation and Thrombosis in COVID-19

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Abstract

Although thrombosis frequently occurs in infectious diseases, the coagulopathy associated with COVID-19 has unique characteristics. Compared with bacterial sepsis, COVID-19-associated coagulopathy presents with minimal changes in platelet counts, normal prothrombin times, and increased D-dimer and fibrinogen levels. These differences can be explained by the distinct pathophysiology of the thromboinflammatory responses. In sepsis-induced coagulopathy, leukocytes are primarily responsible for the coagulopathy by expressing tissue factor, releasing neutrophil extracellular traps, multiple procoagulant substances, and systemic endothelial injury that is often associated with vasoplegia and shock. In COVID-19-associated coagulopathy, platelet activation is a major driver of inflammation/thrombogenesis and von Willebrand factor and platelet factor 4 are deeply involved in the pathogenesis. Although the initial responses are localized to the lung, they can spread systemically if the disease is severe. Since the platelets play major roles, arterial thrombosis is not uncommon in COVID-19. Despite platelet activation, platelet count is usually normal at presentation, but sensitive biomarkers including von Willebrand factor activity, soluble P-selectin, and soluble C-type lectin-like receptor-2 are elevated, and they increase as the disease progresses. Although the role of antiplatelet therapy is still unproven, current studies are ongoing to determine its potential effects.

Keywords
- COVID-19
- platelet
- thrombosis
- von Willebrand factor
- platelet factor 4
- P-selectin
- C-type lectin-like receptor 2
- antiplatelet

Soon after the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic infection, arterial thrombosis emerged as a complication in coronavirus disease 2019 (COVID-19) and was further publicized in early 2021 when Oscar-winning actress Cloris Leachman died due to a stroke associated with COVID-19 (https://www.usatoday.com/story/entertainment/celebrities/2021/02/19/cloris-leachman-cause-of-death-stroke-covid-19/4509045001/). Besides stroke and ischemic cardiovascular diseases, relatively rare arterial thrombosis such as aortic arch thrombus, mesenteric artery thrombus, and ischemic limbs were also reported,[1–4] along with unusual findings in young, healthy adults.[5,6] The COVID-19-specific and nonspecific coagulopathy, inflammation, and endothelial injury are important contributors to this pathophysiologic prothrombotic condition.[7] Both venous thrombosis and thromboembolism are frequently seen in COVID-19, especially in severe cases. A meta-analysis reported the deep vein thrombosis occurred in 19.8% of patients (95% confidence interval [CI]: 10.5–34.0%) and pulmonary embolism in 18.9% (95% CI: 14.4–24.3%) even with pharmacological thromboprophylaxis.[8] However, this high prevalence of thromboembolic complications compared with septic patients is puzzling because global coagulation biomarkers are more prominent in sepsis[9] compared with COVID-19-associated coagulopathy where platelet count and prothrombin time changes are less remarkable on initial presentation and fibrinogen levels increased.[10] Consequently, patients with COVID-19 rarely

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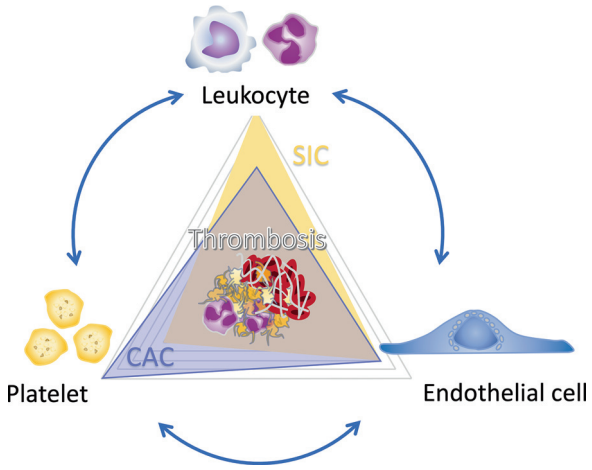

