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INTRODUCTION

In December 2019, hospitals in Wuhan, China, admitted patients with a diagnosis of pneumonia from an unknown etiology, describing a new infection named coronavirus disease 2019 (COVID-19) secondary to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As the pandemic spread, more than half of infected people were found to be asymptomatic or develop mild symptoms with a rapid recovery. Nevertheless, a significant proportion of patients require hospitalization among whom critical cases and death are not rare. The infection has a significant impact on the cardiovascular system and patients with pre-existing cardiovascular disease have an increased risk of developing severe symptoms and death. A majority of admission cases suffered from thromboinflammatory manifestations on admission to the intensive care unit (ICU) including exaggerated cytokine release; profound, progressive hypoxemia; and coagulation abnormalities, leading to thrombotic complications and finally, for the worst cases, multiorgan failure and death. Elevated biomarkers in critically ill COVID-19 patients include C-reactive protein (CRP), procalcitonin, ferritin, erythrocyte sedimentation rate, D-dimers, and many pro-inflammatory cytokines that are elevated or increase during the infection, and for some, highly correlate with fatal outcomes. This clinical picture points out a pathomechanism involving an outburst of the immune system leading to thrombotic manifestations. Coagulation and fibrinolytic parameter abnormalities during COVID-19 encompass elevated D-dimer and fibrinogen and thrombocytopenia, but moderate changes in prothrombin and activated partial thromboplastin time. The COVID-19 pandemic extended all around the world causing millions of deaths. In addition to acute respiratory distress syndrome, many patients with severe COVID-19 develop thromboembolic complications associated to multiorgan failure and death. Here, we review evidence for the contribution of neutrophils, platelets, and extracellular vesicles (EVs) to the thromboinflammatory process in COVID-19. We discuss how the immune system, influenced by pro-inflammatory molecules, EVs, and neutrophil extracellular traps (NETs), can be caught out in patients with severe outcomes. We highlight how the deficient regulation of the innate immune system favors platelet activation and induces a vicious cycle amplifying an immunothrombogenic environment associated with platelet/NET interactions. In light of these considerations, we discuss potential therapeutic strategies underlining the modulation of purinergic signaling as an interesting target.
hematology data also evidence an increase in white blood cell count with a high neutrophil-to-leukocyte ratio (NLR), characteristic of a strong inflammatory process. The analysis of this landscape is coherent with a participation of platelets and neutrophils in COVID-19 immune response, contributing to endothelial dysfunction (ED) and thrombosis, this being particularly pronounced in the pulmonary vascular field.9 The catchall notions of thromboinflammation or immunothrombosis are key in COVID-19. These are hard-to-define concepts that involve a variety of systems and pathways such as CRP, factor (F)XII, complement, neutrophils and neutrophil extracellular traps (NETs), extracellular vesicles (EVs), von Willebrand factor (VWF), thrombin, and cytokines, and make a bridge between inflammation and thrombosis. This review presents data evidencing the cross-talk between neutrophils and platelets, in particular through their EVs, and how they accentuate the thromboinflammatory process in COVID-19. In the light of these considerations, we discuss potential therapeutic strategies that particularly target some of these immunothrombotic pathways.

2 | IMMUNE RESPONSE IN COVID-19; THE CYTOKINE STORM AS A TRIGGER OF THROMBOTIC COMPLICATIONS

Antiviral responses are classically mediated through the type I interferon molecule (IFN α and β) associated with strong cytokine production. The major role of type I IFN is to activate cytotoxic natural killer (NK) cells and CD8+ T cells that eliminate infected cells through the action of perforin and granzyme. In a controlled antiviral response, myeloid cells (granulocytes and monocytes) are attracted and activated by the cytokine secretion/environment. In particular, interleukin 2 (IL-2), an early cytokine produced locally, is responsible for both chemotaxis and activation of neutrophils, initiating a strong pro-inflammatory pathway with occasional NET formation (also called NETosis). In a second step, plasmacytoid dendritic cells (pDCs) drive antiviral response with a robust production of IFNα and β that decreases IL-8 production.10 It is noteworthy that in severe cases of COVID-19, the number of NK, CD8+ T cells as well as pDCs, but also plasmatic levels of IFNα2, were shown to be reduced due to low levels of type I IFN.11 These observations point to a deficient antiviral response in COVID-19 due to decreased T cell number and disproportionate innate activity. In the absence of this efficient adaptive antiviral immune response, the blood neutrophil count stays elevated with sustained pro-inflammatory cytokine concentration. Together with the decrease of lymphocyte count, the NLR is particularly high in severe patients and has emerged as a robust predictor of bad outcomes.12

