Platelet Function in Viral Immunity and SARS-CoV-2 Infection

Afaf Allaoui, MSc¹ Akif A. Khawaja, PhD² Oussama Badad, MSc^{1,3} Mariam Naciri, PhD¹
Marie Lordkipanidzé, BPharm, PhD^{4,5} Fadila Guessous, PhD^{6,7} Younes Zaid, PhD^{1,8}

¹ Department of Biology, Faculty of Sciences, Mohammed V University, Rabat, Morocco

² National Heart and Lung Institute, Imperial College London, London, United Kingdom

³ Department of Plant, Southern Illinois University, Carbondale, Illinois

⁴ Research Center, Montreal Heart Institute, Montréal, Quebec, Canada

⁵ Faculty of pharmacy, Université de Montréal, Montréal, Québec, Canada

⁶ Microbiology, Immunology and Cancer Biology, School of Medicine, University of Virginia, Charlottesville, Virginia

⁷ Department of Biological Sciences, Faculty of Medicine, Mohammed VI University of Health Sciences, Casablanca, Morocco

⁸ Research Center of Abulcasis University of Health Sciences, Cheikh Zaïd Hospital, Rabat, Morocco

Address for correspondence Younes Zaid, PhD, Department of Biology, Faculty of Sciences, Mohammed V University in Rabat, 10000, 4 Ibn Battouta Avenue, Rabat, Morocco (e-mail: y.zaid@um5s.net.ma).

Semin Thromb Hemost 2021;47:419–426.

Abstract

Keywords

- ▶ platelets
- ▶ hemostasis
- ▶ inflammation
- ▶ SARS-CoV-2
- ▶ COVID-19
- ▶ viral infections

Platelets, as nonnucleated blood components, are classically recognized for their pivotal role in hemostasis. In recent years, however, accumulating evidence points to a nonhemostatic role for platelets, as active participants in the inflammatory and immune responses to microbial organisms in infectious diseases. This stems from the ability of activated platelets to secrete a plethora of immunomodulatory cytokines and chemokines, as well as directly interplaying with viral receptors. While much attention has been given to the role of the cytokine storm in the severity of the coronavirus disease 2019 (COVID-19), less is known about the contribution of platelets to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Here, we give a brief overview on the platelet contribution to antiviral immunity and response during SARS-CoV-2 infection.

Infectious diseases have significant impact globally, with high mortality and morbidity rates reported each year by the World Health Organization.¹ Over the past few decades, new challenges associated with infectious diseases have placed additional burdens on health care due to the emergence of antimicrobial resistance,² and viral pandemics including Ebola,³ human immunodeficiency virus (HIV),⁴ and most recently severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is causing the ongoing coronavirus disease 2019 (COVID-19) pandemic outbreak.⁵

Platelets are small anucleate cells derived from megakaryocytes, and are traditionally known for their role in preven-

tion of bleeding and minimizing vascular injury.⁶ While vital for hemostasis, there has been an increasing awareness that platelets also contribute to various human pathologies, including autoimmunity,⁷ cancer,⁸ and infectious diseases.⁹ Thus, in addition to their key contribution to thrombus formation, there is increasing consensus that platelets would play important roles in modulating immune and autoimmune responses.^{10,11}

The ability of platelets to participate in the immune response is in part due to their ability to release a myriad of inflammatory and bioactive molecules stored within their granules. These mediators are able to attract and modulate

published online
April 13, 2021

Issue Theme Maintaining Hemostasis and Preventing Thrombosis in COVID-19—Part II; Guest Editors: Emmanuel J. Favaloro, PhD, FFSc (RCPA) and Giuseppe Lippi, MD.

© 2021. Thieme. All rights reserved.
Thieme Medical Publishers, Inc.,
333 Seventh Avenue, 18th Floor,
New York, NY 10001, USA

DOI <https://doi.org/10.1055/s-0041-1726033>.
ISSN 0094-6176.

the activities of circulating leukocytes, important in orchestrating localized immune responses to pathogens.¹¹ Platelets have also been found to elicit direct effector functions, so as to be considered independent immune effector cells.¹² Indeed, megakaryocytes and platelets have been shown to express several immune-associated molecules and receptors, including Fc receptors,¹³ complement receptors,¹⁴ chemokine receptors,^{15,16} and an array of toll-like receptors (TLRs).^{17–25} The expression of these functional immune receptors raises the question whether platelets can also engage viruses and contribute to antiviral immunity. In this short narrative review, we explore how viruses engage circulating platelets and how they contribute to viral pathology.

The Role of Platelets in Viral Immunity

Viral immunity has traditionally focused on the roles of leukocytes, given their direct involvement in viral spread and antiviral responses. Clinically, platelet hyperactivity has been recognized as a hallmark of many viral infections, including dengue virus,²⁶ HIV,^{27–29} influenza virus,³⁰ and SARS-CoV-2.³¹ Given the prominent clinical presentations of platelet-driven events, along with their emerging immune role, having a better understanding of the role of platelets in viral infections may disclose and highlight novel therapeutic targets.