Fig. 1 The predominant role of platelets in COVID-19-associated coagulopathy. Leukocytes, platelets, and endothelial cells are involved in thrombus formation. In sepsis, monocyte/macrophage express tissue factor and initiate coagulation. Activated leukocytes damage endothelial cells and turn their anti-thrombogenic surface to procoagulant. Platelets are also involved in the pathogenesis of thromboinflammation. In COVID-19, SARS-CoV-2 stimulates platelets to release the prothrombotic substances from the granules, and activate leukocytes and endothelial cells. Therefore, the roles of leukocytes are more significant in sepsis-induced coagulopathy (SIC) and the roles of platelets are more dominant in COVID-19-associated coagulopathy (CAC).

develop disseminated intravascular coagulation (DIC) in their initial clinical presentation.[11] As a result, we considered that factors other than coagulation abnormalities are important for thrombus formation in COVID-19, and platelets may be critical in the pathogenesis (►**Fig. 1**).

Methods

This is a narrative review focused on the platelet activity in COVID-19. We performed a literature search on platelet thrombosis in COVID-19 in PubMed, Scopus, and Web of Science. Two authors (T.I. and H.W.) independently searched the literature using the following subject headings: "COVID-19," "platelet," and "thrombosis." We also examined related articles, press releases, announcements, and home pages of the medical societies on the websites. Each electronic database's search strategy was modified using database-specific search terms, field names, and syntax. We investigated the relevant articles published between March 2020 through December 2021. In addition, relevant literature regarding "von Willebrand factor," "platelet factor 4 (PF4)," "P-selectin," "C-type lectin-like receptor 2," and "antiplatelet" was identified in the electronic resources.

Platelet Activation in COVID-19

The postmortem evaluation of COVID-19 patients reports extensive inflammatory microvascular thrombi consisting of neutrophil extracellular traps (NETs) associated with platelets and fibrin in the lung, kidney, and heart.[12] Despite platelet activation, clinically used measures of platelet counts cannot assess the severity of the disease. The platelet count decreases only when the consumption and destruction overcome the production; consequently, the platelet count is not suitable to evaluate platelet activation in COVID-19.

Platelets are activated through two different mechanisms in COVID-19 that include an inflammation-mediated pathway and a SARS-CoV-2-specific pathway.[13] In the former pathway, cytokine storm stimulates platelets to express tissue factor and release procoagulant microvesicles.[14] Tissue factor-initiated coagulation leads to the generation of thrombin that plays a central role in evoking coagulation and inflammation, endothelial injury, and platelet activation.[15] Inflammation-provoked tissue factor expression on monocytes also facilitates platelet–monocyte interaction, and the platelet–monocyte aggregates play essential roles in the progressive organ injury of COVID-19.[16] In addition, inflammation elicited neutrophil activation also facilitates the formation of neutrophil–platelet aggregates that characterize immunothrombosis. NETs, damage-associated molecular patterns, oxygen radicals, and many other factors are involved in this pathway.[17]

In the SARS-CoV-2 specific pathway, the reaction is initiated by the binding of virus spike protein to its receptor. SARS-CoV-2 infects host cells by binding spike protein to angiotensin-converting enzyme 2 (ACE2), expressed on alveolar epithelial cells, enterocytes, monocytes/macrophages, platelets, and vascular endothelial cells.[18] The binding of endothelial cells, leukocytes, and platelets to spike protein-ACE2 shifts their functions to procoagulant and thrombogenic as part of a thromboinflammatory response.[19] SARS-CoV-2 uses ACE2 as a receptor to attach cells, and the endothelial cell reduces ACE2 expression after the binding that decreases angiotensin II conversion to angiotensin 1 to 7. Increased angiotensin II stimulates vascular constriction, and decreased angiotensin 1 to 7 reinforces the proinflammatory reaction and thrombogenicity via vasoconstriction and leucocyte-platelet adhesion.[20] SARS-CoV-2 also infects platelets and megakaryocytes by binding to ACE2 on their cellular membranes,[21] Further, spike protein-ACE2 binding stimulates platelet release of α and dense granules' contents that include von Willebrand factor (VWF), PF4, adenosine diphosphate, and serotonin that increase platelet aggregation and enhance thrombus formation.[18] When an anti-PF4 IgG antibody is formed as part of inflammatory responses, the PF4-anti-PF4 IgG complex binds platelets, macrophages, and neutrophils via the binding to Fcγ receptor IIA, and upregulates cell–cell interaction and aggregation.[22] SARS-CoV-2 stimulated platelets are also known to facilitate the release of coagulation factors, inflammatory cytokines, and express the procoagulant phosphatidylserine to facilitate prothrombotic effects[23] (►**Fig. 2**).