Efficient antiviral response is associated with the release of pro-inflammatory cytokines to recruit and activate the immune system. Cytokines from which secretion is increased in COVID-19 defining the so-called “cytokine storm” have been reviewed by Costela-Ruiz et al.13 These include an increase of macrophage colony-stimulating factor (M-CSF), granulocyte CSF (G-CSF), and granulocyte-macrophage CSF (GM-CSF) that participate in myeloid cell activation and survival for antigen processing and presentation to T cells. Also increased are the interleukins IL-2, IL-4, IL-13, IL-12, and IL-7 that participate in T cell maintenance, activation, and polarization. IL-4 and IL-13 target Th2 function and favor B cell activation and immunoglobulin production against viral molecules. IL-12 induces Th1 response and IFNγ production. Together, IL-17 and IFNγ maintain a pro-inflammatory state to prime myeloid cells and activate chemokine release and adherence molecules from infected epithelia. IL-10, by contrast, decreases Th1 cell activation and is anti-inflammatory.13,14 Notably, some of these cytokines have been shown to directly or indirectly contribute to exert prothrombotic activity and correlate with bad outcomes.15 In particular, IL-1β, IL-8, IL-6, and tumor necrosis factor alpha (TNF-α), which participate in neutrophil, endothelial cell, and platelet activation and the release of pro-inflammatory molecules, and favor the recruitment of immune cells and in turn thrombosis.

Altogether, this argues in favor of a causal relationship among the immune imbalance (due to insufficient type I IFN production) encountered in COVID-19, a specific cytokine environment =, and the pathological involvement of neutrophils. Many of the above-mentioned cytokines interact, activate, or can be secreted by neutrophils and platelets. Table 1 presents how the main cytokines are produced following neutrophil and platelet activation and participate in their activation in the context of COVID-19.

3 | NEUTROPHILS TO NETS, A BRIDGE FOR THROMBOINFLAMMATION IN COVID-19

3.1 | Neutrophils and NETosis in COVID-19

Neutrophils are the most abundant circulating leukocytes (40–70% of white blood cells) and the first responders to migrate toward the site of inflammation to fight infection and launch the immune response. They neutralize microbial agents through tissue-degrading and microbial-killing hydrolytic proteases and reactive oxygen species (ROS) production. Together with activated epithelium, neutrophils also produce chemokines and pro-inflammatory cytokines to enhance local inflammation and in the case of sustained activation, the release of NETs. In the context of COVID-19, primary pulmonary type-II pneumocyte viral infection initiates the inflammatory response including the secretion of inducers of neutrophil colony formation (GM-CSF 2 and 3) and CXC cytokines (including CXCL8/IL-8) potent neutrophils chemoattractant. This response, coupled with the weak type I IFN production (as detailed above) leads to early neutrophil recruitment and activation.16 If neutrophil involvement in COVID-19 patients is evidenced by an elevated NLR and indirectly by neutrophil-derived cytokines such as IL-8, CCL2/MCP-1 (monocyte chemotactic protein-1), and CXCL10 (Table 1), their excessive, not to say terminal, activation has mostly been evidenced by NET formation.17 NETosis is a kamikaze way of dying for neutrophils,
### TABLE 1  Roles of various mediators on platelets and neutrophils in COVID-19

| Molecules | Secreted by | Roles on: | Pathophysiological effects | References |
|-----------|-------------|-----------|---------------------------|------------|
| **Cytokines** | | | | |
| IL-6 | Myeloid cells, Neutrophils, Macrophages, DCs, Lymphocytes (B, T), EC, Virus-infected cells | + trafficking + neutrophil-mediated pulmonary inflammation (mouse model of colitis) | Abnormalities Thrombogenesis | | 168, 169, 170, 171, 172 |
| TNF-α | Macrophages, Macrophages Lung epithelial cell, T cells | + activation + recruitment + apoptosis (NETs) | | 173, 174, 175, 176 |
| IL-1 | Myeloid cells, Macrophages, Neutrophils, DCs, Epithelial cells, Platelets, EC | + NETs + recruitment | | 177, 178, 179, 180, 181 |
| **Chemokines** | | | | |
| IL-8/CXCL8 | Epithelial cells, EC, Myeloid cells | + recruitment + activation + NETs | ND | | 182, 183 |
| CCL2/MCP-1 | Epithelial cells, Platelets, EC, myeloid cells | + recruitment | + activation | Blocking CCL2 reduces monocyte recruitment and deep vein thrombosis | 184, 185 |
| COVID-19 spikes | | + NETs | + activation | Direct effect of the virus | 18, 63 |

