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Review

Platelet and extracellular vesicles in COVID-19 infection and its vaccines

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ABSTRACT

Platelets are at the crossroads between thrombosis and inflammation. When activated, platelets can shed bioactive extracellular vesicles [pEVs] that share the hemostatic potential of their parent cells and act as bioactive shuttles of their granular contents. In a viral infection, platelets are activated, and pEVs are generated with occasional virion integration. Both platelets and pEVs are engaged in a bidirectional interaction with neutrophils and other cells of the immune system and the hemostatic pathways. Severe COVID-19 infection is characterized by a stormy thromboinflammatory response with platelets and their EVs at the center stage of this reaction. This review sheds light on the interactions of platelets, pEVS and SARS-CoV-2 infection and prognostic and potential therapeutic role of pEVs. The review also describes the role of pEVs in the rare adenovirus-based COVID-19 vaccine-induced thrombosis thrombocytopenia.

1. Introduction

The membranes of all viable cells, including megakaryocytes, platelets, red blood, white blood cells and endothelial cell lining, are continuously shedding into the circulation a heterogeneous population of cell-derived extracellular vesicles [EVs]. They circulate under physiological conditions in the blood at a concentration of $> 10^9$/mL, whereas their diameter ranges from approximately 50 nm to 1 μm [1–3]. Local cytoskeletal rearrangement changes result in plasma membrane budding and EVs production. Pathological conditions such as inflammation, coagulation or complement cascade activation and increased shear stress enhance EVs shedding [4].

EVs have a bi-layered phospholipid structure that exposes coagulant-active phosphatidylserine (PS) and expresses various membrane bioactive receptors that interplay with the coagulation, the complement and the immune systems. They serve as cell-to-cell shuttles that transmit signals via their bioactive molecules such as lipids, growth factors, microRNAs, and mitochondria [1]. Described 40 years ago as cell dust, platelet-derived EVs [pEVs] are considered a critical hemostatic response component. As the platelet membrane is highly reactive, pEVs constitute the primary source of circulating, short-half life, procoagulant "microparticles" that also behave as sensors of the hemostatic response [5,6].

Platelets were traditionally recognized only for their central role in hemostasis, but we now know that they also play critical immunomodulatory roles. The presence of chemotactic factors, chemokines, adhesion, and growth factor molecules in their granules and membranes, extended their role beyond the innate immune response through Toll-like receptors (TLRs) and the release of inflammatory cytokines to involve the adaptive immune response through the expression of critical costimulatory and major histocompatibility complex (MHC) molecules capable of activating T cells. These functions are amplified by the vast amounts of pEVs generated when platelets are activated and pEVs have the same hemostatic and immunomodulatory potential [7]. Platelets and pEVs are, therefore, at the crossroad between hemostasis, inflammation, the complement and the immune systems with their various limbs. On the one hand, their role is vital in the defense processes but can potentially degenerate into life-threatening pathological processes on the other [8].

The coagulant response is amplified at the platelet surface as platelets become activated. Similarly, pEVs have negatively charged procoagulant surface and PS that can support the binding of coagulation factors contributing to their increased 50–100-times prothrombinase and tenase complexes generation capacity compared to resting platelets

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[3,9] Through their interactions with membrane surface markers like CD61 + (GPIIIa), pEVs may even reinforce the polymerization and strengthening of the fibrin clot [10]. Furthermore, via their P-selectin (CD62P) receptor, they can interact with the P-selectin glycoprotein ligand-1 (PSGL-1) present on leukocytes [11]. Their interaction is not limited to the one leukocyte subset as pEVs activate and aggregate monocytes and stimulate the release of EVs expressing tissue factor (TF) from these cells [12]. pEVs are a reservoir of biological response modifiers (anti-leukocyte antibodies and lipids) in particular soluble (s) CD40L that can interact with CD40 and prime polymorphenuclear leukocytes inducing endothelial damage [13].

pEVs can be detected by various methods, including physical techniques; flow cytometry, microscopy, dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), and tunable resistive pulse sensing (TRPS) or immunological and procoagulant-based assays or even cellular-based functional and internalization assays [3].

In a biological system, cells convey information to one another via hormones or cytokines. One "instrument" employed to transmit biological information and constituents to specific (target) cells is EVs. The EVs cargo could be DNA, mRNA, microRNA, receptors, metabolites, cytoplasmic proteins, or pathological molecules. Therefore, EVs exert different functions upon endocytosis in recipient cells. Recently, EVs have been unveiled to act as essential participants in various pathologies, including infection and atherogenesis [14]. Together with soluble P-selectin, pEVs correlate with the degree of platelet activation in peripheral arterial disease [15] and other inflammatory conditions. Therefore, pEVs may contribute to disease pathology and can be used as biomarkers reflecting platelet activation.