A key antiviral platelet response is to sequester viral particles, thus limiting viral spread within the host environment. Evidence of such activity has been seen in HIV, where platelets bind and endocytose HIV virions,^{32–35} which is believed to help clearance of viral particles from circulation.³⁶ In addition to engaging viruses, platelets are also able to exert direct antiviral properties. During platelet activation, α -granules are trafficked to the cell surface and externalized, so releasing a wide spectrum of bioactive molecules, including platelet factor 4 (PF4; also referred to as the chemokine CXCL4). As well as being an important chemotactic agent for leukocytes, PF4 has direct antiviral activity, being found to suppress HIV infection of T cells.^{37–39} Interestingly, platelets may also help control infection through the secretion of platelet antimicrobial peptides, such as PD1-PD4, which have been shown to have antiviral activity against the vaccinia virus.⁴⁰ A recent study has also presented data demonstrating that platelets contain virus-specific immunoglobulin G (IgG), which is able to potentially neutralize in vitro and in vivo viral infection against human cytomegalovirus (HCMV) and influenza A virus.⁴¹ Platelet-derived IgG localizes to α -granules,⁴² suggesting that megakaryocytes are able to take up IgG, where they are stored in α -granules for later secretion by mature platelets. Interestingly, IgG released from platelets was found to be more efficient at neutralizing virus compared with equal amounts of plasma IgG,⁴¹ the biological significance of which is unclear.

Platelets are also able to orchestrate local immune responses to viral infection. HCMV can be recognized by platelet TLR-2. This engagement leads to platelet degranulation, leukocyte chemotaxis, and formation of platelet aggre-

gates with neutrophils, monocytes, B cells, T cells, and dendritic cells.⁴³ Through these platelet-leukocyte interactions, platelets present viral antigens to leukocytes via major histocompatibility complex class I,⁴⁴ as well as providing costimulatory signals to antigen-presenting cells,⁴⁵ both of which can prime and mount an antiviral leukocyte response. Similar inflammatory activities have been observed in dengue virus infection, whereby dengue-infected platelets were able to induce monocyte activation.⁴⁶

While platelets can exert a degree of antiviral immunity, viruses have evolved mechanisms of evading platelet recognition. Viruses are able to engage with receptors at platelet surfaces; for example, dengue virus and HIV both bind surface lectin receptors and dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN) on platelets.⁴⁷ Such interactions lead to internalization of viral particles, where viruses such as HIV, HCMV, and hepatitis C virus (HCV) can continue to replicate and generate productive viruses within both megakaryocytes and platelets.^{48–50} In addition to using platelets as a site of replication, some viruses use circulating platelets as cellular carriers to evade immune detection, such as the influenza virus⁵¹ and HIV,^{52,53} essentially forming latent viral reservoirs within the circulation. Interestingly, HCV is believed to utilize circulating platelets to transport itself to the liver, where enhanced platelet-hepatocyte interactions prolong the time for potential viral infection.⁵⁴

Thrombocytopenia is a common feature among various viral infections, which is associated with more severe diseases.⁵⁵ Viruses have developed several mechanisms to target and reduce platelet production and/or integrity. A classic example can be seen with neuraminidase activity of influenza virus, which reduces platelet life span by targeting them for rapid clearance in the liver and spleen.⁵⁶ In addition to targeting platelets for destruction, neuraminidase activity also alters megakaryocyte ploidy, morphology, and subsequent platelet size.⁵⁷ Human herpes viruses have also adopted similar mechanisms, and can interfere with thrombopoietin activity, thus reducing megakaryocyte colony formation⁵⁸ and impairing megakaryocyte survival and differentiation.⁵⁹ Defective megakaryocyte differentiation can also be achieved by altering cytokine expression in the bone marrow, which has been found in dengue virus infection.⁶⁰ By targeting these megakaryocytic developmental checkpoints, abnormal platelet activation, mitochondrial dysfunction, reduced cellular integrity, and increased apoptosis are often seen in patients infected with dengue,^{61–63} encephalomyocarditis virus,²³ and HIV.^{64,65}

In addition to impacting platelet integrity, viral infection can also affect platelet function.⁶⁶ Coxsackievirus B virus (CVB) binds and enters platelets via the Coxsackie-Adeno receptor.⁶⁷ While unable to replicate inside the platelet, CVB modulates activity and enhances P-selectin release and phosphatidylserine exposure, which collectively promote platelet-leukocyte interactions and ultimately leads to platelet destruction and thrombocytopenia,⁶⁷ driving viral pathology. While vaccinia virus is known to bind and enter platelets,⁶⁸ the significance of this interaction to disease is

not completely understood. Early studies reported reduced *in vitro* platelet aggregation, but increased serotonin release in vaccinia-infected platelets,⁶⁸ which would suggest that platelet function may be suppressed. In contrast, *in vivo* models found vaccinia virus infection led to fatal intravascular coagulation,⁶⁹ implicating an augmented platelet response. This disparity may highlight that the vaccinia virus may impact endothelial function, which is known to be critical in regulating *in vivo* platelet responses.^{70,71}

Given the complex nature of the immune network, the existence of indirect effects of viruses on platelets is unsurprising. Platelet hyperactivity in influenza infection can be partially attributed to influenza's impact on monocyte cytokine release, which then activates platelets.⁷² Adaptive immune responses to HCV, HIV, HCMV, herpes viruses, and coronaviruses result in the production of antibodies targeting viral glycoproteins to help neutralize and suppress viral spread. These antiviral glycoprotein antibodies can, however, cross-react with platelet integrins and trigger platelet auto-antibody-induced thrombocytopenia in several viral settings.⁷³ Viruses have also been found to infect the endothelium, with indirect effects on platelet function. For example, dengue virus and hantaviruses infect endothelial cells, promoting endothelial activation, endothelial-platelet interactions, and increasing vascular permeability.^{74,75} This disruption of vascular integrity is thought to contribute to the increased platelet reactivity observed in virally infected patients and may represent one mechanism of enhanced platelet clearance.

An additional consideration of chronic viral infection, like HIV, is that patients require permanent therapeutic intervention to suppress viral replication. Some cohort studies have found that certain antiretroviral drugs are associated with increased risk of myocardial infarction,^{76,77} which have subsequently been found to enhance platelet activation and aggregation.^{78,79} These effects can be further enhanced by vascular endothelium, which is also impacted by antiretroviral drugs in ways that increase platelet reactivity.^{80,81} Such data demonstrate that both viral infection and therapeutic measures to control infection can impact platelet reactivity.