Abbreviations: DC, dendritic cells; EC, epithelial cell; IL, interleukin; NET, neutrophil extracellular trap; mAb, monoclonal antibody; MCP, monocyte chemotactic protein; TNF, tumor necrosis factor.
characterized by degranulation and release of their nuclear content that aims at trapping pathogens and favors their engulfment by phagocytes. Moreover, NETs display an important procoagulant and prothrombotic activity (discussed below). Both suicidal and vital NETosis were reported.\(^\text{19}\) The IL-8-rich and the pro-oxidant environments, typically encountered in COVID-19, promote suicidal NETosis while vital NETosis rather occurs in the case of moderate bacterial infection involving Toll-like receptor (TLR) pathways.\(^\text{19}\)

Suicidal NETosis is initiated by oxidative stress-dependent signaling and leads to activation of peptidylarginine deiminase 4 (PAD4), histone 3/4 citrullination, and nucleosome dismantling.\(^\text{20}\) In vitro studies evidenced that SARS-CoV-2 potentiated ROS and IL-8 production by healthy neutrophils and NETosis, suggesting that the virus triggers neutrophil activation through direct binding.\(^\text{21}\) Corroborating these data, SARS-CoV-2 was shown to infect directly circulating neutrophils through ACE2-serine protease (viral cellular receptor) binding, virus replication, and PAD4 signaling.\(^\text{18}\) Cytokines known to enhance NETosis such as IL-8, IL-1β, TNFα, and IL-6, were all found to be elevated in COVID-19.\(^\text{13}\)

Attesting for NETosis in COVID-19 patients, early papers described an increase in free DNA, myeloperoxidase (MPO)-DNA complex, and citrullinated histone H3 in COVID-19 patients.\(^\text{22}\) Infiltrated neutrophils emitting NETs were found in COVID-19 lung and heart samples after autopsy.\(^\text{23,24}\) It is noteworthy that NET-containing platelet microthrombi were evidenced in pulmonary autopsies of COVID-19 patients.\(^\text{17}\) Importantly, NETs highly correlate with CRP, neutrophil count, and lactate dehydrogenase, three predictors of death at admission and, overall, with COVID-19 severity.\(^\text{5}\) During disease progression, NET markers strongly associate to clinical outcomes, coagulation, fibrinolysis, and inflammatory markers and importantly returned to basal levels in convalescent patients.\(^\text{25}\) Altogether, these data point to a strong prognostic value of neutrophil activation markers in COVID-19 severity that may be explained by their causative role, and this of NETs in particular, in the pathogenesis of acute respiratory distress syndrome (ARDS) on one hand and immunothrombosis on the other.

Neutrophil extracellular traps are released together with proteolytic enzymes such as MPO, proteinase 3 (PR3), neutrophil elastase (NE), and cathepsin G (CG). These enzymes cleave targets at the surface of viruses and bacteria and contribute to permeabilizing epithelial barriers favoring ED, immune cell infiltration, and tissue inflammation.\(^\text{26}\)

Neutrophil activation is also associated with the release of danger-associated molecular patterns (DAMPs) such as ATP and ADP, histones (H3), the chromatin-associated protein high–mobility group box 1 (HMGB1), and S100 protein. Both NETs and DAMPs such as HMGB1 were proposed as valuable targets for treatment strategies to dampen thromboinflammation in the context of COVID-19.\(^\text{27}\)