Arterial Thrombus in COVID-19

Early in the pandemic, COVID-19 patients were noted to present with unusual arterial thrombi.[1–6] A Swedish matched cohort study including over 86,000 COVID-19 patients demonstrated the incidence rate ratios for acute

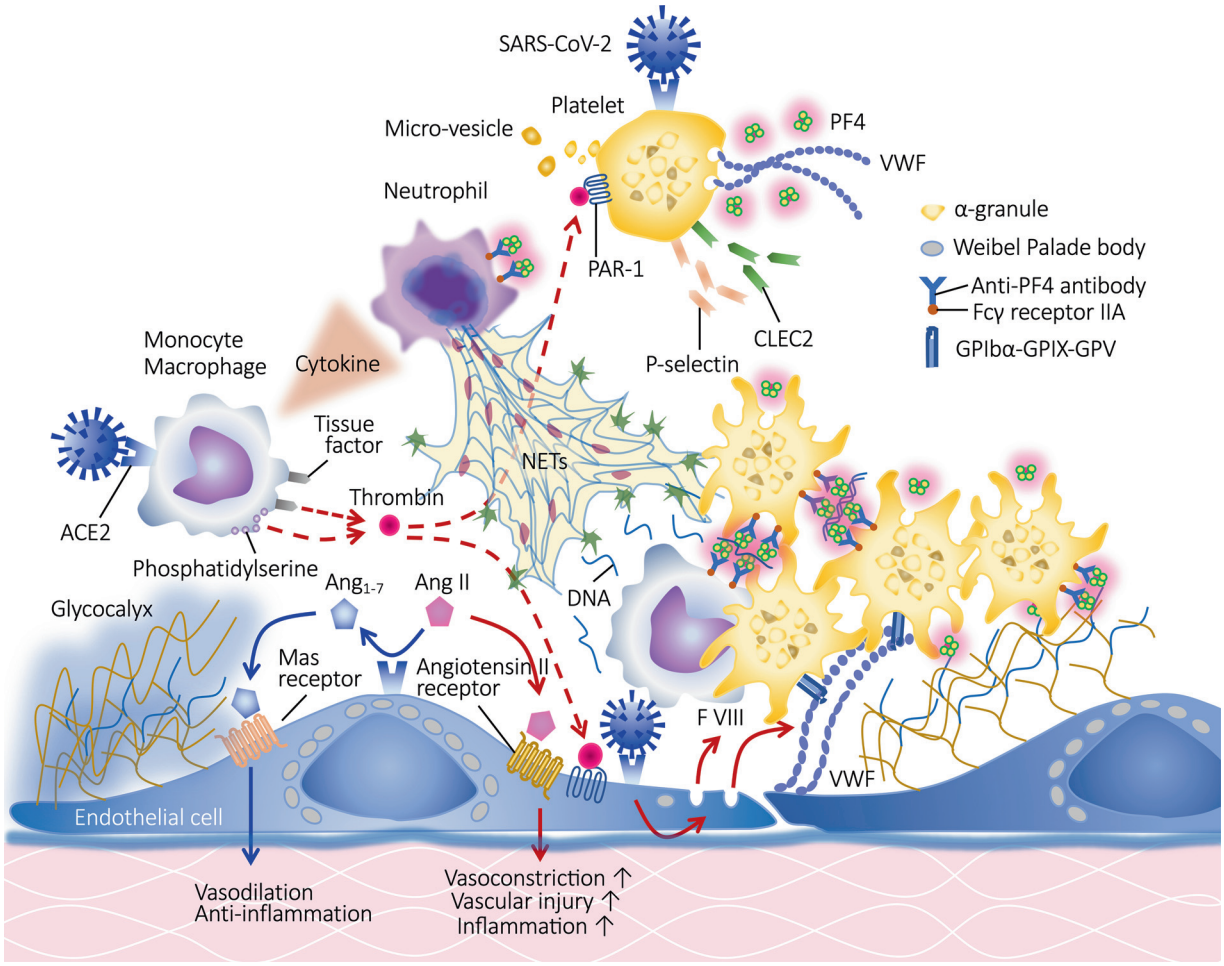

Fig. 2 Thrombus formation in COVID-19. SARS-CoV-2 attaches macrophage/monocyte via the binding to angiotensin-converting enzyme-2 (ACE2) and stimulate cytokine release to activate neutrophils and initiate coagulation. Generated thrombin stimulates platelets to become thrombogenic through the activation of protease activated receptor 1 (PAR-1). Activated neutrophil release neutrophil extracellular traps (NETs) that are composed of DNA and granule proteins. SARS-CoV-2 also infects platelets to release the contents of α-granule such as von Willebrand factor (VWF) and platelet factor 4 (PF4). At the same time, the P-selectin and C-type lectin-like receptor 2 (CLEC-2) are expression on the surface. PF4 binds DNA or other polyanion and upregulate the production of anti-PF4/polyanion antibody. This antibody attaches to the monocyte/macrophage, neutrophil, and platelets by binding to the Fcγ receptor IIA and facilitates cell–cell interaction. The spike protein of SARS-CoV-2 suppresses the conversion of angiotensin II (Ang II) to angiotensin $_{1-7}$ (Ang $_{1-7}$) and induces vasoconstriction, vascular injury, and inflammation.