The Platelet Response during SARS-CoV-2 Infection

Dual activation of inflammation and coagulation pathways, combined with an excessive recruitment and activation of immune cells to sites of infection, is known as "immunothrombosis," a concept that was initially conceptualized by Engelmann and Massberg, in 2013, to accurately define the crosstalk between hemostasis and the innate immune system.⁸² Given that aberrant platelet activation has been documented with other viral infections, researchers have begun to explore the potential contributory role of platelets to SARS-CoV-2 infection.

The core pathology of COVID-19 is pulmonary, with epithelial cell infection by SARS-CoV-2 ultimately resulting in pulmonary leukocyte infiltration and an excessive inflammatory response.⁸³ Clinical evidence supports this model,

with several reports detailing signs of epithelial and endothelial inflammation, leukocyte recruitment, and platelet activation in the lung of COVID-19 patients.^{84–86} Poorer prognoses in patients are shown to associate with abnormal coagulation parameters, primarily D-dimer, fibrinogen, fibrin degradation product levels, reduced mitochondrial depolarization, and phosphatidylserine exposure,^{87–89} suggesting that thrombosis may be important to COVID-19 pathophysiology. Severe pulmonary inflammation and obstructive immunothrombosis in the lung microvascular network of COVID-19 patients, leading to pulmonary thrombosis/thromboembolism, underlie multiple organ failure and mortality in patients with advanced stages of illness.^{90–92}

Elevated plasma levels of proinflammatory cytokines such as interleukin (IL)-1 α , IL-1 β , IL-6, IL-12, monocyte chemoattractant protein-1, interferon- γ , and tumor necrosis factor- α have been found in patients with COVID-19.^{93–95} While there is evidence of elevated proinflammatory cytokines, it is important to note that there are also reports finding similar or lower levels of proinflammatory cytokines when compared with patients with COVID-19-unrelated acute respiratory distress syndrome or other cytokine release syndromes.^{96–98} In addition, reports have also found elevated D-dimer concentrations in patients with COVID-19,⁹⁹ which is consistent with the observed systemic inflammation and macrovascular thrombotic complications seen in patients with SARS-CoV-2 infection,^{100,101} and may therefore be linked to coagulation activation and diffuse macro- and microvascular thrombosis.^{102,103}

While SARS-CoV-2 messenger RNA (mRNA) can be detected in platelets isolated from patients with COVID-19,^{94,104–106} it is not clear whether SARS-CoV-2 is internalized by the platelets via receptor-mediated endocytosis. Although it is widely accepted that SARS-CoV-2 infects host cells via binding angiotensin-converting enzyme 2 (ACE2),¹⁰⁷ it is not known whether platelets express this protein. While some studies have shown that neither ACE2 mRNA nor protein could be detected in platelets,^{94,104} others have reported robust ACE2 expression in platelets, associated to direct platelet activation by SARS-CoV-2 via spike/ACE2 interactions.^{108,109} The reason for this disparity is unclear, but may stem from differences in washed platelet preparation given that one study used sodium citrate-evacuated blood tubes, while others used an acid/citrate/dextrose anticoagulant. It is also feasible that genetic differences between cohort populations may account for differences in ACE2 expression or protein polymorphisms. These discordant results were clearly highlighted in a recent review.¹⁰⁶ Interestingly, while Zaid et al demonstrated that platelets only associate with SARS-CoV-2 RNA, they reported substantial alterations in the platelet transcriptome and proteome profiles,¹⁰⁴ as well as platelet hyperreactivity.^{94,104} The abilities of viruses including SARS-CoV-2 to associate and internalize with platelets are listed in ►Table 1.

These studies indicate that platelet activation contributes to COVID-19 pathophysiology. Autopsy studies found evidence of extensive thrombosis in multiple organs,⁸⁴

Table 1 Associations and/or internalizations between platelets and different viruses

Virus		Platelet		References
Type	Nomenclature	Association	Internalization	
DNA	Herpes simplex virus type 1 (HSV-1)	Yes	?	108
	Human cytomegalovirus (HCMV)	Yes	?	118
	Vaccinia virus (VACV or VV)	Yes	?	43
RNA	Human immunodeficiency virus (HIV)	Yes	Yes	68
	Hepatitis C virus (HCV)	Yes	Yes	32
	Dengue virus (DENV)	Yes	Yes	119
	Influenza virus (flu virus)	Yes	Yes	120
	Coxsackievirus B (CVB)	Yes	Yes	121
	Encephalomyocarditis virus (EMCV)	Yes	Yes	67
	Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)	Yes	?	94

suggesting enhanced platelet reactivity may be a driver of thrombosis in severe COVID-19. Abnormal platelet morphology has also been reported in COVID-19 patients, with evidence of large, hyperchromatic, and vacuolated platelets.¹¹⁰ A recent study documented COVID-19 patients as having enhanced platelet hyperreactivity relative to non-COVID-19 patients and controls subjects.¹¹¹ Greater levels of platelet-monocyte and platelet-granulocyte aggregates can be seen in patients with COVID-19 pneumonia,¹¹² highlighting greater levels of systemic platelet activation. Further phenotypic analysis revealed that resting platelets in COVID-19 patients had similar levels of P-selectin expression as control platelets activated with collagen.¹¹² Taus et al also demonstrate that COVID-19 platelets contribute to the increased fibrinogen, von Willebrand factor, and factor XII reported in patients, while facilitating accelerated factor XII-dependent coagulation.¹¹² Moreover, platelets isolated from patients with severe COVID-19 were able to induce ex vivo tissue factor expression in monocytes isolated from health controls,⁸⁸ indicating platelet crosstalk into other circulating cells. Interestingly, normal platelet function is restored in patients who have recovered from SARS-CoV-2 infection, which suggests that platelet hyperreactivity may be a direct consequence of SARS-CoV-2 infection.¹¹¹ Together, these data suggest that in COVID-19, platelets are primed to spread proinflammatory and procoagulant activities within the systemic circulation.