### 3.2 | NETs and thrombogenicity

Unleashed neutrophil activation and excessive NET formation can worsen the preexisting pathological environment driving ARDS in the lungs, and atherosclerosis and aortic aneurysms in the vascular system. Moreover, NETosis is commonly associated with acute organ failure through promoting thrombosis.\(^\text{28}\) In the microcirculation of patients with a severe form of COVID-19, NET aggregates were found to enter into the composition of occlusive thrombi composed of platelets and neutrophils\(^\text{29}\) and to co-stained with neutrophil granular enzymes.\(^\text{24}\) In a small case series of patients with myocardial infarction, analyses of coronary thrombosis revealed a higher NET density in COVID-19 versus non COVID-19 patients.\(^\text{30}\)

The histology of thrombi did not evidence signs of increased plaque rupture suggesting a role of NETs in the pathogenesis of myocardial infarction during COVID-19. Several other papers reported the causative link existing between NET formation and thrombotic risk in COVID-19 patients.\(^\text{31,32}\)

Activated neutrophils, and NETs in particular, contribute to generate a prothrombotic environment through several mechanisms.\(^\text{28,33,34}\) DNA from NETs constitute a scaffold for a forming venous thrombus in addition to fibrin and VWF.\(^\text{35}\) Negatively charged extracellular nucleic acids favor the assembly of coagulation factors and NETs contribute to enhance both intrinsic and extrinsic coagulation pathways. This is in agreement with an increase in FXIa, α1AT, FIIa, and thrombin/anti-thrombin complex on one hand and tissue factor (TF) on the other hand, according to the severity of the disease.\(^\text{26}\) NETs were shown to directly favor the exposition of TF, the physiological activator of the coagulation cascade.\(^\text{37}\) In addition, a previous paper has demonstrated that NETs may represent an assembly platform for neutrophil-derived EVs resulting in increased thrombin generation through the intrinsic pathway of coagulation in a sepsis model in mice.\(^\text{38}\) In COVID-19 patients, high TF expression was measured in neutrophils and on NETs, this effect being dependent, at least in part, on complement,\(^\text{39}\) which could induce directly thrombosis\(^\text{40}\) and NETosis through C5aR1.\(^\text{39}\) Many studies point to exaggerated platelet activation in severe COVID-19 patients (discussed below). Platelet activation by NETs (and histones) has already been reported in viral influenza infection\(^\text{41}\) or in various pathological contexts such as deep vein thrombosis (DVT),\(^\text{42}\) transfusion-related acute lung injury,\(^\text{43}\) or cancer-associated thrombosis.\(^\text{44}\)

### 4 | ALTERATION OF PLATELET HOMEOSTASIS IN COVID-19

During COVID-19, platelets get activated through multiple mechanisms involving the global inflammatory reaction, the dysfunctional endothelium, altered shear stress, increased viscosity, angiotensin II (AngII), thrombin, or plausible direct viral infection. To ensure hemostasis, platelets aggregate and adhere to diseased endothelial cells and activated leukocytes. Secreting their granules that contain soluble activators (ADP, serotonin, calcium) and adhesive proteins (VWF, thrombospondin, and fibrinogen) platelets grow thrombi by recruiting additional platelets. Activated platelets also enhance inflammation and tissue damage through cytokine release and their interaction with innate immune cells,\(^\text{45}\) including neutrophils.\(^\text{46}\)
Through this interaction, platelets have been shown to promote ARDS, systemic inflammatory response syndrome, multiorgan failure, or immune complex disease, all of which have been related to COVID-19. Hence, platelets seem to contribute to COVID-19-associated thrombopathy not only through the constitution of thrombi but also by feeding an amplification thromboinflammatory loop involving neutrophils. 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. Other parameters and markers advocating for platelet activation in COVID-19 patients include increased P-selectin exposure and platelet-leukocyte aggregation, EV release, depolarization of mitochondrial inner transmembrane potential, and transcriptomic and platelet-leukocyte aggregation, EV release, depolarization of mitochondrial inner transmembrane potential, and transcriptomic

Several cytokines also attest for platelet activation in severe COVID-19 patients such as platelet factor-4 (PF4/CXCL4) and RANTES/CCL5, which are stored in platelet-dense granules. Increase in circulating PF4 in COVID-19 patients has been reported. PF4 deposits in the lungs have been found in autopsy studies in patients with COVID-19 and correlate with increased NETosis and microthrombus formation. The IL-1β, an early key cytokine dependent of the inflammasome pathway, is also dramatically increased in COVID-19 patients. Its production, which has been shown to rely on platelets in preclinical models of venous thrombosis and hypoxia, likely involves platelets also in COVID-19 patients.