myocardial infarction and ischemic stroke compared with a matched cohort as 2.89 (95% CI: 1.51–5.55) and 2.97 (1.71–5.15), respectively, in the first week following COVID-19 infection.[13] Compared with bacterial sepsis, the incidence of arterial thrombosis seems to be higher in COVID-19. In bacterial sepsis, activated coagulation and suppressed fibrinolysis are the hallmarks.[11] Comparatively, those changes are less remarkable, and the roles of platelet activation in thrombosis are more dominant in COVID-19.[24] In the largest previously published cohorts on COVID-19, the incidence of arterial thrombus was reported at 3.7%.[25] A systematic review reported the pooled incidence of arterial thrombosis in severe/critically ill patients across five cohort studies as 4.4% (95% CI: 2.8–6.4%). As for the distribution, the arterial thrombosis occurred at a rate of 39% in limb arteries, 24% in cerebral arteries, 19% in great vessels (aorta, common iliac, common carotid, and brachiocephalic trunk), 9% in coronary arteries, and 8% in superior mesenteric arteries.[26]

However, other large studies have reported lower incidences. Glober et al[27] performed a cross-sectional study using the RECOVER database to determine the incidence and location of arterial thromboemboli, including myocardial infarction, ischemic stroke, and peripheral arterial thrombosis. The result was showed among 13,803 COVID-19 patients and the incidence of arterial thromboemboli was 0.13%. In this study, the incidence in patients who were negative for COVID-19 was 0.19%. This result suggests that arterial thromboemboli are less common in patients with COVID-19 than non-COVID-19 patients. Similarly, a multicenter cross-sectional study performed in New York state did not find any association between ischemic stroke and COVID-19.[28] From these mixed results, we cannot conclude whether the SARS-CoV-2 increases or decreases arterial thrombosis.