► **Fig. 1** summarizes the role that the platelet could have following SARS-CoV-2 infection.

Conclusion

COVID-19 is a viral infection with variable clinical outcomes, determined by the amplitude of immunothrombosis response and extent of tissue injury. While hyperinflammation and the “cytokine storm” may be central to the most severe COVID-19 cases,¹¹³ given the clinical spectrum of COVID-19, the absolute centrality of the “cytokine storm” may not be as straightforward. It could be argued that the extent and

importance of increased cytokine release on pathology draws upon multiple factors including genetic, host and viral phenotypic, and environmental.¹¹⁴ Recently, studies using the bronchoalveolar lavage of COVID-19 patients in intensive care, highlighted the presence of a ‘lipid storm’ but were unable to definitively demonstrate whether platelets or other cells are the cause of this altered lipid profile.¹¹⁵ Currently, available studies suggest that the COVID-19 coagulopathy comprises a combination of localized pulmonary platelet consumption, low-grade disseminated intravascular coagulation, and thrombotic microangiopathy.

Of particular interests are the various circulating inflammatory coagulation biomarkers involved directly in clotting, with specific focus on fibrin/fibrinogen, D-dimers, P-selectin, von Willebrand factor multimers, soluble thrombomodulin, and tissue factor, which may amplify inflammation and hypercoagulability in patients with COVID-19. Central to the activity of these biomarkers are their receptors and signaling pathways on endothelial cells, platelets, monocytes, and erythrocytes. Altogether, these collective observations raise the question as to whether the virus acts directly on the hemostatic system or whether hemostatic activation is secondary to the upstream inflammatory process.

Currently, literature remains ambivalent regarding ACE2 expression on platelets. It would therefore be useful to explore whether SARS-CoV-2 directly binds platelets via ACE2 or through alternative pathways. These studies may give better insight into underlying pathways driving the “cytokine storm coagulation” that contributes to the multiple organ dysfunction associated with severe COVID-19. While therapeutic intervention targeting the cytokine storm in severe COVID-19 is gaining increasing attention,¹¹⁶ the use of antiplatelet therapy also warrants further study. Aspirin administration has been associated with a reduced risk of mechanical ventilation, intensive care unit admission, and in-hospital mortality in 412 hospitalized COVID-19 patients.¹¹⁷ A limitation to this study, however, is that it is a retrospective, observational cohort study, which limits its

Fig. 1 Hypothetical model for platelet interaction with SARS-CoV-2. SARS-CoV-2 potentially depends on ACE2 receptor for viral entry through the endothelium and spread in the host. Then various platelet receptors can mediate binding to viral particles; however, SARS-CoV-2 binds to platelets probably via its potential receptor ACE2, and viral hemagglutinin is cleaved by TMPRSS2 to activate internalization of the virus. Such cleavage triggers platelet activation and downstream signaling events leading to cytokine overproduction, platelet aggregation, and leukocyte–platelet aggregate formation. The combination of the cytokine storm, platelet activation, microvesicle shedding, and immunothrombotic events have deleterious consequences such as cellular damage, acute lung injury, and thromboembolism (created with BioRender and Servier). ACE2, angiotensin-converting enzyme 2; CLEC, C-type lectin-like receptor; CXCR, chemokine receptor; DC-SIGN, dendritic cell-specific ICAM-grabbing nonintegrin; EV, extracellular vesicles; FcγRIIa, low-affinity immunoglobulin gamma Fc region receptor II-a; MHC-1, major histocompatibility complex 1; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TLR, toll-like receptor; TNFR, tumor necrosis factor receptor.

strength as clinical evidence. This study does demonstrate that further randomized controlled trials examining the efficacy of antiplatelet therapeutics in treating patients with severe COVID-19 are of clinical value. These future trials would be strengthened by complementary basic and translational studies dissecting the role of platelets in COVID-19 pathophysiology.

Ultimately, a cross-disciplinary approach drawing upon the expertise of biomedical and clinical communities is critical in developing a therapeutic arsenal to target not only the cytokine storm but also the coagulopathy related to SARS-CoV-2 infection. A deeper understanding of the contributions of platelets to viral immunity will not only allow for better treatment of COVID-19, but also help to be more prepared to manage future viral pandemics.

Authors' Contributions

A.A., A.A.K., O.B., M.N., M.L., F.G., and Y.Z. contributed to literature search and writing of this review. A.A.K., M.L., F.G., and Y.Z. designed the structure and content of this review. Y.Z. provided the figure. All authors approved the submitted version of the manuscript.

Funding

None.

Conflict of Interest

None declared.