In COVID-19 patients, it is likely that platelets are pre-activated owing to the ED. The presence of IL-6 within the cytokine storm enhances the effect of other agonists on platelet aggregation and secretion. While IL-6 alone does not activate platelets in vitro, it potentiates the effect of ADP or TxB2.

Serotonin, co-stored in dense granules, was decreased in platelets while increased in the plasma, attesting here again for increased platelet degranulation in patients. VWF is stored and released from endothelial cells Weibel-Palade bodies and platelet alpha granules upon activation/secretion and is essential for platelet adhesion to the damaged vascular wall. Elevated VWF blood levels (and factor VIII) have been reported in Italian and French cohorts of COVID-19 patients. The VWF causes vascular inflammation because it recruits leukocytes and platelet–leukocyte aggregates at the vascular wall and contributes to the biogenesis of the Weibel-Palade bodies that contain inflammatory players such as P-selectin. Altogether, increase of these cytokines and factors in the plasma of COVID-19 patients reflects platelet degranulation and contributes to inflammation and endothelial dysfunction.

As platelets express several pattern recognition receptors (PRR) and pathogen associated molecular patterns (PAMPs), it has been proposed that they could constitute first-line sentinels in detecting virus in the vasculature. This function includes virus recognition and platelet-dependent cytokine production, such as IL-1β, able to fuel, together with ATP and the NLRP3/inflammasome pathway, innate immune cells and shape/strengthen IL-1β inflammatory response.

Direct viral infection of platelets and/or megakaryocytes was another mechanism suggested to underlie COVID-19–associated platelet activation. The presence of SARS-CoV-2 RNA in platelets has been reported by some authors but not others. Moreover, the expression of SARS-CoV-2 receptors is a matter of debate and...
whether platelets express sufficient ACE2 for SARS-CoV-2 entry has still to be established despite emerging alternative mechanisms of SARS-CoV-2 entry independent of ACE2. According to Zhang et al., the binding of S viral protein to platelet ACE2 is sufficient to induce IL-8, IL-1β, TNFα, and CXCL4/PF4. Additionally, isolated SARS-CoV-2 RNA was found to induce platelet activation providing an alternative direct mode of platelet activation. AngII was also demonstrated to stimulate platelets to express TF. Even if this may likely result from an uptake of TF− EVs, this mechanism may have direct implications in COVID-19 procoagulant state in which AngII levels are increased as a result of the upregulation of the ACE1/ACE2 ratio.

Cytokine storm (Table 1), ED, thrombin generation, complement activation (C3a), increased viscosity, and hypoxia are most likely the key determinants of platelet activation and aggregation in COVID-19. It has recently been reported that IgG fraction of COVID-19 patients triggers platelet activation through Fcγ receptor IIα-dependent signaling (mitochondrial depolarization, cytosolic Ca2+, and phosphatidylserine [PS] externalization). Considering the correlations between apoptotic, procoagulant platelets and D-dimer levels, the authors proposed that antibody-mediated procoagulant platelets contribute to increased thromboembolic risk in COVID-19 patients.

Importantly, platelets also amplify EV emission and TF expression on monocytes via the interaction of P-selectin with P-selectin glycoprotein ligand-1, its counter-receptor on monocytes and neutrophils. Interaction with neutrophils and the dysfunctional endothelium is a critical point for full platelet but also neutrophil activation Figure 1.

5 | ENDOTHELIAL DYSFUNCTION IN COVID-19 AS A TRIGGER OF PLATELET ACTIVATION

The origin of ED reported in COVID-19 seems to be multifactorial. Whether it results from direct endothelial cell viral infection or a secondary event remains unclear. Endothelial activation was evidenced by specific secretory profile (IL-8, VWF, PAI1, soluble thrombomodulin, angiopoietin-2), increased viscosity, and hypoxia. The loss of endothelial cells by apoptosis is associated with a disruption of the endothelial barrier by cleavage of adherent junctions and extracellular matrix by proteolytic enzymes exposing subendothelium to platelets and leukocytes. Even if endothelial TF production is controversial, it has been shown that NETs could directly induce the production of TF as well as adhesion molecule (VCAM-1 and ICAM-1) by the endothelium, through IL-1α and cathepsin G-dependent mechanisms.