Platelet-Related Biomarkers

Platelet Count and Mean Platelet Volume

Platelet count is routinely monitored as a clinical indicator of the worsening of infectious diseases during hospitalization and is also associated with an increased risk of severe disease and mortality in COVID-19. A meta-analysis of nine studies reported significantly lower platelet count in patients with severe COVID-19 (weighted mean difference: -310^9/L; 95% CI: -35 to -29×10^9/L).[29] Furthermore, a lower platelet count was associated with increased mortality. However, the platelet count is usually initially maintained in a normal range. Zhou et al[30] reported the median platelet counts in non-survivors and survivors as 165.5×10^9/L (interquartile range [IQR]: 107.0–229.0) and 220.0×10^9/L (IQR: 168.0–271.0), respectively ($p <$ 0.0001). Based on these findings, platelet consumption seems to be compensated in COVID-19 even in severe cases. Comparatively, mean platelet volume (MPV) is a more sensitive reflection of platelet turnover, and the presence of large platelets in the circulation indicates the intense prothrombotic tendency as they are more metabolically active and have a greater prothrombotic potential.[31] The pooled analysis results of 18 studies noted that the MPV values in severe COVID-19 patients were increased by 6.3% (95% CI: 3.6–9.0%) compared with non-severe cases.[32] However, the changes were relatively small and heterogeneity among the studies was high ($I^2 = 91\%$). As a result, MPV may not be useful in clinical practice as neither platelet count nor MPV will be sensitive or practical enough to evaluate the platelet activation.

von Willebrand Factor

VWF, a multimeric glycoprotein stored in α-granules of platelets and in Weibel-Palade bodies of vascular endothelial cells, is critical for platelet aggregation via receptor binding on glycoprotein Ib-IX-V complex and integrin αIIbβ3 on platelets.[33] The multimeric size and VWF activity are regulated by ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) and the presence of ultralarge VWF due to the shortage of ADAMTS-13 results in the increased risk of thrombosis.[34] VWF is released into the circulation upon platelet and endothelial activation and/or injury. Further, ADAMS-13 activity decreases in COVID-19 and VWF activity increases more than five times higher than normal.[35] As a result, VWF/ADAMTS-13 ratio that reflects the thrombogenicity rises considerably in COVID-19.[36] Ward et al[37] reported median VWF collagen-binding activity in patients with severe COVID-19 of 509.1 IU/dL compared with controls of 94.3 IU/dL. Furthermore, increased levels of VWF are associated with disease severity and mortality in COVID-19.[35] However, it should be reminded that although the VWF activity level reflects platelet activation, levels are also affected by other factors such as endothelial cell stimulation and ADAMS-13 activity.[37]

Platelet Factor 4

PF4 is a CXC chemokine that is also stored in the α-granule of the platelets. The accumulated platelets release PF4 at the sites of inflammation and promote monocyte and neutrophil adhesion onto the endothelial cell, leading to thrombogenesis. The physiological role of PF4 is the neutralization of heparin-like molecules, a major component of the glycocalyx, on the vascular endothelial surface, promoting coagulation and inhibiting the local antithrombogenicity.[38] Rampotas and Pavord[39] reported increased platelet aggregates and macrothrombocytes on the blood peripheral smears obtained from COVID-19 patients. Similarly, Middleton et al[40] found elevated levels of PF4 and platelet–neutrophil aggregates in COVID-19 patients. They speculated that released PF4 binds to NETs that consist of DNA web released from neutrophils, and the PF4-NETs binding contributes to thrombosis in COVID-19 patients. This reaction is further enhanced by the presence of anti-PF4 antibodies. Extremely high levels of anti-PF4 antibodies are found in vaccine-induced immune thrombotic thrombocytopenia but that type of unusual production is observed only in the specific occasion following virus-vectored vaccine administration.[41] Cacciola et al[42] measured PF4 in control, mild COVID-19 patients, and moderate COVID-19 patients and reported levels of 4 ± 1 IU/mL, 3 ± 1 IU/mL, and 158 ± 63 IU/mL, respectively, levels that were significantly higher in moderate COVID-19 patients compared with the healthy controls and mild disease ($p < 0.05$, respectively). Platelet activation with PF4 release occurs in COVID-19 due to spike protein binding to the ACE2 platelet receptors, mechanisms that also are involved in thrombotic thrombocytopenia pathogenesis after COVID-19 vaccination.[24]