References

- 1 World Health Organization. Disease outbreaks. Accessed February 5, 2021 at: <https://www.who.int/emergencies/diseases/en/>
- 2 Howard DH, Scott RD II, Packard R, Jones D. The global impact of drug resistance. *Clin Infect Dis* 2003;36(Suppl 1):S4–S10
- 3 Delamou A, Delvaux T, El Ayadi AM, et al. Public health impact of the 2014–2015 Ebola outbreak in West Africa: seizing opportunities for the future. *BMJ Glob Health* 2017;2(02):e000202
- 4 Piot P, Bartos M, Ghys PD, Walker N, Schwartzländer B. The global impact of HIV/AIDS. *Nature* 2001;410(6831):968–973
- 5 Hiscott J, Alexandridi M, Muscolini M, et al. The global impact of the coronavirus pandemic. *Cytokine Growth Factor Rev* 2020; 53:1–9
- 6 Machlus KR, Italiano JE Jr. The incredible journey: from megakaryocyte development to platelet formation. *J Cell Biol* 2013; 201(06):785–796
- 7 Liu X, Gorzelanny C, Schneider SW. Platelets in skin autoimmune diseases. *Front Immunol* 2019;10:1453
- 8 Haemmerle M, Stone RL, Menter DG, Afshar-Kharghan V, Sood AK. The platelet lifeline to cancer: challenges and opportunities. *Cancer Cell* 2018;33(06):965–983
- 9 Middleton E, Rondina MT. Platelets in infectious disease. *Hematology (Am Soc Hematol Educ Program)* 2016;2016(01):256–261
- 10 Guo L, Rondina MT. The era of thromboinflammation: platelets are dynamic sensors and effector cells during infectious diseases. *Front Immunol* 2019;10:2204
- 11 Ribeiro LS, Migliari Branco L, Franklin BS. Regulation of innate immune responses by platelets. *Front Immunol* 2019;10:1320
- 12 Liu JZ, van Sommeren S, Huang H, et al; International Multiple Sclerosis Genetics Consortium International IBD Genetics Consortium. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat Genet* 2015;47(09):979–986

- 13 Riaz AH, Tasma BE, Woodman ME, Wooten RM, Worth RG. Human platelets efficiently kill IgG-opsonized *E. coli*. *FEMS Immunol Med Microbiol* 2012;65(01):78–83
- 14 Martel C, Cointe S, Maurice P, et al. Requirements for membrane attack complex formation and anaphylatoxins binding to collagen-activated platelets. *PLoS One* 2011;6(04):e18812
- 15 Clemetson KJ, Clemetson JM, Proudfoot AE, Power CA, Baggiolini M, Wells TN. Functional expression of CCR1, CCR3, CCR4, and CXCR4 chemokine receptors on human platelets. *Blood* 2000;96(13):4046–4054
- 16 Rivière C, Subra F, Cohen-Solal K, et al. Phenotypic and functional evidence for the expression of CXCR4 receptor during megakaryocytopoiesis. *Blood* 1999;93(05):1511–1523
- 17 Shiraki R, Inoue N, Kawasaki S, et al. Expression of toll-like receptors on human platelets. *Thromb Res* 2004;113(06):379–385
- 18 Keane C, Tilley D, Cunningham A, et al. Invasive *Streptococcus pneumoniae* trigger platelet activation via Toll-like receptor 2. *J Thromb Haemost* 2010;8(12):2757–2765
- 19 Aslam R, Speck ER, Kim M, et al. Platelet Toll-like receptor expression modulates lipopolysaccharide-induced thrombocytopenia and tumor necrosis factor- α production in vivo. *Blood* 2006;107(02):637–641
- 20 D'Attri LP, Etulain J, Rivadeneyra L, et al. Expression and functionality of toll-like receptor 3 in the megakaryocytic lineage. *J Thromb Haemost* 2015;13(05):839–850
- 21 Clark SR, Ma AC, Tavener SA, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med* 2007;13(04):463–469
- 22 Andonegui G, Kerfoot SM, McNagny K, Ebbert KV, Patel KD, Kubes P. Platelets express functional toll-like receptor-4. *Blood* 2005;106(07):2417–2423
- 23 Koupenova M, Vitseva O, MacKay CR, et al. Platelet-TLR7 mediates host survival and platelet count during viral infection in the absence of platelet-dependent thrombosis. *Blood* 2014;124(05):791–802
- 24 Thon JN, Peters CG, Machlus KR, et al. T granules in human platelets function in TLR9 organization and signaling. *J Cell Biol* 2012;198(04):561–574
- 25 Panigrahi S, Ma Y, Hong L, et al. Engagement of platelet toll-like receptor 9 by novel endogenous ligands promotes platelet hyperreactivity and thrombosis. *Circ Res* 2013;112(01):103–112
- 26 Ojha A, Nandi D, Batra H, et al. Platelet activation determines the severity of thrombocytopenia in dengue infection. *Sci Rep* 2017;7:41697
- 27 Baker JV. Chronic HIV disease and activation of the coagulation system. *Thromb Res* 2013;132(05):495–499
- 28 Satchell CS, O'Halloran JA, Cotter AG, et al. Increased platelet reactivity in HIV-1-infected patients receiving abacavir-containing antiretroviral therapy. *J Infect Dis* 2011;204(08):1202–1210
