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The roles of platelets in COVID-19-associated coagulopathy and vaccine-induced immune thrombotic thrombocytopenia

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ABSTRACT

In coronavirus disease 2019 (COVID-19), multiple thromboinflammatory events contribute to the pathophysiology, including coagulation system activation, suppressed fibrinolysis, vascular endothelial cell injury, and prothrombotic alterations in immune cells such as macrophages and neutrophils. Although thrombocytopenia is not an initial presentation as an infectious coagulopathy, recent studies have demonstrated the vital role of platelets in COVID-19-associated coagulopathy SARS-CoV-2 and its spike protein have been known to directly or indirectly promote release of prothrombotic and inflammatory mediators that lead to COVID-19-associated coagulopathy. Although clinical features of vaccine-induced immune thrombotic thrombocytopenia include uncommon locations of thrombosis, including cerebral venous sinus, we speculate coronavirus spike-protein-initiated prothrombotic pathways are involved in the pathogenesis of vaccine-induced immune thrombotic thrombocytopenia, as current evidence suggests that the spike protein is the promotor and other cofactors such as perturbed immune response and inflammatory reaction enhance the production of anti-platelet factor 4 antibody.

Introduction

Platelets are critical components of hemostasis but also participate in the host defense mechanisms of bacterial infections. Platelets are known to play vital roles as immune modifiers as well as the progenitor cells of clot formation in collaboration with the other immune cells and the coagulation system [1,2]. In invertebrates, platelets have been shown to directly kill pathogens. Meanwhile, platelets induce neutrophil extracellular traps (NETs) release and indirectly dispose of pathogens in vertebrates [3,4]. These responses to infection are elicited by the interaction between soluble mediators, including thrombin, damage-associated molecular patterns (DAMPs), and their membrane surface receptors [5]. Hemocytes, an ancestor cell that serves platelet functions in invertebrates have evolved to important modulators of host defense, inflammation, and hemostasis [3]. Platelets detect pathogens and deploy host-defense peptides such as platelet factor 4, a major platelet-derived CXC chemokine, to recruit and facilitate leukocyte responses in the context of the innate immune system [6]. Platelets also act as liaisons to promote T cell-B cell crosstalk, critical interactions required in adaptive immunity [7]. However, the extent to which platelets are the major response initially in the host defense against viral infection in humans has not been determined.

Microthrombosis, a prominent pathological feature of coronavirus disease 2019 (COVID-19), has been extensively documented by autopsy reports demonstrating extensive platelet-fibrin clot formation in the pulmonary microvasculature in 80–100% of lungs examined [8]. In this review, we discuss the roles of platelets in the pathogenesis of COVID-19-associated coagulopathy (CAC) and vaccine-induced immune thrombotic thrombocytopenia (VITT).

Platelets in COVID-19

Although platelet counts are within the normal range in most patients who initially present with COVID-19, platelet activation plays a vital role in thrombosis development. For instance, the cytokine storm described in COVID-19 stimulates platelets to express tissue factor on their surface and release procoagulant microvesicles [9]. As a result, increased levels of tissue factor-positive platelets and microvesicles are reported in COVID-19 patients [10]. The increase in von Willebrand factor, especially its ultra-high-molecular-weight form, occurs in the early stages of COVID-19 and platelet clumping occurs [11,12]. The immunothrombus formed mainly by platelets and leukocytes is also a hallmark of intravascular clotting in COVID-19 [8,13]. Manne et al. [14] re-

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ported both platelet aggregates and circulating platelet-neutrophil, -monocyte, and -T-cell aggregates increased significantly in COVID-19 patients. Virus-induced sensitization of platelets is observed with increased platelet responsiveness by thromboxane generation to low-dose agonist stimulation. Thrombin, a potent platelet agonist, also generated early in COVID-19 to further activate platelets. Platelets also release various cytokines that modulate inflammation (Interleukin [IL]-1α, IL-1β, IL-4, IL-10, IL-13, interferon-α, and interferon-γ), chemokines (monocyte chemoattractant protein 1), and growth factors (vascular endothelial growth factor) [15].

The cytokine storm and macrophage activating syndrome play important roles in the pathogenesis of COVID-19. Excessive cytokines that are released are important in further orchestrating the systemic hemodynamic alterations and cardiovascular injury, and the mortality rate increases with higher cytokine levels [16]. In COVID-19, elevated levels of biomarkers for cardiac injuries, such as natriuretic peptides, troponins, myoglobin were observed along with the increased levels of C-reactive protein, IL-2, and IL-6. The possible mechanisms of cardiovascular injury include direct toxicity through the viral invasion to the cardiac myocytes, ACE2 receptor-mediated cardiovascular injury, microthrombosis, and cytokine release syndrome [17]. Among various cytokines, IL-17 attracted much attention recently. IL-17 is a multifunctional cytokine that shows a mixed immunopathological effect (from pro to opposite antiinflammatory) depending on the condition [18]. In COVID-19, IL-17 activates platelets and the severity of disease presentation was shown to positively correlate with levels of IL-17 and other T-helper-17 lymphocytes-producing pro-inflammatory cytokines such as IL-1, IL-6, IL-15 tumor necrosis factor, and interferon-γ [19]. Although its efficacy is still inconclusive, the inhibitory effect of IL-17 has been examined in a pilot study [20].