2. Platelets, pEVs and infection

Platelets are positioned at the nexus of inflammation and host defense. Many bacteria are capable of interacting with platelets, inducing their aggregation. This interaction may be a direct interaction between a bacterial surface protein and platelet receptors like low-affinity type 2 receptor for the Fc portion of IgG, a receptor for immune complexes (FcγRIIA), GPIb, αIIbβ3, TLR2 and TLR4, or may be an indirect interaction enabled by proteins like fibrinogen and von Willebrand factor that binds to the bacterial surface and subsequently to a platelet receptor. Although some secreted bacterial products and toxins like polysaccharides are capable of platelet activation and can cause intravascular platelet aggregation with the generation of pEVs, a secondary co-signal is often needed for the process to occur. Furthermore, platelet granules contain many proteins that modulate the immune response and microbicidal agents [16,17]. They also prime macrophages and monocytes and are critical in recruiting and activating neutrophils, triggering intravascular thrombosis. In addition, migrating platelets can collect and bundle bacteria and exert direct antimicrobial effects [18].

The mechanism through which viral infections induce platelet activation seems more complex as it involves the host microenvironment. The host inflammatory response mediators and the generated antigen-antibody complexes released in a pro-coagulant environment interact with megakaryocytes and activate platelets [18], pEVs are shed with platelet/pEVs-induced immune modulation. These processes explain the noted initial inflammatory reactive thrombocytosis or thrombocytopenia in the context of viral infections, which results from either immune destruction or platelet activation, consumption and removal [19].

Following the H1N1 influenza A virus infection epidemic, it was demonstrated that platelets display thrombin-dependent and independent activation markers. Independently of thrombin, the virus scaffolds with immunoglobulin G (IgG) to form immune complexes that promote platelet activation through stimulation of FcγRIIA or can activate platelets via thrombin formation, independently of complement and FcγRIIA [20].

Approximately 10% of the population is infected by the influenza A virus (IAV) each year. In severe respiratory IAV infection leads to excessive inflammation and tissue pathology. Platelet aggregation and profound neutrophil recruitment, with neutrophil extracellular trap (NET) production and thrombin activation within the microvasculature of the lung, are often noted. Platelets have been shown to induce NET (Netosis) formation, and, in turn, NET components further regulate platelet and neutrophil function. The generation of NET and NET release in the extracellular space can either occur with cell death and membrane damage, "suicidal," or while the structural integrity of the cell is preserved, "vital."

Specific cytokines or some microbial pathogens trigger suicidal NETosis in an NADPH-oxidase-dependent manner, leading to nuclear content release and mixing with the granular and cytosolic proteins. The outer membrane eventually ruptures after 1–4 h releasing NETs into the extracellular space. The vital NETosis, on the other hand, induced by TLR-4-mediated platelet activation and its interaction with CD11a on neutrophils, is characterized by a rapid release of NETs (5–60 min) while preserving the cell integrity. NETosis leads to trapping pathogens and favors their engulfment by phagocytes. NETs also induce endothelial cell activation TF expression through Interleukin-1α and cathepsin G. NETs affect platelet function. Direct evidence demonstrates that the platelet-NET axis is by no means a one-way axis. Thrombin-stimulated platelets, with pEVs shedding, play a critical role in the activation/recruitment of neutrophils' NET release and reciprocally, NETs release recruits more platelet with further pEVs generation directly contributing to lung injury [21].

Interestingly, the amount of generated microparticles, particularly pEVs, has served as disease severity marker and helped guide treatment in patients with certain viral infections like Dengue fever. Not only do pEVs contribute to the pathology, but they also can serve as biomarkers of disease progression and severity [22,23].

3. Platelets, pEVs and EVs in COVID-19

Coronavirus Disease-19 (COVID-19), an acute respiratory illness in humans caused by coronavirus 2 (SARS-CoV-2), can produce severe symptoms and has caused millions of deaths, especially in older adults and those with underlying health conditions. This complex disease with multiorgan effect characterized by severe inflammation and thrombotic risks with multiorgan failure, became pandemic in 2020 [24,25].

Plasmacytoid dendritic cells drive an antiviral response with type I interferon molecule (IFN α and β) production in response to viral infections. They activate cytotoxic natural killer (NK) cells and CD8 + T cells to eliminate infected cells and are associated with secondary solid cytokine production [24]. Granulocytes and monocytes are attracted to the inflammatory milieu and activated, initiating a robust pro-inflammatory pathway related to NETosis. COVID-19 infection includes the secretion of inducers of neutrophil colony formation (GM-CSF 2 and 3) and CXC cytokines, including CXCL8/IL-8, a potent neutrophil chemoattractant. Moreover, NETs display an important procoagulant and prothrombotic activity. Both suicidal and vital NETosis are reported [26].