- 29 Mayne E, Funderburg NT, Sieg SF, et al. Increased platelet and microparticle activation in HIV infection: upregulation of P-selectin and tissue factor expression. *J Acquir Immune Defic Syndr* 2012;59(04):340–346
- 30 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
- 31 Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med* 2020;383(02):120–128
- 32 Youssefian T, Drouin A, Massé JM, Guichard J, Cramer EM. 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
- 33 Boukour S, Massé JM, Bénit L, Dubart-Kupperschmitt A, Cramer EM. Lentivirus degradation and DC-SIGN expression by human platelets and megakaryocytes. *J Thromb Haemost* 2006;4(02):426–435
- 34 Banerjee M, Huang Y, Joshi S, et al. Platelets endocytose viral particles and are activated via TLR (toll-like receptor) signaling. *Arterioscler Thromb Vasc Biol* 2020;40(07):1635–1650
- 35 Beck Z, Jagodzinski LL, Eller MA, et al. Platelets and erythrocyte-bound platelets bind infectious HIV-1 in plasma of chronically infected patients. *PLoS One* 2013;8(11):e81002
- 36 Torre D, Pugliese A. Platelets and HIV-1 infection: old and new aspects. *Curr HIV Res* 2008;6(05):411–418
- 37 Solomon Tsegaye T, Gnirß K, Rahe-Meyer N, et al. Platelet activation suppresses HIV-1 infection of T cells. *Retrovirology* 2013;10:48
- 38 Auerbach DJ, Lin Y, Miao H, et al. Identification of the platelet-derived chemokine CXCL4/PF-4 as a broad-spectrum HIV-1 inhibitor. *Proc Natl Acad Sci U S A* 2012;109(24):9569–9574
- 39 Parker ZF, Rux AH, Riblett AM, et al. Platelet factor 4 inhibits and enhances HIV-1 infection in a concentration-dependent manner by modulating viral attachment. *AIDS Res Hum Retroviruses* 2016;32(07):705–717
- 40 Mohan KV, Rao SS, Atreya CD. Antiviral activity of selected antimicrobial peptides against vaccinia virus. *Antiviral Res* 2010;86(03):306–311
- 41 Schrottmaier WC, Salzmann M, Badrnya S, et al. Platelets mediate serological memory to neutralize viruses in vitro and in vivo. *Blood Adv* 2020;4(16):3971–3976
- 42 George JN, Saucerman S, Levine SP, Knieriem LK, Bainton DF. Immunoglobulin G is a platelet alpha granule-secreted protein. *J Clin Invest* 1985;76(05):2020–2025
- 43 Assinger A, Kral JB, Yaiw KC, et al. Human cytomegalovirus-platelet interaction triggers toll-like receptor 2-dependent proinflammatory and proangiogenic responses. *Arterioscler Thromb Vasc Biol* 2014;34(04):801–809
- 44 Chapman LM, Aggrey AA, Field DJ, et al. Platelets present antigen in the context of MHC class I. *J Immunol* 2012;189(02):916–923
- 45 Czapiga M, Kirk AD, Lekstrom-Himes J. Platelets deliver costimulatory signals to antigen-presenting cells: a potential bridge between injury and immune activation. *Exp Hematol* 2004;32(02):135–139
- 46 Barbosa-Lima G, Hottz ED, de Assis EF, et al. Dengue virus-activated platelets modulate monocyte immunometabolic response through lipid droplet biogenesis and cytokine signaling. *J Leukoc Biol* 2020;108(04):1293–1306
- 47 Chaipan C, Soilleux EJ, Simpson P, et al. DC-SIGN and CLEC-2 mediate human immunodeficiency virus type 1 capture by platelets. *J Virol* 2006;80(18):8951–8960
- 48 Chelucci C, Federico M, Guerriero R, et al. Productive human immunodeficiency virus-1 infection of purified megakaryocytic progenitors/precursors and maturing megakaryocytes. *Blood* 1998;91(04):1225–1234
- 49 Crapnell K, Zanjani ED, Chaudhuri A, Ascensao JL, St Jeor S, Maciejewski JP. In vitro infection of megakaryocytes and their precursors by human cytomegalovirus. *Blood* 2000;95(02):487–493
- 50 Li X, Jeffers LJ, Garon C, et al. Persistence of hepatitis C virus in a human megakaryoblastic leukaemia cell line. *J Viral Hepat* 1999;6(02):107–114
- 51 Terada H, Baldini M, Ebbe S, Madoff MA. Interaction of influenza virus with blood platelets. *Blood* 1966;28(02):213–228
- 52 Simpson SR, Singh MV, Dewhurst S, Schifitto G, Maggirwar SB. Platelets function as an acute viral reservoir during HIV-1 infection by harboring virus and T-cell complex formation. *Blood Adv* 2020;4(18):4512–4521
- 53 Baumer Y, Weatherby TM, Mitchell BI, et al. Hiding in plain sight - platelets, the silent carriers of HIV-1. *Platelets* 2020 (e-pub ahead of print). Doi: 10.1080/09537104.2020.1849606
- 54 Zahn A, Jennings N, Ouwehand WH, Allain JP. Hepatitis C virus interacts with human platelet glycoprotein VI. *J Gen Virol* 2006;87(Pt 8):2243–2251
- 55 Larsen JB, Pasalic L, Hvas AM. Platelets in coronavirus disease 2019. *Semin Thromb Hemost* 2020;46(07):823–825