Except for D-dimer and fibrinogen levels, standard coagulation tests including prothrombin time and activated partial thromboplastin time are often normal. However, additional biomarkers of thrombin generation or activation, including thrombin-antithrombin complex (TAT) and prothrombin fragment 1.2 are markedly elevated in COVID-19 [21]. As mentioned, thrombin activation stimulates protease-activated receptor-1 (PAR1), a critical mechanism in platelet aggregation and upregulation of coagulation [22]. Although in most patients, platelet counts initially are normal, the count is lower in non-survivors than in survived patients with COVID-19, and platelet count less than 150,000 /μL is a predictor of worse outcome [23].

Platelet size is an indicator of platelet recycling, and the mean platelet volume is known to correlate with platelet activation and increases in septic patients with thrombosis. Comer et al. [24] reported elevated mean platelet volume in COVID-19 higher levels in critically ill patients, while Zhang et al. [25] reported increased mean platelet volume correlated with platelet count decreases. Moreover, single-cell transcriptome analysis showed megakaryocyte progenitors increased up to 5% of $CD34^+$ cells in all symptomatic COVID-19 patients, while such increase was absent in healthy or asymptomatic patients [26].

Platelet susceptibility to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still controversial, but Campbell et al. [27] reported platelets express angiotensin-converting enzyme 2 (ACE2), and the spike protein binding directly enhances platelet aggregation, α-granule secretion, and spike protein-induced thrombus formation (Fig. 1) although Manne et al. [14] reported ACE2 was not detected by messenger RNA (mRNA) or protein levels in the platelets. The discussion on SARS-CoV-2 infection via ACE2 is still ongoing, and Campbell et al. [27] suggested that platelets may express different SARS-CoV-2 receptors.

In bacterial infections, microcirculatory thrombus is formed as a host defense mechanism that inhibits microbial dissemination. However, systemic microthrombosis impairs tissue circulation and leading to organ dysfunction [28] that is initially localized in the lung, platelet counts do not decrease. Nevertheless, platelets are functionally activated and contribute to thrombus formation, and thrombotic sequela. Platelet activation is also determined by additional factors that include *P*-selectin expression, platelet-leukocyte aggregate formation, and altered nitric oxide/prostacyclin synthesis [10,29]. For further understanding of CAC, additional indicators of platelet function such as mean platelet volume, platelet factor 4, and *P*-selectin beyond platelet counts should be considered. It is also important to remind clinicians that the prothrombotic shift depends on the mutual effect between platelets and other factors including activated coagulation, vascular dysfunction, neutrophils, and the complement system [30,31].

Platelet factor 4 in COVID-19

Platelet factor 4, a CXC chemokine stored in α-granule of the platelet, is released from activated platelets during aggregation, and promotes further thrombogenesis via platelet clumping and NETs release [32,33]. Platelet factor 4 is also known as a heparin-binding protein, and the physiological role of platelet factor 4 is to neutralize heparan sulfate on the endothelial surface, thereby inhibiting the local antithrombotic properties [34]. In addition, the positively charged platelet factor 4 binds negatively charged bacterial surfaces to promote opsonization. In this fashion, platelet factor 4 contributes to the host-defense against bacterial infection [24]. Platelets provide an essential role in bacteria-killing and immunothrombus formation in sepsis. Activated platelets release platelet factor 4 that stimulates platelets, macrophage, and neutrophils via the interaction with IgG and its receptor FcγRIIA on the cellular membrane [35]. platelet factor 4 is also known to bind polyanions charge-dependently and undergoes a conformational change. This change provokes the exposure of antigenic epitope that induces the production of anti-platelet factor 4/polyanions antibodies. As a result, anti- platelet factor 4/polyanion antibodies also helps to eliminate various pathogens from the host [36], but also can induce heparin-induced thrombocytopenia (HIT) without heparin, a response known as spontaneous HIT [37]. HIT is an immune complication of heparin therapy caused by antibodies to platelet factor 4/heparin conjugates (also known as HIT antibody), and spontaneous HIT can occur without prior heparin exposure. The clinical feature of HIT is represented by thrombosis with thrombocytopenia and the mortality exceeds 20% [38].