The platelet–neutrophil interactions are at the center of the pathology of the COVID-19 inflammatory response and multiorgan failure with platelet-neutrophil aggregation, NETosis, activation and pEVs generation mediating its severe pathology. A bidirectional axis is generated, and the platelet aggregates formed due to the NET–platelet interaction bind to neutrophils via glycoprotein Ib (GPIb), further amplifying NETosis [27]. It is now documented that COVID-19 induces a hyperactive phenotype of platelets associated with a dramatic increase in platelet dense granule secretion [28]. The abnormal levels of platelet Factor-4 (PF4/CXCL4) and RANTES/CCL5 and other cytokines and chemokines also attest to platelet activation in severe COVID-19 patients [29,30]. As expected, increased pEV formation has been associated with systemic inflammation in COVID-19 patients [31].

As previously mentioned, TF contributing to thrombogenesis is induced, in part, by NETosis but also in various cell types during viral

infection. TF-positive EVs (TF+EVs) shedding from TF-expressing cells can be assayed using an extracellular vesicle tissue factor (EVTF) activity capture test. As previously demonstrated in animal models, in humans, TF and TF+EVs were observed in the peripheral blood mononuclear cells, platelet-monocyte aggregates, and neutrophils in severely affected COVID-19 patients [32]. EVTF activity and raised D-Dimer correlated with severity, thrombosis, and mortality in COVID-19 patients [32]. Early and intense platelet activation characterizes COVID-19 and contributes, therefore, to the thromboinflammatory manifestations of the disease.

Interestingly, platelets exposed in vitro to SARS-CoV-2 undergo activation. This observation was replicated using SARS-CoV-2 pseudo-viral particles or purified recombinant SARS-CoV-2 spike protein S1 subunits. Human platelets express CD147, a putative co-receptor for SARS-CoV-2. In the presence of anti-CD147 antibodies, spike-dependent platelet activation, aggregation, and granule release and expression of soluble P-selectin and pEVs are suppressed [33].

When the number and surface characteristics of EVs were determined in the plasma of 41 adult PCR positive, COVID-19 patients and compared to the results of 37 sex- and age-matched healthy controls, the number of EVs was significantly higher in patients compared to controls ($p < 0.001$). Patients exhibited substantially higher numbers of pEVs, and EVs derived from endothelial cells (eEVs), leukocytes, or neutrophils than controls. TF-expressing EVs and angiotensin-converting 2 enzyme-positive EVs (ACE2 +EVs) were observed equally in both groups [34].

In another study, platelet activity was assessed by the expression and distribution of HMGB1 and von Willebrand factor and the accumulation of pEVs and HMGB1 + pEVs in the plasma. P-selectin upregulation was not detectable on the platelet surface in 55% of patients; conversely, the concentration of soluble P-selectin was increased in plasma. The plasma concentration of HMGB1 + pEVs at admission was an independent predictor of the clinical outcome [33].

In a detailed longitudinal study, EVs originating from various cells were measured using flow cytometry and phospholipid-dependent clotting time (PPL) in hospitalized COVID-19 patients and trended in association with clinical outcomes within 48 h of hospital admission at discharge and 30 days after that [34]. A broad spectrum of EVs was quantitated and included eEVs, pEVs, leukocyte-derived EVs and TF+EVs, ACE2 +EVs, platelet-derived growth factor receptor-β (PDGF-β)+ and SARS-CoV-2-nucleoprotein (NP)+ were also assayed. From baseline to 30-days post-discharge, significantly decreased plasma concentrations of eEVs (E-Selectin+), endothelium-derived bearing TF (E-Selectin+ TF+), endothelium-derived bearing ACE2 (E-Selectin+ACE2 +) and leukocyte-EVs bearing TF (CD45 +TF+) were observed respectively. In contrast, pEVs (P-Selectin+) and leukocyte-derived EVs (CD45 +), as well as PPL, increased from baseline to 30-days post-discharge. During the observation period, TF+EVs, ACE2 +EVs, PDGF-β + , and SARS-CoV-2-NP+ were unchanged. Interestingly, a cut-off of a P-Selectin + EVs > 1054/μL was associated with thrombotic events ($p = 0.024$) and an E-Selectin + EVs ≤ 531/μL with poor outcome and death (p 0.026). At 30-days P-Selectin+ and CD45 + EVs abnormalities were associated with persistence of the symptoms ($p < 0.0001$) [35].

From all published and observed data, it is evident that aging and the presence of comorbidities confer a worse COVID-19 prognosis. Intimate crosstalk exists between malignancy and the platelets with the generation of pEVs [36,37]. Malignancy, therefore, contributes to the generation of EVs as well as pEVs, and one would expect an amplified EVs response in the context of COVID-19 infection. Twenty-three malignant patients with a positive PCR test were recruited and compared to 19 COVID-19 non-malignant patients and 20 healthy volunteers concerning total EVs, pEVs, eEVs, CD62 activated platelets and CD41 platelet marker. Even though COVID-19 malignant patients had significantly lower platelets counts than COVID non-malignant ones, their total EVs and eEVs were considerably higher, with no significant difference in pEVs between both groups [38]. A considerable accumulation of total EVs, pEVs, eEVs, and activated platelets was observed in COVID-19 - affected patients compared to healthy controls. This highlights the importance and need for prevention in the context of cancer and the value of using biomarkers capable of identifying severe cases.