- 56 Sørensen AL, Rumjantseva V, Nayeb-Hashemi S, et al. Role of sialic acid for platelet life span: exposure of beta-galactose results in the rapid clearance of platelets from the circulation by asialoglycoprotein receptor-expressing liver macrophages and hepatocytes. *Blood* 2009;114(08):1645–1654
- 57 Stenberg PE, Levin J, Baker G, Mok Y, Corash L. Neuraminidase-induced thrombocytopenia in mice: effects on thrombopoiesis. *J Cell Physiol* 1991;147(01):7–16
- 58 Isomura H, Yoshida M, Namba H, et al. Suppressive effects of human herpesvirus-6 on thrombopoietin-inducible megakaryocytic colony formation in vitro. *J Gen Virol* 2000;81(Pt 3):663–673
- 59 Gonelli A, Mirandola P, Grill V, Secchiero P, Zauli G. Human herpesvirus 7 infection impairs the survival/differentiation of megakaryocytic cells. *Haematologica* 2002;87(11):1223–1225
- 60 Zapata JC, Cox D, Salvato MS. The role of platelets in the pathogenesis of viral hemorrhagic fevers. *PLoS Negl Trop Dis* 2014;8(06):e2858
- 61 Hottz ED, Oliveira MF, Nunes PC, et al. Dengue induces platelet activation, mitochondrial dysfunction and cell death through mechanisms that involve DC-SIGN and caspases. *J Thromb Haemost* 2013;11(05):951–962
- 62 Hottz ED, Medeiros-de-Moraes IM, Vieira-de-Abreu A, et al. Platelet activation and apoptosis modulate monocyte inflammatory responses in dengue. *J Immunol* 2014;193(04):1864–1872
- 63 Trugilho MRO, Hottz ED, Brunoro GVF, et al. Platelet proteome reveals novel pathways of platelet activation and platelet-mediated immunoregulation in dengue. *PLoS Pathog* 2017;13(05):e1006385
- 64 Wang J, Zhang W, Nardi MA, Li Z. HIV-1 Tat-induced platelet activation and release of CD154 contribute to HIV-1-associated autoimmune thrombocytopenia. *J Thromb Haemost* 2011;9(03):562–573
- 65 Pastori D, Esposito A, Carnevale R, et al. HIV-1 induces in vivo platelet activation by enhancing platelet NOX2 activity. *J Infect* 2015;70(06):651–658
- 66 Page MJ, Pretorius E. A champion of host defense: a generic large-scale cause for platelet dysfunction and depletion in infection. *Semin Thromb Hemost* 2020;46(03):302–319
- 67 Negrotto S, Jaquenod de Giusti C, Rivadeneyra L, et al. Platelets interact with Cocksackieviruses B and have a critical role in the pathogenesis of virus-induced myocarditis. *J Thromb Haemost* 2015;13(02):271–282
- 68 Bik T, Sarov I, Livne A. Interaction between vaccinia virus and human blood platelets. *Blood* 1982;59(03):482–487
- 69 Sottnek HM, Campbell WG Jr, Cassel WA. The pathogenesis of Vaccinia virus toxicity. II. An electron microscopic study. *Lab Invest* 1975;33(05):522–532
- 70 Tymvios C, Moore C, Jones S, Solomon A, Sanz-Rosa D, Emerson M. Platelet aggregation responses are critically regulated in vivo by endogenous nitric oxide but not by endothelial nitric oxide synthase. *Br J Pharmacol* 2009;158(07):1735–1742
- 71 Moore C, Tymvios C, Emerson M. Functional regulation of vascular and platelet activity during thrombosis by nitric oxide and endothelial nitric oxide synthase. *Thromb Haemost* 2010;104(02):342–349
- 72 Bouwman JJ, Visseren FL, Bosch MC, Bouter KP, Diepersloot RJ. Procoagulant and inflammatory response of virus-infected monocytes. *Eur J Clin Invest* 2002;32(10):759–766
- 73 Goeijenbier M, van Wissen M, van de Weg C, et al. Review: viral infections and mechanisms of thrombosis and bleeding. *J Med Virol* 2012;84(10):1680–1696
- 74 Gavrilovskaya IN, Gorbunova EE, Mackow ER. Pathogenic hantaviruses direct the adherence of quiescent platelets to infected endothelial cells. *J Virol* 2010;84(09):4832–4839
- 75 Hottz ED, Lopes JF, Freitas C, et al. Platelets mediate increased endothelium permeability in dengue through NLRP3-inflammatory activation. *Blood* 2013;122(20):3405–3414
- 76 Choi AI, Vittinghoff E, Deeks SG, Weekley CC, Li Y, Shlipak MG. Cardiovascular risks associated with abacavir and tenofovir exposure in HIV-infected persons. *AIDS* 2011;25(10):1289–1298
- 77 Sabin CA, Reiss P, Ryom L, et al; D:A:D Study Group. Is there continued evidence for an association between abacavir usage and myocardial infarction risk in individuals with HIV? A cohort collaboration. *BMC Med* 2016;14:61
- 78 Collado-Díaz V, Andujar I, Sanchez-Lopez A, et al. Abacavir induces arterial thrombosis in a murine model. *J Infect Dis* 2018;218(02):228–233
- 79 Taylor KA, Smyth E, Rauzi F, et al. Pharmacological impact of antiretroviral therapy on platelet function to investigate human immunodeficiency virus-associated cardiovascular risk. *Br J Pharmacol* 2019;176(07):879–889
- 80 Alvarez A, Rios-Navarro C, Blanch-Ruiz MA, et al. Abacavir induces platelet-endothelium interactions by interfering with purinergic signalling: a step from inflammation to thrombosis. *Antiviral Res* 2017;141:179–185
- 81 Khawaja AA, Taylor KA, Lovell AO, et al. HIV antivirals affect endothelial activation and endothelial-platelet crosstalk. *Circ Res* 2020;127(11):1365–1380
- 82 Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. *Nat Rev Immunol* 2013;13(01):34–45
- 83 Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. *Eur Respir J* 2020;55(04):2000607
- 84 Schurink B, Roos E, Radonic T, et al. Viral presence and immunopathology in patients with lethal COVID-19: a prospective autopsy cohort study. *Lancet Microbe* 2020;1(07):e290–e299
- 85 Bradley BT, Maioli H, Johnston R, et al. Histopathology and ultrastructural findings of fatal COVID-19 infections in Washington state: a case series. *Lancet* 2020;396(10247):320–332
- 86 Borczuk AC, Salvatore SP, Seshan SV, et al. COVID-19 pulmonary pathology: a multi-institutional autopsy cohort from Italy and New York City. *Mod Pathol* 2020;33(11):2156–2168
- 87 Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost* 2020;18(04):844–847
- 88 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
- 89 Denorme F, Manne BK, Portier I, et al. COVID-19 patients exhibit reduced procoagulant platelet responses. *J Thromb Haemost* 2020;18(11):3067–3073
- 90 Gu SX, Tyagi T, Jain K, et al. Thrombocytopenia and endotheliopathy: crucial contributors to COVID-19 thromboinflammation. *Nat Rev Cardiol* 2021;18(03):194–209
- 91 Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020;8(04):420–422
- 92 Ciceri F, Beretta L, Scandroglio AM, et al. Microvascular COVID-19 lung vessels obstructive thromboinflammatory syndrome (MicroCLOTS): an atypical acute respiratory distress syndrome working hypothesis. *Crit Care Resusc* 2020;22(02):95–97
- 93 Thwaites RSUrchurtu ASS, Siggins M, et al; on behalf of the ISARIC4C investigators. Elevated antiviral, myeloid and endothelial inflammatory markers in severe COVID-19. *medRxiv* 2020. Doi: 10.1101/2020.10.08.20209411
- 94 Zaid YPuhm F, Allaey I, et al. Platelets can associate with SARS-CoV-2 RNA and are hyperactivated in COVID-19. *Circ Res* 2020;127(11):1404–1418
- 95 Parra-Izquierdo I, Aslan JE. Perspectives on platelet heterogeneity and host immune response in coronavirus disease 2019 (COVID-19). *Semin Thromb Hemost* 2020;46(07):826–830
- 96 Sinha P, Calfee CS, Cherian S, et al. Prevalence of phenotypes of acute respiratory distress syndrome in critically ill patients with