Multiple platelet biomarkers are increased in COVID-19 patients [24,39], including platelet factor 4, soluble *P*-selectin, and thrombopoietin. Comer et al. reported agonist-induced ADP release from platelets was 30- to 90-fold higher in COVID-19 compared to controls and unrelated to platelet counts. The prevalence of IgG HIT antibodies in COVID-19 patients is high, and a prospective study demonstrated an incidence of 11% IgG HIT antibodies after hospitalization unrelated to a HIT diagnosis [40]. These observations suggest the SARS-CoV-2 can potentiate the platelet factor 4 release, and the circulating platelet factor 4-induced platelet activation may contribute to the pathogenesis of CAC.

COVID-19 vaccines and platelets

Vaccine-induced immune thrombotic thrombocytopenia (VITT)

In the early communication from the European Medicines Agency (https://www.ema.europa.eu/en/documents/prac-recommendation/signal-assessment-report-embolic-thrombotic-events-smq-covid-19-vaccine-chadox1-s-recombinant-covid_en.pdf), 30 cases of thromboembolic events had been reported by March 11, 2021 among approximately 5.5 million ChAdOx1, an adenovirus-vectored vaccine, recipients. At that time, the thromboembolic

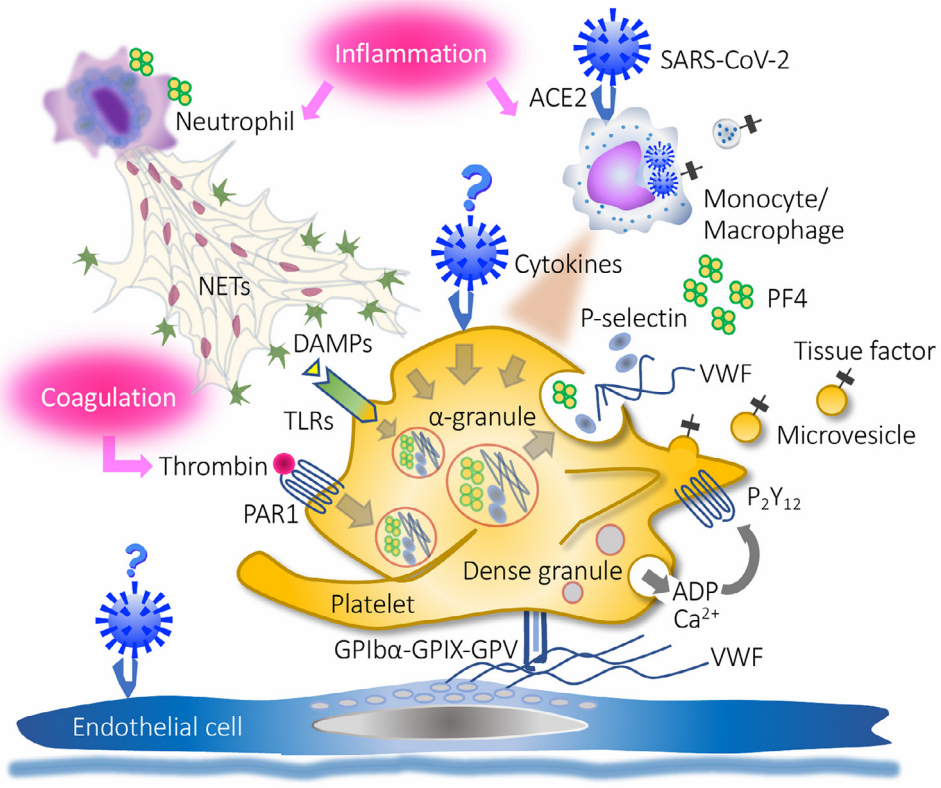

Fig. 1. Platelet activation in COVID-19.
Both inflammation and coagulation are important promotors of clot formation in coronavirus disease 2019 (COVID-19). Other than severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binding to angiotensin-converting enzyme 2 (ACE2), damage-associated molecular patterns (DAMPs) formed as the consequence of inflammation and thrombin generated in the course of coagulation stimulate platelets to release platelet factor 4, *P*-selectin, and von Willebrand Factor from α-granule, and adenosine diphosphate (ADP) release from dense granule, and the tissue factor-expressing microvesicle release from platelets. Platelet factor 4 can stimulate neutrophil extracellular traps (NETs) ejection and propagate the inflammation. These responses altogether facilitate platelet aggregation and clot formation. TLRs: Toll-like receptors, PAR1: protease activated receptor 1, GP: glycoprotein, PF4: platelet factor 4, VWF: von Willebrand factor

events were rare, and the risk did not seem to increase beyond the expected rate [41]. However, soon after, the number of cases increased, and as of April 4, 2021, 222 cases with cerebral venous sinus thrombosis (CVST) were reported to the European drug safety database out of 34 million vaccinations with ChAdOx1 (estimated incidence of 1:150 000) [42], and this thrombotic event has been recognized as rare but serious complication and named vaccine-induced immune thrombotic thrombocytopenia (VITT) or thrombotic thrombocytopenic syndrome (TTS).