Since thromboinflammation is the hallmark of severe COVID-19 infection, it often results in T cell exhaustion and lymphopenia. Lymphopenia and its severity levels may serve as reliable predictive factors for COVID-19 clinical outcomes, including mortality, need for intensive care, and oxygen requirements [39]. In a study on PS, a marker of dying cells, activated platelets and pEVs, during the clinical course of COVID-19 infection, Rausch et al. [40] found a high number of blood cells loaded with PS+ pEVs for weeks after the initial COVID-19 diagnosis. PS+ pEVs are preferentially bound to CD8 + T cells interfering with the programmed death-ligand expression rather than memory T cells. The level of these markers correlated strongly with increased disease severity [40]. Other studies have also highlighted the role of vascular cell adhesion molecule 1 and annexin A5 [41].

4. Platelet programmed cell death

Virion internalization, viral RNA sensing, and their consequent impact on platelet function have been studied recently [32,42]. The increased incidence of micro thrombosis with hyperactive platelets sporadically containing viral RNA in COVID-19 infected patients attracted, therefore, the attention of many investigators. Transmission electron microscopy of platelets incubated with purified SARS-CoV-2 virions demonstrated rapid internalization and digestion, leading to distinct morphological changes and release of pEVs. Koupenova et al. characterized the direct SARS-CoV-2–platelet interactions using in vitro studies with purified infectious virions and samples from infected patients and showed the presence of fragmented viral genome in all patients with COVID-19 [43]. Furthermore, platelets interacting in vitro with SARS-CoV-2, SARS-CoV-2 pseudo-viral particles or purified recombinant SARS-CoV-2 spike protein S1 subunits undergo activation [32]. Immunofluorescent imaging of platelets from patients with COVID-19 confirmed the presence of SARS-CoV-2 proteins, whereas there was no detection of viral RNA by real-time quantitative polymerase chain reaction. SARS-CoV-2 elicits unconventional CD147-dependent platelet activation in COVID-19, a process that could be abated in the presence of anti-CD147 antibodies [33]. Platelets, therefore, seem to internalize SARS-CoV-2 virions directly or through the attachment to microparticles. This leads to rapid digestion, programmed cell death, and pEVs release [43].

5. Therapeutic targets

Targeting neutrophils and the neutrophil inflammation-dependent cascade, platelet activation, and aggregates could restrain thromboinflammation and limit COVID-19 consequences. A broad palette of medications including steroids, anti-inflammatory agents, anti-platelet agents, anti-cytokines and anticoagulants are now offered to COVID-19 patients [25].

As it is now clear that platelets and pEVs are at the crossroads of thrombosis and inflammation in COVID-19, inhibitors of platelet function seem to be appealing tools. A retrospective analysis has recently emphasized that among COVID-19–positive patients, pre-diagnosis low-dose aspirin prescription was associated with a 2–fold decrease in 14–day and 30–day overall mortality [44]. P2Y12 receptor inhibitors have been shown to display anti-inflammatory effects in many, and current studies evaluate their benefit in COVID-19.

As many as 40% of patients admitted to the hospital due to COVID-19 have acute kidney injury, with coagulation abnormalities, the leading cause of impaired function. Through inhibition of PS-mediated coagulopathy, early anticoagulation allows maintenance of unobstructed blood circulation [45]. Therefore, many meta-analyses and guidelines

supported the use of anticoagulation and thromboprophylaxis for hospitalized patients with COVID-19 infection [46,47].

The scientific community spared no effort to rapidly develop vaccines in the pandemic. Some of the novel approaches looked into the potential of EVs as potential targets or tools. Ongoing EVs-based strategies for treating COVID-19, including mesenchymal stem cell (MSC)-EVs, drug-EVs, vaccine-EVs, platelet-EVs, and others, have been contemplated and are currently being studied [48].

Furthermore, interfering with SARS-CoV-2 entry into a host cell using soluble or EV-bound seems promising in vitro. Considering their crucial role in COVID-19 pathology, targeting the platelets and their pEVs appears to be a very reasonable approach [25]. In an attempt to eliminate the harmful cytokines and probably the EVs in patients with severe COVID-19 disease, plasma exchange was considered with some success in limited series. Inflammatory cytokine levels (TNF-α, IFN-γ, IL-1, IL-6, and IL-17), and acute-phase reaction proteins including ferritin and CRP had a significant decrease following plasma excahnge courses [49,50]. However, the role of plasma exchange in eliminating pEVs is only hypothetical and has not been studied.