- COVID-19: a prospective observational study. *Lancet Respir Med* 2020;8(12):1209–1218
- 97 Kox M, Waalders NJB, Kooistra EJ, Gerretsen J, Pickkers P. Cytokine levels in critically ill patients with COVID-19 and other conditions. *JAMA* 2020;324(15):1565–1567
 - 98 Leisman DE, Ronner L, Pinotti R, et al. Cytokine elevation in severe and critical COVID-19: a rapid systematic review, meta-analysis, and comparison with other inflammatory syndromes. *Lancet Respir Med* 2020;8(12):1233–1244
 - 99 Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* 2020;323(11):1061–1069
 - 100 Klok FA, Kruip MJHA, van der Meer NJM, et al. Confirmation of the high cumulative incidence of thrombotic complications in critically ill ICU patients with COVID-19: an updated analysis. *Thromb Res* 2020;191:148–150
 - 101 Hanley B, Naresh KN, Roufosse C, et al. Histopathological findings and viral tropism in UK patients with severe fatal COVID-19: a post-mortem study. *Lancet Microbe* 2020;1(06):e245–e253
 - 102 Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020;395(10223):497–506
 - 103 Rapkiewicz AV, Mai X, Carsons SE, et al. Megakaryocytes and platelet-fibrin thrombi characterize multi-organ thrombosis at autopsy in COVID-19: a case series. *EClinicalMedicine* 2020; 24:100434
 - 104 Manne BK, Denorme F, Middleton EA, et al. Platelet gene expression and function in patients with COVID-19. *Blood* 2020;136(11):1317–1329
 - 105 Bury L, Camilloni B, Castronari R, et al. Search for SARS-CoV-2 RNA in platelets from COVID-19 patients. *Platelets* 2020;32(02): 284–287
 - 106 Campbell RA, Boilard E, Rondina MT. Is there a role for the ACE2 receptor in SARS-CoV-2 interactions with platelets? *J Thromb Haemost* 2021;19(01):46–50
 - 107 Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020;181(02):271.e8–280.e8
 - 108 Zhang S, Liu Y, Wang X, et al. SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19. *J Hematol Oncol* 2020;13(01): 120
 - 109 Sahai ABhandari R, Koupenova M, et al. SARS-CoV-2 receptors are expressed on human platelets and the effect of aspirin on clinical outcomes in COVID-19 patients. *Res Sq* [preprint] 2020; rs.3.rs-119031. Doi: 10.21203/rs.3.rs-119031/v1
 - 110 Zini G, Bellesi S, Ramundo F, d'Onofrio G. Morphological anomalies of circulating blood cells in COVID-19. *Am J Hematol* 2020;95(07):870–872
 - 111 Zaid Y, Guessous F, Puhm F, Elhamdani W, et al. Platelet reactivity to thrombin differs between patients with COVID-19 and those with ARDS unrelated to COVID-19. *Blood Adv* 2020;5(03): 635–639
 - 112 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
 - 113 Vadasz Z, Brenner B, Toubi E. Immune-mediated coagulopathy in COVID-19 infection. *Semin Thromb Hemost* 2020;46(07): 838–840
 - 114 Lippi G, Plebani M. Cytokine “storm”, cytokine “breeze”, or both in COVID-19? *Clinic Chem Lab Med* 2020; (e-pub ahead of print)
 - 115 Archambault ASZY, Rakotoarivelo V, Doré E, et al. Lipid storm within the lungs of severe COVID-19 patients: extensive levels of cyclooxygenase and lipoxygenase-derived inflammatory metabolites. *MedRxiv* 2021. Doi: 10.1101/2020.12.04.20242115
 - 116 Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJHLH Across Speciality Collaboration, UK. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020;395(10229):1033–1034
 - 117 Chow JH, Khanna AK, Kethireddy S, et al. Aspirin use is associated with decreased mechanical ventilation, ICU admission, and in-hospital mortality in hospitalized patients with COVID-19. *Anesth Analg* 2020;132(04):930–941
 - 118 Forghani B, Schmidt NJ. Association of herpes simplex virus with platelets of experimentally infected mice. *Arch Virol* 1983;76(03):269–274
 - 119 de Almeida AJ, Campos-de-Magalhães M, Brandão-Mello CE, et al. Detection of hepatitis C virus in platelets: evaluating its relationship to antiviral therapy outcome. *Hepatogastroenterology* 2009;56(90):429–436
 - 120 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(02): 253–262
 - 121 Danon D, Jerushalmy Z, De Vries A. Incorporation of influenza virus in human blood platelets in vitro. *Electron microscopical observation. Virology* 1959;9:719–722