Greinacher et al. [43] demonstrated an essential role of anti-platelet factor 4 antibody in the development of VITT, and hypothesized the mechanism resembles that of HIT. Recently, Huynh et al. [44] determined the binding sites of anti- platelet factor 4 antibody obtained from VITT patients were all located in the heparin-binding site of platelet factor 4 and indicated that pathogeneses of VITT and HIT should highly overlap. The pathogenic HIT antibodies bind cellular FcγRIIA, a low-affinity receptor for the Fc-fragment of immunoglobulin G, on platelets and monocytes to upregulate aggregation and propagate inflammation [45]. However, since most of the VITT cases were not exposed to heparins, a similar mechanism with heparin-independent spontaneous HIT is suspected. In VITT, free DNA in the vector vaccine or DNA from other sources is assumed to bind platelet factor 4 and triggers the HIT antibody production [46]. McGonagle et al. [47] suggested viral DNA- platelet factor 4 interplay is a part of antiviral immunity, akin to the innate immunity for bacterial infection. Another possible source of DNA is the exaggerated immune reaction of the leukocytes. The inflammatory stimulus after vaccination can cause NETs formation with the DNA release from macrophages and neutrophils [48]. Other than above, hypersulfated glycosaminoglycan, chondroitin sulfate, is known to trigger spontaneous HIT after trauma and knee surgery [46]. Chondroitin sulfate, a major component of the glycocalyx on the endothelial cell that is easily injured and released into the circulation during inflammation, can be another candidate of the polyanion source [49] (Fig. 2).

During the course of anti- platelet factor 4 antibody production, the involvement of spike protein is significant. Similar to SARS-CoV-2, the spike protein will stimulate the release of platelet factor 4 from platelets, activate immune cells to induce inflammatory reaction, and turn the antithrombotic properties of the vascular lumen toward the opposite side through the binding to ACE2 [50]. Therefore, although VITT was initially reported as the complication of the virus-vectored vaccine, it can be also found after the vaccination with mRNA vaccine [51–53].

Spike protein and anti-platelet factor 4 antibody

As described previously, the spike protein of coronavirus upregulates the inflammatory response and injures the vascular endothelium by binding to ACE2 [54]. ACE2 converts angiotensin II to angiotensin 1–7, a molecule counterbalancing the vasoconstrictive, proinflammatory, and pro-coagulant effects of angiotensin II [55]. Therefore, decline of angiotensin 1–7 on the cellular membrane by COVID-19 infection, as well as loss of the endothelial anticoagulant effects, leads to microcirculatory disturbance by vasoconstriction, profound inflammation, and activated coagulation [56]. The