6. pEVs and vaccines

Numerous vaccines have been widely used in nearly half of the world population to combat the pandemic and reduce hospitalization rates. The use of the adenovirus-based vaccines was marked with sporadic but devastating disease. Vaccine-induced thrombotic thrombocytopenia (VITT) resulting from platelet activation and aggregation was reported in rare instances following the first injections of SARS-CoV-2 vaccines (Ad26. COV2. S and ChAdOx1 nCoV-19). This condition, occurring in only a small subset of individuals who produce IgG specific anti-PF4/P, leads to accelerated thrombin generation caused by antigen-antibody complexes consisting of PF4, polyanion (P), and IgG anti-PF4/P-reactive antibodies, linked to a genetic variation of class II HLA [51].

VITT or vaccine-induced immune thrombocytopenia or thrombosis with thrombocytopenia syndrome (TTS) (CDC and FDA nomenclature) is characterized by a triad of venous or arterial thrombosis; mild to severe thrombocytopenia and positive antiplatelet PF4–polyanion antibodies or anti-PF4-heparin antibodies detected by the HIT (heparin-induced thrombocytopenia) ELISA assay. It develops five to 24 days after ChAdOx1 nCoV-19 or Ad26. COV2. S vaccination [52]. As in heparin-induced thrombocytopenia (HIT), PF4 antibodies are thought to activate platelets via binding to the FcγRIIA receptor leading to their aggregation and pEVs shedding [53]. Similar to HIT, and with the contribution of pEVs, the process is associated with an increased risk of thrombosis [54]. The culprit component of the vaccine has not been identified [55]. Nevzorova et al. confirmed that PF4-containing pathogenic immune complexes lead to platelet activation, PS and P-selectin exposure on the outer leaflet of the platelet plasma membrane together with the shedding of procoagulant pEVs expressing PS [56]. In addition, HIT Ab complexes induced TF expression by monocytes and the release of TF-EVs. Therefore, it is likely that the pathogenesis of VITT involves FcγRIIA receptor pathways, the same pathway implicated in SARS-CoV-2 induced thrombogenesis, with circulating PF4 antibodies complexes binding platelets with the release of procoagulant pEVs and direct activation of the endothelium by HIT antibody complexes with enhanced thrombogenicity [55]. The resultant enhanced PS and TF expression, platelet recruitment and pEVs amplification and subsequent thrombin generation are more likely to occur in the cerebral venous system [57]. Interestingly, mRNA-based vaccines do not seem to share these properties and do not seem to confer an increased risk of clot formation [58].

7. Conclusions

COVID-19 caused by SARS-CoV-2 with its thromboinflammatory consequences and mortality has highlighted the need for a better understanding of the crucial role played by the platelets and their pEVs at the crossroads of thrombosis and inflammation. EVs and pEVs will probably be used shortly as prognostic markers that could affect the decision-making process and will likely be used as targets or vehicles to develop future therapeutic tools.

References

[1] Burnouf T, Chou ML, Goubran H, Cognasse F, Garraud O, Seghatchian J. An overview of the role of microparticles/microvesicles in blood components: Are they clinically beneficial or harmful? Transfus Apher Sci 2015;53(2):137–45. https://doi.org/10.1016/j.transci.2015.10.010. Epub 2015 Oct 27.PMID: 26596959.

[2] Simak J, Gelderman MP. Cell membrane microparticles in blood and blood products: potentially pathogenic agents and diagnostic markers. Transfus Med Rev 2006;20:1–26.

[3] Burnouf T, Goubran HA, Chou ML, et al. Platelet microparticles: detection and assessment of their paradoxical functional roles in disease and regenerative medicine. Blood Rev 2014;28:155–66.

[4] Goubran HA, Burnouf T, Stakiw J, et al. Platelet microparticle: a sensitive physiological “fine tuning” balancing factor in health and disease. Transfus Apher Sci 2015;52:12–8.

[5] Morel O, Morel N, Freyssinet JM, Toti F. Platelet microparticles and vascular cells interactions: a checkpoint between the haemostatic and thrombotic responses. Platelets 2008;19(1):9–23. https://doi.org/10.1080/09537100701817232.

[6] Rank A, Nieuwland R, Crispin A, et al. Clearance of platelet microparticles in vivo. Platelets 2011;22:111–6.

[7] Kerris EWJ, Hoptay C, Calderon T, Freishtat RJ. Platelets and platelet extracellular vesicles in hemostasis and sepsis. J Investig Med 2020;68(4):813–20. https://doi.org/10.1136/jim-2019-001195. Epub 2019 Dec 15.PMID: 31843956.