Soluble P-Selectin

P-selectin is a type-1 transmembrane single-chain glycoprotein expressed on platelets and endothelial cells that functions as a cell adhesion molecule. In platelets, P-selectin is stored in α granules, and in Weibel-Palade bodies in endothelial cells.[43] Circulating levels of P-selectin increase in various atherothrombotic disorders and are considered to reflect platelet activation.[44] Karsli et al[45] measured soluble P-selectin in patients with COVID-19 and reported levels of 1.7 ng/mL in the healthy control, 6.24 ng/mL in mild-to-moderate pneumonia, and 6.72 ng/mL in severe pneumonia. As for diagnostic performance, soluble P-selectin was 76.9% sensitive and 51.9% specific at the level of 6.12 ng/mL ($p = 0.005$) to predict the need for intensive care treatment. Comer et al[46] reported PF4 circulating levels did not differ between severe and non-severe COVID-19, although soluble P-selectin was higher among the severe COVID-19 group. Since the median platelet count in mild-to-moderate and severe pneumonia is kept in the normal range (192.5 and 233.0×10^9/L, respectively), soluble P-selectin is more suitable for evaluating the platelet activation in COVID-19, but also reflect endothelial injury.

Soluble C-Type Lectin-Like Receptor 2

A platelet activation receptor, C-type lectin-like receptor 2 (CLEC-2), was identified as a receptor for platelet-activating snake venom. CLEC-2 is involved in vascular/lymphatic development, maintenance of vascular integrity, tissue regeneration, and blood coagulation.[47] Furthermore, recent studies reported the upregulation of CLEC-2 ligands in

inflamed tissues that highlight its role in inflammatory thrombus formation. CLEC-2 and its ligands have been considered to be a molecular bridge between platelets and immune cells that explains the interaction between inflammation and thrombosis.[48] CLEC-2 protein expressed on platelets/megakaryocytes is released into the circulation,[49] and the circulating levels of CLEC-2 have been introduced as a new biomarker of platelet activation.[50] The elevated plasma levels of soluble CLEC-2 have been reported in patients with thrombotic microangiopathy and DIC,[51,52] as well as in patients with arterial thrombosis such as acute coronary syndrome[53] and acute cerebral infarction.[54] Wada et al[55] reported that the plasma levels of CLEC-2 in COVID-19 patients were significantly higher than in those with bacterial infections, and the levels reflected the severity of illness. Notably, the platelet counts were maintained within the normal range in those patients. Therefore, it is important to evaluate specific biomarkers of platelet activation in COVID-19.

Antiplatelet Therapy

Although antiplatelet therapy administration to prevent arterial thrombosis may offer a therapeutic benefit, its effectiveness in COVID-19 is still under investigation.[56] An observational cohort study of 412 hospitalized adult COVID-19 cases demonstrated the association between aspirin use and less mechanical ventilation (aspirin group: 35.7% vs. non-aspirin group: 48.4%, $p = 0.03$), but there was no mortality difference reported (aspirin group: 26.5% vs. non-aspirin group: 23.2%, $p = 0.51$).[57] Salah and Mehta[58] performed a meta-analysis using three cohort studies that included 1,054 patients and reported mortality differences with aspirin treatment in patients with COVID-19. Similarly, Wang et al[59] performed a meta-analysis using a total of nine articles that included 5,970 cases and concluded that antiplatelet agents were not associated with a reduced risk of severe COVID-19 disease (OR: 0.98, 95% CI: 0.64–1.50, $p = 0.94$; $I^2 = 65\%$), and the result was also confirmed in an adjusted analysis (OR: 0.65, 95% CI: 0.40–1.06, $p = 0.498$; $I^2 = 0\%$). Recently the RECOVERY study, a randomized, open-label trial in hospitalized COVID-19 patients, evaluated the effect of 150 mg daily aspirin doses. The aspirin group reported no reductions in 28-day mortality or risk of progressing to mechanical ventilation.[60] ACTIV-4B is another randomized controlled trial that compared the effect of 81 mg once daily aspirin, prophylactic- or therapeutic-dose of apixaban to placebo. The primary end point was a composite of all-cause mortality, symptomatic venous, or arterial thromboembolism, myocardial infarction, stroke, or hospitalization for the cardiovascular or pulmonary cause. However, there were no differences between the groups.[61] Currently, the National Institute of Health guidelines recommend against the use of antiplatelets for the prevention of venous thromboembolism or arterial thrombosis (https://www.covid19treatmentguidelines.nih.gov/therapies/antithrombotic-therapy/). Other large-scale studies that included more than 500 cases are summarized in ►**Table 1**.