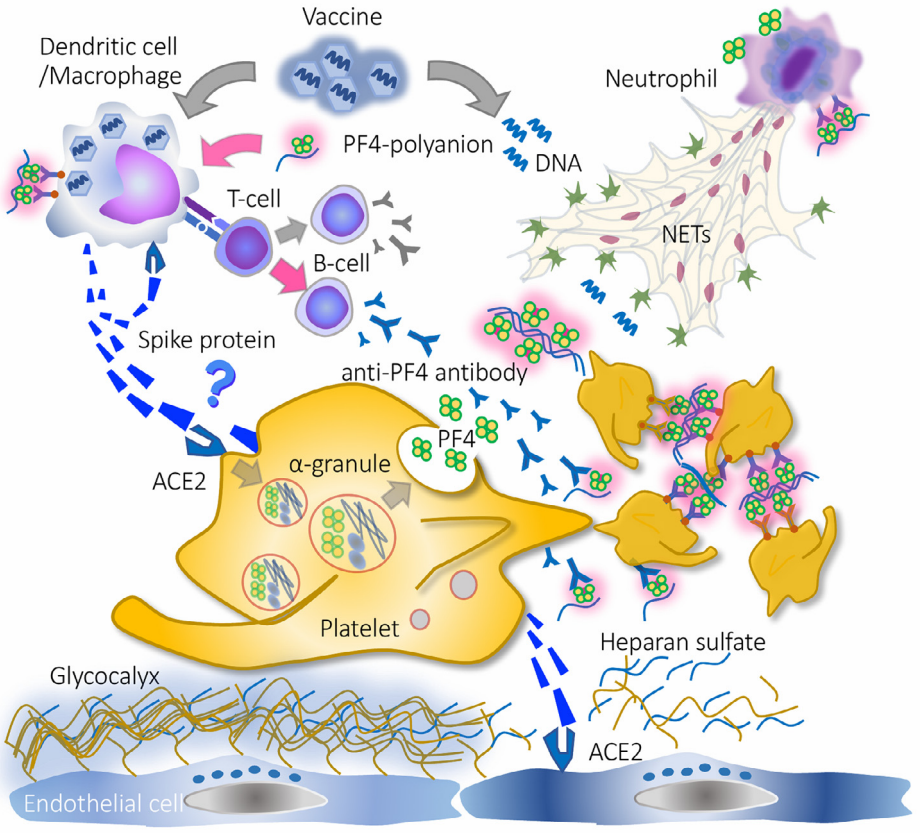

Fig. 2. Platelet activation in vaccine-induced thrombotic thrombocytopenia (VITT).
The spike protein produced after vaccination stimulates dendritic cells/macrophages to initiate the production of anti-spike protein antibody as well as elicit inflammatory reactions. The spike protein also stimulates platelets and vascular endothelial cells directly via angiotensin-converting enzyme 2 (ACE2) binding or indirectly through inflammation and coagulation. Platelet factor 4 released from α-granule of the platelets binds to polyanions, *i.e.*, free-DNA in the virus-vectored vaccine, free-DNA released from neutrophil extracellular traps (NETs), and heparan sulfate released from glycocalyx. Platelet factor 4/polyanion binding expresses immunogenicity and stimulates the anti-platelet factor 4 antibody production. Platelet factor 4/polyanion-antibody binding promotes platelet aggregation and further NETs release from the leukocytes. PF4: platelet factor 4.

vaccine-induced spike protein also may induce the similar reactions and may serve the underlying condition of thrombogenesis. Colunga Biancatelli et al. [57] reported the S1 subunit of SARS-CoV-2 spike protein alone could produce acute lung injury. So far, the amount of produced spike protein and the productivity difference between the vaccines have not been examined; however, the immunogenicity of the spike protein may not be the same among the vaccines. Kowarz et al. [58] reported the spike protein is immunogenic, and the immunogenicity of the spike protein generated by virus-vectored vaccine is more potent than that of mRNA vaccine. They also reported the virus-vectored vaccine can produce variant spike protein by splicing, and that may cause the intensified inflammation and hypercoagulation.

Although the immunogenicity is considered higher in virus-vectored vaccine, the induction of neutralizing antibody is more potent in mRNA vaccines, and neutralization level is reportedly highest in mRNA vaccines followed by convalescent serum, and the levels were lower in virus-vectored vaccines [59]. Thus, the production of anti- platelet factor 4 antibodies does not correlate with the generation of anti-spike neutralizing antibodies.

With respect to the prevalence of anti- platelet factor 4/polyanion antibody after vaccination, Thiele et al. [60] examined the 281 vaccinated samples by enzyme-linked immunoassay and reported that induction of anti- platelet factor 4/polyanion antibody was recognized in both after mRNA- and adenovirus-vectored vaccinations, and the positive rate was 5.6% (95% confidence interval [CI]: 2.9–10.7) with BNT162b2 (mRNA vaccine) and 8.0% (95% CI: 4.5–13.7%) with ChAdOx1. However, the optical densities were low in most cases (range: 0.5–1.0 units), and none of those samples induced platelet activation. Ant-platelet factor 4 antibody is also produced in COVID-19. Even though the optical density was also low and 0.752 (interquartile range: 0.48–1.31), IgG anti- platelet factor 4 antibody was detected in 7.6% of hospitalized COVID-19 patients not received heparin therapy [61]. These observations suggest that anti- platelet factor 4 antibody production is the consequence of the immune reaction evoked by the spike protein [60]. However, in many cases, the platelet activation test was negative, and the antibody is functionally inactive [62]. Therefore, anti- platelet factor 4 antibodies and other factors are necessary for VITT development. The current evidences suggest spike-protein can trigger the production of anti- platelet factor 4 antibody, and the immunogenicity of vectored vaccine is more potent in terms of anti- platelet factor 4 antibody production, but other missing links should be found to explain the higher prevalence of VITT in the vectored vaccines.