[8] Rolla R, Puricelli C, Bertoni A, Boggio E, Gigliotti CL, Chiocchetti A, et al. Platelets: "multiple choice" effectors in the immune response and their implication in COVID-19 thromboinflammatory process. Int J Lab Hematol 2021;43(5):895–906. https://doi.org/10.1111/ijlh.13516. Epub 2021 Mar 22. PMID: 33749995.

[9] Sinauridze EI, Kireev DA, Popenko NY, Pichugin AV, Panteleev MA, Krymskaya OV, et al. Platelet microparticle membranes have 50-to 100- fold higher specific procoagulant activity than activated platelets. Thromb Haemost 2007;97:425–34.

[10] Zubairov LD, Nabiullina RM, Nagaswami C, Zuev YF, Mustafin IG, Litvinov RI, et al. Circulating microparticles alter formation, structure and properties of fibrin clots. Sci Rep 2015;5:17611.

[11] Burnouf, T., Cognasse, F., Goubran, H. Cell-derived microvesicles/microparticles in blood components: consequences for transfusion recipients. Hematology Education, the education program of the Annual Congress of the European Haematology Association- 2017, June: 11(1).

[12] Jy W, Ricci M, Shariatmadar S, Gomez-Marin O, Horstman LH, Ahn YS. Microparticles in stored red blood cells as potential mediators oftransfusion complications. Transfusion 2011;51:886–93.

[13] Khan SY, Kelher MR, Heal JM, Blumberg N, Boshkov LK, Phipps R, et al. Soluble CD40 ligand accumulates in stored blood components, primes neutrophils through CD40, and is a potential cofactor in the development of transfusion-related acute lung injury. Blood 2006;108:2455–62.

[14] Georgescu A, Simionescu M. Extracellular vesicles: versatile nanomediators, potential biomarkers and therapeutic agents in atherosclerosis and COVID-19-related thrombosis. Int J Mol Sci 2021;22(11):5967. https://doi.org/10.3390/ijms22115967.

[15] Tan KT, Tayebjee MH, Lynd C, Blann AD, Lip GY. Platelet microparticles and soluble P selectin in peripheral artery disease: relationship to extent of disease and platelet activation markers. Ann Med 2005;37(1):61–6. https://doi.org/10.1080/07853890410018943.

[16] Deppermann C, Kubes P. Platelets and infection. Semin Immunol 2016;28(6):536–45. https://doi.org/10.1016/j.smim.2016.10.005. Epub 2016 Oct 18. PMID: 27769639.

[17] Ali RA, Wuescher LM, Dona KR, Worth RG. Platelets mediate host defense against Staphylococcus aureus through direct bactericidal activity and by enhancing macrophage activities. J Immunol 2017;198:344–51.

[18] Nicolai L, Massberg S. Platelets as key players in inflammation and infection. Curr Opin Hematol 2020;27(1):34–40. https://doi.org/10.1097/MOH.0000000000000551.

[19] Assinger A. Platelets and infection - an emerging role of platelets in viral infection. Front Immunol 2014;5:649. https://doi.org/10.3389/fimmu.2014.00649. eCollection 2014. PMID: 25566260.

[20] Boilard E, Paré G, Rousseau M, Cloutier N, Dubuc I, Lévesque T, et al. Influenza virus H1N1 activates platelets through FcgammaRIIA signaling and thrombin generation. Blood 2014;123(18):2854–63. https://doi.org/10.1182/blood-2013-07-515536. Epub 2014 Mar 24. PMID: 24665136.

[21] Kim SJ, Carestia A, McDonald B, Zucoloto AZ, Grosjean H, Davis RP, et al. Release amplifies coagulopathy and drives lung pathology during severe influenza infection. Front Immunol 2021;12:772859. https://doi.org/10.3389/fimmu.2021.772859. eCollection 2021. PMID: 34858432.

[22] Punyadee N, Mairiang D, Thiemmeca S, Komoltri C, Pan-Ngum W, Chomanee N, et al. Microparticles provide a novel biomarker to predict severe clinical outcomes of dengue virus infection. J Virol 2015;89(3):1587–607. https://doi.org/10.1128/JVI.02207-14. Epub 2014 Nov 19. PMID: 25410854.