Table 1 Summary of the large-scale or randomized controlled studies evaluated the effect of antiplatelet therapy

Author/Group	Country	Design	Number of patients	Results	Interpretation
Osborn et al[62]	United States	Retrospective database review	12,600	OR for 14-d mortality = 0.38 (95% CI: 0.33–0.45)	Pre-diagnosis aspirin prescription was associated with decreased mortality rates.
Meizlish et al[63]	United States	Retrospective database review	638	HR for in-hospital death = 0.522 (95% CI: 0.34–0.81)	In-hospital aspirin was associated with a significantly lower incidence of in-hospital death compared to no antiplatelet therapy.
Fröhlich et al[64]	Germany	Retrospective database review	6,637	OR for all-cause mortality or need for invasive or non-invasive ventilation or extracorporeal membrane oxygenation= 1.10 (95% CI: 0.88–1.23)	Antiplatelet therapy was not associated with lower mortality or requirement of intensive care.
Ho et al[65]	United States	Retrospective database review	2,876	OR for emergency department visit, inpatient hospitalization, ICU stay, VTE, mechanical ventilation, and mortality = 0.89 (95% CI: 0.64–1.24)	Chronic antiplatelet use was not associated with a lower risk of the requirement of intensive treatment.
RECOVERY study[60]	UK, Indonesia, Nepal	Randomized, open-label trial	2,521	RR of invasive mechanical ventilation = 0.96, (95% CI: 0.90–1.03; $p = 0.23$)	Aspirin was not associated with reductions in 28-d mortality or in the risk of progressing to invasive mechanical ventilation.
ACTIV-4B trial[61]	United States	Randomized controlled trial	280	Risk difference compared with placebo for the primary end point was 0.0%	Aspirin did not improve the composite outcome (all-cause mortality, arterial and venous thromboembolism) compared with placebo.

Abbreviations: HR, hazard ratio; ICU, intensive care unit; OR, odds ratio; RR, risk ratio; VTE, venous thromboembolism.

Except for aspirin, there is no reliable data with regard to the effect of other antiplatelet agents such as clopidogrel and ticagrelor.

Summary and Conclusion

In the early phase of the COVID-19 pandemic, despite the minimal changes in routine coagulation tests associated with acute infections, a higher rate of thromboembolism and frequent complications of arterial thrombosis were noted. Thrombocytopenia and prothrombin time prolongation are commonly seen in bacterial sepsis-induced coagulopathy but rarely occur in initial presentations of COVID-19, at least in moderate and mild disease. Instead, SARS-CoV-2 upregulates thrombogenesis by activating platelets and endothelial cells through the binding to ACE2 receptors. As a result, platelets release VWF and PF4 from α-granules and express P-selectin and CLEC2. In addition, endothelial cell injury further promotes prothrombotic effects by the release of VWF and factor VIII. These changes altogether contribute to the generation of the prothrombotic state of COVID-19. For monitoring COVID-19-associated coagulopathy, sensitive biomarkers such as VWF activity, PF4, soluble P-selectin, and soluble CLEC-2 are useful but not readily available for clinicians. With respect to the treatment, the effectiveness of antiplatelet therapy is still under investigation (https://doi.org/10.1182/bloodadvances.2021005945). One of the major problems regarding antiplatelet therapy in COVID 19 is despite the potential role of platelet activation, combining anticoagulants with antiplatelet agents may further risk the potential of bleeding. Future studies targeting platelet inhibition will need to evaluate the following factors: which patients, what therapeutic agent and dosing, when to start, and the duration of administration.

Conflict of Interest
T.I. has received a research grant from Japan Blood Products Organization and JIMRO. H.W. received grants and personal fees from Asahi Kasei Pharma Corporation and Japan Blood Products Organization. J.H.L. serves on the Steering or Advisory Committees for Instrumentation Laboratories, Merck, Octapharma. This work was supported in part by a Grant-in-Aid for Special Research in Subsidies for ordinary expenses of private schools from the Promotion and Mutual Aid Corporation for Private Schools of Japan.

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