Virus-vectored vaccine and mRNA vaccine

The virus-vectored vaccine elicits poly clonal antibodies that make the virus neutralization possible driving other antibody-dependent effector functions as well as potent T cell responses [63]. The splicing occurs only in virus-vectored vaccines, and the immunogenicity of vectored vaccine to induce anti- platelet factor 4 antibody seems higher, however, VITT-like adverse events are also rarely found in mRNA vaccines. Cari et al. [64] reported

the adverse events expressed by bleeding and clot with thrombocytopenia were 3.3 and 15.1/100,000 doses in mRNA and virus-vectored vaccine recipients, respectively. The severe cases were documented 0.4 and 3.0 cases, and death rate was 0.04 and 0.48/100,000 doses for mRNA and vectored vaccine recipients, respectively. In other report, an observed risk of thrombocytopenic events after ChAdOx1 vaccination was higher than the expected risk (relative risk [RR]: 2.80, 95% CI: 1.39–5.67) [65]. These observations suggest thrombocytopenia and thrombosis after vaccination is the result following spike protein production and subsequent platelet factor 4 release, and the incidence is higher in vectored vaccine. The mechanism of the different reactions among vaccines should be examined more thoroughly to reduce similar adverse events in the future.

Whether spike protein-elicited thrombogenicity can explain the different prevalence of VITT still remains to be determined. As for the possibility of spike protein-induced thrombogenicity, SARS-CoV-2 is known to use ACE2 to infect lung epithelial cells and elicit intra-alveolar inflammation and peri-alveolar thrombogenicity [66]. It is generally accepted that SARS-CoV-2 attach to endothelial cells by binding to ACE2 expressed on the surface [67]. However, some researchers doubt whether the spike protein of SARS-CoV-2 directly alters endothelial cell functions [68,69] as the presence of ACE2 on endothelial cell membranes is uncertain. Nascimento Conde et al. [70] demonstrated that primary human endothelial cells lack ACE2 at both protein and RNA levels, and suggested SARS-CoV-2 is incapable of infect endothelial cells. If endothelial cell expresses ACE2, and the spike protein produced by the vectored vaccines upregulates the endothelial prothrombotic reaction more vigorously, the higher prevalence of VITT is understandable. The splicing variant can also be the breakthrough of this issue [51], but the presence of ACE2 on endothelium is the precondition of this theory.

Another possible explanation of the different VITT prevalence rates is the participation of specific components in the vectored vaccine that facilitate the formation of anti-platelet factor 4/polyanion antibody. Negatively charged extracellular DNA in the vector virus binds platelet factor 4 and DNA- platelet factor 4 engagement may lead to platelet factor 4-directed thrombosis [47]. In this case, the exaggerated inflammatory response induced by free DNA-Toll-like receptor on platelets may also be involved in the hyperinflammation and the release of platelet factor 4. Other than above, not only the humoral factors *i.e.*, dendritic cell-B lymphocytes system but also the cellular immunity that includes dendritic cells, cytotoxic *T* cells, and helper T cells may contribute to the different prevalence in VITT [71].

The perplexing mystery in VITT is why the thrombosis frequently occurs in cerebral venous sinus. According to the data prior to COVID-19 pandemic, thrombocytopenia was observed in 8.4% of total CVST, and heparin-induced thrombocytopenia was only 0.1% [72]. Consequently, even if the incidence of CVST in 2021 does not increase compared to anticipated risk, a strong connection should exist with vaccination. The incidence of CVST in COVID-19 was reportedly 0.02% (20/100,000, 95% CI: 4–60/100,000) however, these patients did not show thrombocytopenia and anti-platelet factor 4 antibodies in this report [73]. Meanwhile, Ostovan et al. [74] reported 6 COVID-19 cases with CVST, and 3 of them were complicated with thrombocytopenia. Unfortunately, anti-platelet factor 4 antibody was not checked in these patients. Therefore, CVST with thrombocytopenia can occur in COVID-19 and perhaps after the vaccination with mRNA vaccine. However, since the prevalence is much lower, some essential factors in the vectored vaccines or produced by vaccination are necessary to complicate CVST. Some unknown events that occur after vaccination in the intracranial space must be figured out to explain the high prevalence of CVST. Pons et al. [68] delineated the ACE2 expression on the blood vessels of the brain, and the different distribution of ACE2 can be the hint to solve the mystery [75].

Are there any specific backgrounds or comorbidities related to the risk of VITT? Are there any genetic risks for the recipients? These additional questions also need to be answered.