[23] Patil R, Bajpai S, Ghosh K, Shetty S. Microparticles as prognostic biomarkers in dengue virus infection. Acta Trop 2018;181:21–4. https://doi.org/10.1016/j.actatropica.2018.01.017. Epub 2018 Feb 3. PMID: 29378163.
[24] Puhm F, Flamand L, Boilard E. Platelet extracellular vesicles in COVID-19: Potential markers and makers. J Leukoc Biol 2022;111(1):63–74. https://doi.org/10.1002/JLB.3MIR0221-100R. Epub 2021 Nov 3. PMID: 34730839.
[25] Caillon A, Trimaille A, Favre J, Jesel L, Morel O, Kauffenstein G. Role of neutrophils, platelets, and extracellular vesicles and their interactions in COVID-19-associated thrombopathy. J Thromb Haemost 2022;20(1):17–31. https://doi.org/10.1111/jth.15566. Epub 2021 Nov 9. PMID: 34672094.
[26] Yipp BG, Kubes P. NETosis: how vital is it? Blood. 2013;122:2784–94.
[27] Rossaint J, Kühne K, Skupski J, et al. Directed transport of neutrophil-derived extracellular vesicles enables platelet-mediated innate immune response. Nat Commun 2016;7 (13464).
[28] Comer, et al. provided an elegant demonstration that COVID-19 induces a hyperactive phenotype on platelets associated to a dramatic increase in platelet dense granule secretion. PLOS Biol 2021;19:e3001109.
[29] Gruber CN, Patel RS, Trachtman R, et al. Mapping systemic inflammation and antibody responses in multisystem inflammatory syndrome in children (MIS-C). Cell 2020;183:982–95. e14.
[30] Middleton EA, He X-Y, Denorme F, et al. Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. Blood 2020;136:1169–79.
[31] Zaid Y, Puhm F, Allaeys I, et al. Platelets can associate with SARS-Cov-2 RNA and are hyperactivated in COVID-19. Circ Res 2020;127(11):1404–18.
[32] Mackman N, Grover SP, Antoniak S. Tissue factor expression, extracellular vesicles, and thrombosis after infection with the respiratory viruses influenza A virus and coronavirus. J Thromb Haemost 2021;19(11):2652–8. https://doi.org/10.1111/jth.15509. Epub 2021 Sep 3. PMID: 34418279.
[33] Maugeri N, De Lorenzo R, Clementi N, Antonia Diotti R, Criscuolo E, Godino C, et al. Unconventional CD147-dependent platelet activation elicited by SARS-CoV-2 in COVID-19. J Thromb Haemost 2022;20(2):434–48. https://doi.org/10.1111/jth.15575. Epub 2021 Nov 16. PMID: 34710269.
[34] Traby L, Kollars M, Kussmann M, Karer M, Šinkovec H, Lobmeyr E, et al. Extracellular vesicles and citrullinated histone H3 in coronavirus disease 2019 patients. Thromb Haemost 2022;122(1):113–22. https://doi.org/10.1055/a-1522-4131. Epub 2021 Jun 2. PMID: 34077977.
[35] Campello E, Radu CM, Simion C, Spiezia L, Bulato C, Gavasso S, et al. Longitudinal trend of plasma concentrations of extracellular vesicles in patients hospitalized for COVID-19. Front Cell Dev Biol 2022;9:770463. https://doi.org/10.3389/fcell.2021.770463. eCollection 2021. PMID: 35111751.
[36] Goubran H, Sabry W, Kotb R, Seghatchian J, Burnouf T. Platelet microparticles and cancer: an intimate cross-talk. Transfus Apher Sci 2015;53(2):168–72. https://doi.org/10.1016/j.transci.2015.10.014. Epub 2015 Oct 27. PMID: 26542350.
[37] Goubran HA, Stakiw J, Radosevic M, Burnouf T. Platelet-cancer interactions. Semin Thromb Hemost 2014;40(3):296–305. https://doi.org/10.1055/s-0034-1370767. Epub 2014 Mar 3. PMID: 24590421.
[38] Zahran AM, et al. Circulating microparticles and activated platelets as novel prognostic biomarkers in COVID-19; relation to cancer. PLOS One 2021.
[39] Niu J, Sareli C, Mayer D, Visbal A, Sareli A. Lymphopenia as a predictor for adverse clinical outcomes in hospitalized patients with COVID-19: a single center retrospective study of 4485 cases. J Clin Med 2022;11(3):700. https://doi.org/10.3390/jcm11030700. PMID: 35160150.
[40] Rausch L, Lutz K, Schifferer M, Winheim E, Gruber R, Oesterhaus EF, et al. Binding of phosphatidylserine-positive microparticles by PBMCs classifies disease severity in COVID-19 patients. J Extracell Vesicles 2021;10(14):e12173. https://doi.org/10.1002/jev2.12173. PMID: 34854246.
[41] Garnier Y, Claude L, Hermand P, Sachou E, Claes A, Desplan K, et al. Plasma microparticles of intubated COVID-19 patients cause endothelial cell death, neutrophil adhesion and netosis, in a phosphatidylserine-dependent manner. Br J Haematol 2021. https://doi.org/10.1111/bjh.18019. Online ahead of print. PMID: 34962643.