Immune thrombocytopenic purpura (ITP) and thrombotic thrombocytopenic purpura (TTP) after vaccination

SARS-CoV-2 can alter the host immune system, and the increased prevalence of autoantibodies such as antinuclear antibodies and antigen-specific autoantibodies in COVID-19 patients is reported [76]. The vaccination has been known to induce various autoimmune disorders like immune thrombotic thrombocytopenic purpura (ITP) [77] and Guillain–Barre syndrome [78], and the pathogenic autoantibody generation due to the immunologic deregulation is highly suspected. The chimpanzee adenovirus-vectored Ebola vaccine is reported to cause thrombocytopenia without thrombosis however, the mechanism has not been resolved [79]. Regarding COVID-19 vaccine, ITP is listed as another autoimmune thrombocytopenic disease [80]. ITP is mediated by antiplatelet autoantibodies against membrane glycoprotein (GP) complexes such as GPIIb/IIIa and GPIb/IX [81]. However, the detection of these antibodies is not commonly performed, and the clinical diagnosis is made by excluding other thrombocytopenic diseases [82]. Recently, a prospective cohort study surveyed the association between ChAdOx1 vaccination and ITP. The result reported the incidence was 1.13 (95%CI: 0.62–1.63) cases per 100,000 doses. The study concluded that the ChAdOx1 vaccination is associated with small increased risk of ITP with small increased risks arterial thromboembolic and hemorrhagic events [65]. Meanwhile, according to Vaccine Adverse Event Reporting System (VAERS), the prevalence of thrombocytopenia was higher and 8.0 per 100,000 doses for both mRNA and vectored vaccine [80]. Kuter [83] prospectively followed fifty-two consecutive chronic ITP patients after vaccination and reported a platelet count drop of 96% within 2–5 days after vaccination in 12% of the patients. The events could occur in both types of vaccines and the platelet counts recovered to more than 30,000 /μL after the treatment with corticosteroids and intravenous immunoglobulin (IVIG).

Other than ITP, cases are even rare but the complication of thrombotic thrombocytopenic purpura was reported after receiving either Ad26.COV2-S or mRNA-based anti-COVID-19 vaccine [84,85]. Thus, when the patients demonstrate unexpected thrombocytopenia shortly after the vaccination, the possibility of these diseases should be considered.

Antithrombotic therapy for COVID-19

Anticoagulant therapy for COVID-19

SARS-CoV-2 infection induces a process known as immunothrombosis, in which activated neutrophils and monocytes interact with platelets and the coagulation systems, leading to intravascular clot formation from small to large vessels [86]. Therapeutic approach to mitigate immunothrombus may theoretically be useful, and anticoagulant and antiinflammatory agents have been proposed as therapeutic candidates. However, high-quality evidence does not exist to support the use of anticoagulants. An observational study demonstrated a better survival (Hazard ratio [HR]: 0.73, 95% CI: 0.66–0.81) in the patients treated with prophylactic dose of anticoagulants within 24 h after admission compared to the patients treated without anticoagulation [87]. In contrast, therapeutic dose of anticoagulation with rivaroxaban or enoxaparin followed by rivaroxaban did not improve the clinical outcomes

but increased bleeding compared with prophylactic anticoagulation [88]. A systematic review and meta-analysis revealed that the overall odds of mortality comparing anticoagulated to non-anticoagulated patients were similar. Of note was the mortality was lower with prophylactic dose (Odds ratio [OR]: 0.83, 95% CI: 0.73–0.95) and higher with intermediate-to-therapeutic dose (OR: 1.60, 95% CI: 1.11–2.31). The major bleeding of higher dose anticoagulation is more frequent compared to prophylactic dose (OR: 3.33, 95% CI: 2.34–4.72) [89]. In an open-label, adaptive, multiplatform, randomized clinical trial (REMAP-CAP, ACTIV-4a, and ATTACC Clinical Trials), therapeutic-dose anticoagulation with heparin did not result in a greater probability of survival or a greater number of days free of cardiovascular or respiratory organ support than usual thromboprophylaxis [90]. In summary, the anticoagulation will be beneficial however, intensive anticoagulation may increase the bleeding risk and potentially harmful for the COVID-19 patients. The patients who may benefit from the intensive anticoagulation should be carefully selected.

Antiinflammatory therapies for COVID-19

Regarding the efficacy of antiinflammatory therapy, series of randomized controlled trials (RCTs) examined the effects of an anti–interleukin-6 receptor monoclonal antibody. In the early RCT, tocilizumab failed to prevent the intubation or death in moderately ill COVID-19 patients required oxygenation [91]. Next RCT performed in COVID-19 pneumonia patients treated without mechanical ventilation showed reduced progression to the composite outcome of mechanical ventilation or death [92]. Following these, a large RCT examined the effect of tocilizumab in critically ill patients receiving organ support in ICU. The treatment with the tocilizumab and sarilumab improved outcomes including survival [93]. Most recent RCT was done in hospitalized patients with severe COVID-19 pneumonia, and the use of tocilizumab did not result in significantly better clinical status or lower mortality than placebo at 28 days [94]. Some other RCTs reported mixed results, and further study is necessary to obtain the definitive result.