[42] Bote J, Corkrey HA, Koupenova M. Human platelets and influenza virus: internalization and platelet activation. Platelets 2022;33(2):184–91. https://doi.org/10.1080/09537104.2021.1961710. Epub 2021 Aug 8. PMID: 34369285.
[43] Koupenova M, Corkrey HA, Vitseva O, Tanriverdi K, Somasundaran M, Liu P, et al. SARS-CoV-2 initiates programmed cell death in platelets. Circ Res 2021;129(6):631–46. https://doi.org/10.1161/CIRCRESAHA.121.319117. Epub 2021 Jul 23. PMID: 34293929 1.
[44] Osborne TF, Veigulis ZP, Arreola DM, Mahajan SM, Röösli E, Curtin CM. Association of mortality and aspirin prescription for COVID-19 patients at the veterans health administration. PLOS One 2021;16:e0246825.
[45] Wang C, Yu C, Novakovic VA, Xie R, Shi J. Circulating microparticles in the pathogenesis and early anticoagulation of thrombosis in COVID-19 with kidney injury. Front Cell Dev Biol 2022;9:784505. https://doi.org/10.3389/fcell.2021.784505. eCollection 2021. PMID: 35118071.
[46] Ge J, Ma Y, Wu Z, Jin J, Sun X. Anticoagulation treatment for patients with coronavirus disease 2019 (COVID-19) and its clinical effectiveness in 2020: a meta-analysis study. Medicine 2021;100(47):e27861. https://doi.org/10.1097/MD.0000000000027861.
[47] Cuker A, Tseng EK, Nieuwlaat R, Angchaisuksiri P, Blair C, Dane K, et al. American society of hematology 2021 guidelines on the use of anticoagulation for thromboprophylaxis in patients with COVID-19. Blood Adv 2021;5(3):872–88. https://doi.org/10.1182/bloodadvances.2020003763.
[48] Yan YY, Zhou WM, Wang YQ, Guo QR, Zhao FX, Zhu ZY, et al. The potential role of extracellular vesicles in COVID-19 treatment: opportunity and challenge. Front Mol Biosci 2021;8:699929. https://doi.org/10.3389/fmolb.2021.699929. eCollection 2021. PMID: 34368228.
[49] Hashemian SM, Shafigh N, Afzal G, Jamaati H, Tabarsi P, Marjani M, et al. Plasmapheresis reduces cytokine and immune cell levels in COVID-19 patients with acute respiratory distress syndrome (ARDS). Pulmonology 2021;27(6):486–92. https://doi.org/10.1016/j.pulmoe.2020.10.017. Epub 2020 Dec 4. PMID: 33358260.
[50] Hassaniazad M, Vahedi MS, Samimagham HR, Gharibzadeh A, Beyranvand S, Abbasi H, et al. Improvement of clinical outcome, laboratory findings and inflammatory cytokines levels using plasmapheresis therapy in severe COVID-19 cases. Respir Med 2021;189:106669. https://doi.org/10.1016/j.rmed.2021.106669. Epub 2021 Oct 26. PMID: 34757278.
[51] Bouayad A. HLA and PF4 antibody production after adenoviral vector SARS-CoV-2 vaccination. Curr Res Transl Med 2021;69(4):103312. https://doi.org/10.1016/j.retram.2021.103312. Epub 2021 Sep 17. PMID: 34563798.
[52] Thiele T, Ulm L, Holtfreter S, Schönborn L, Kuhn SO, Scheer C. Frequency of positive anti-PF4/polyanion antibody tests after COVID-19 vaccination with ChAdOx1 nCoV-19 and BNT162b2. Blood 2021;138(4):299–303.
[53] Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination. N Engl J Med 2021;384(22):2092–101.
[54] Campello E, Radu CM, Duner E, Lombardi AM, Spiezia L, Bendo R, et al. Activated platelet-derived and leukocyte-derived circulating microparticles and the risk of thrombosis in heparin-induced thrombocytopenia: a role for PF4-bearing microparticles? Cytom B Clin Cytom 2018;94(2):334–41.
[55] Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT. Thrombosis and thrombocytopenia after ChAdOx1 nCoV-19 vaccination. N Engl J Med 2021;384(22):2124–30.
[56] Nevzorova TA, Mordakhanova ER, Daminova AG, Ponomareva AA, Andrianova IA, Le Minh G, et al. Platelet factor 4-containing immune complexes induce platelet activation followed by calpain-dependent platelet death. Cell Death Discov 2019;5:106.
[57] Marchandot B, Carmona A, Trimaille A, Curtiaud A, Morel O. Procoagulant microparticles: a possible link between vaccine-induced immune thrombocytopenia (VITT) and cerebral sinus venous thrombosis. J Thromb Thrombolysis 2021;52(3):689–91. https://doi.org/10.1007/s11239-021-02505-4. Epub 2021 Jun 15. PMID: 34129181.
[58] Nicholson, M., Goubran, H., Chan, N., Siegal, D. No Apparent association between mRNA COVID-19 vaccination and venous thromboembolism. Under review, 2022.