Other than anti-interleukin-6 therapy, the effect of non-specific antiinflammatory therapy with corticosteroid have been reported. In RECOVERY trial, an open-label randomized trial performed in hospitalized patients with COVID-19, the use of dexamethasone resulted in lower 28-day mortality in the patients receiving oxygen or ventilated at randomization [95]. In another trial, the efficacy of 7-day fixed-dose of hydrocortisone or shock-dependent dosing of hydrocortisone was compared with no hydrocortisone. The results showed 93 and 80% probabilities of superiority with regard to the improvement in organ support-free days within 21 days; however, neither treatment strategy met prespecified criteria for statistical superiority [96]. While the definitive answer needs further investigation, the present evidence suggests that moderate to severely ill COVID-19 patients may benefit from corticosteroid therapy.

Antiplatelet therapy for sepsis and COVID-19

Since platelets contribute to the thrombotic and inflammatory response in sepsis, it is logical to think antiplatelet therapy might be beneficial. However, the reported efficacy of antiplatelet therapy in septic patients is inconsistent, and therapeutic approaches to inhibit platelet activation and microthrombosis formation need further investigation. Platelet-activating factor (PAF) is an endogenous proinflammatory mediator implicated in the pathogenesis of sepsis. The clinical effect of lexipafant, a PAF receptor antagonist, was examined in a small-size double-blind RCT but failed to show the improvement of survival [97]. Similarly, the effect of recombinant PAF acetylhydrolase that degrades PAF was examined in a phase III trial, but this agent also could not show a positive impact in terms of survival [98]. Much later, the effects of more common antiplatelet agents such as aspirin and $P2Y_{12}$ inhibitors, including clopidogrel, prasugrel, and ticagrelor were examined in an observational study, and the use of these agents was shown to associate with the reduced mortality in sepsis (OR: 0.82, 95% CI: 0.81–0.83) [99]. In contrast, in a prospective setting, 100 mg once daily of aspirin did not reduce the mortality in sepsis (HR: 1.08, 95% CI: 0.82–1.43) [100].

Similarly, positive and negative results are mixed concerning the effectiveness of antiplatelet therapy for COVID-19. A retrospective, observational cohort study that included 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%, $P = 0.03$). However, the mortality was not different between the groups (aspirin group: 26.5% vs. non-aspirin group: 23.2%, $P = 0.51$) [101]. In the following open-labeled RCT, 15,000 hospitalized COVID-19 were randomized to the aspirin group (150 mg once a day) and control (usual care alone) group. The result showed a subtle difference, and although aspirin was associated with a small increase in the likelihood of being discharged alive, the difference was not statistically significant (RR: 0.96, 95% CI: 0.89–1.04) [102].

Treatments for VITT

In the event of major thrombotic events 4 to 30 days after COVID-19 vaccination, VITT should be considered, and the infusion of high-dose IVIG (1 g/kg followed by a second dose 24 h later) in combination with non-heparin anticoagulants are recommended. The objective of immunoglobulin therapy is to neutralize the causative anti-platelet factor 4/polyanion antibody. Steroids or plasma exchange are also options to reduce the autoantibodies [103,104]. Vayne et al. [105] reported platelet activation was suppressed by IV.3, a monoclonal antibody that binds FcγRIIA receptors and also by IdeS (IgG-degrading enzyme derived from Streptococcus pyogenes).

Conclusions

Thrombosis is a major pathological driver in COVID-19. Evolving evidence suggests that in addition to the activated leukocytes and derangement of antithrombotic property of endothelial cells, hyperactive platelets participate in thrombogenesis. The direct and indirect effects of SARS-CoV-2 spike protein on platelets stimulate the release of platelet factor 4. The spike protein also upregulates inflammation and coagulation through the binding to ACE2 on macrophages/monocytes, lung epithelial cells, and possibly vascular endothelial cells, reactions that lead to micro and macro circulatory clotting known as CAC. In this regard, CAC is not just a result of SARS-CoV-2 infection but the major facilitator of COVID-19. The virus vectored vaccines provide a certain amount of DNA that binds platelet factor 4 released from platelets. DNA-platelet factor 4 binding induces the antigenicity of platelet factor 4, and the newly generated anti-platelet factor 4/polyanion antibody causes VITT. Other than the adenovirus-vectored vaccine origin DNA, polyanion can be originated from NETs and endothelial glycocalyx. As for the treatment of CAC, there is no high-quality evidence that supports the use of antiplatelet therapy. For VITT, high-dose IVIG is recommended to neutralize anti-platelet factor 4 antibody-FcγRIIA binding. It may be rational to think that the thrombotic events in CAC and VITT occur not separately but rather some common mechanisms exist. Understanding the mechanism is extremely important to develop safer vaccines.

Declaration of Competing Interest

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CRediT authorship contribution statement

Toshiaki Iba: Writing – original draft, Writing – review & editing. **Jerrold H. Levy:** Writing – review & editing.

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