6 | EXTRACELLULAR VESICLES

Extracellular vesicles are released by activated leukocytes, platelets, and endothelium. They spread noxious biological functions, inflammation, and coagulation, contributing to tissue damage and thrombosis in many cardiovascular diseases and likely contribute to worsening vascular condition in COVID-19. Before being reliable biomarkers of vascular damage, EVs are essential for blood clot formation by promoting thrombin generation through the exposure of TF and negatively charged phospholipids such as PS. TF allows complexation of coagulation factors VII/VIIa (extrinsic pathway) while the exposure of PS allows tenase and prothrombinase complexes assembly (intrinsic pathway), both pathways ultimately leading to thrombin generation and fibrin clot formation.

Several reports documented an increase in circulating EVs from platelets and granulocytes in COVID-19 patients. Increase in PS− platelet EVs has been associated with systemic inflammation in COVID-19 patients. Contradictory data exist in those patients, some reporting an increase, others a decrease in PS exposure on EVs. Such discrepancies might reveal heterogeneity in patient cohorts. However, considering the dramatic increase in circulating EVs in COVID-19 patients, which is not in doubt, this may result in a net increase in PS and procoagulant phospholipid surface exposure as reported recently.

Compelling data have emphasized a drastic shedding of EVs from leukocytes or platelets harboring TF in COVID-19 patients. It is noteworthy that the main source of blood-borne TF comes from monocytes. It has been proposed that other cells express TF following an incorporation of monocyte-derived EVs through interaction with specific adhesion molecules. TF-bearing platelets and EVs were both found to correlate with plasmatic thrombin generation and the severity of the disease, being higher in COVID-19 patients who require mechanical ventilation. Corroborating these data, new evidence from our group suggested that elevated systemic EV levels are associated with severe pulmonary injury extension (>50% parenchymal extension by CT analysis) and thromboembolic events (Morel et al., in press). At the onset of thrombosis, the accumulation of leukocyte-derived EVs is mandatory because these represent the main source of TF at the vicinity of arterial thrombus.
Beside their direct procoagulant activity, circulating EVs contribute to thromboinflammation through indirect mechanisms. Several studies have emphasized the pro-inflammatory effects of EVs on the vascular wall. Importantly, EVs are able to stimulate the release of pro-inflammatory endothelial cytokines (IL-8, IL-6, and MCP-1) which induce ED, the expression of cyto-adhesins, and diapedesis. EVs were also shown to participate in endothelial senescence leading to an alteration of the vascular condition in a rat model of ischemia reperfusion. EVs enhance platelet adhesion to collagen surfaces and atherosclerotic plaques and lead to monocyte activation and subsequent TF production. Amplification loops may also result from EV-driven cell crosstalk, as suggested in vitro by platelet-derived EV-driven neutrophil aggregation and activation (see Morel et al. for a review). Importantly, platelet EVs were shown to promote NETosis during SARS-CoV-2 infection through TLR2- and CLEC5A-dependent mechanisms. While NET and EV production from activated neutrophils seem to originate independently, they form complexes in which the presence of EVs synergizes with NETs in enhancing neutrophil activation and endothelial adhesion through a HMGB1/TLR pathway.

It is noteworthy that circulating neutrophil EVs constitute a bad prognosis marker for sepsis patients and are responsible of matrix degradation in chronic lung diseases through NE exposure. Altogether these data suggest that EVs likely enhance thromboinflammation in COVID-19 patients and cause major lung dysfunction.

In this context, a drug that reduces the release of EVs and/or their thrombogenic capacity may improve patients' outcomes during severe COVID-19 beyond available current therapeutics (anticoagulants, corticosteroids).

**FIGURE 1** Schematic representation of thromboinflammation that results from the interaction between platelets and neutrophils. On one hand, systemic inflammation induced by SARS-CoV-2 infection is associated with the release of pro-inflammatory cytokines and endothelial dysfunction. On the other hand, increase of innate immune activation without effective adaptive immune system response leads to neutrophil accumulation, which participates in neutrophil extracellular trap (NET) formation and pro-thrombotic mediators' production. Together with platelet activation, extracellular vesicle (EV) release and NET emission neutrophils and platelets aggregate, driving strong and sustained thrombosis. Therapeutic approaches targeting inflammation, NET formation, and platelet aggregation aiming at limiting thromboinflammation are represented in green.

**PLATELET AND NEUTROPHIL INTERACTIONS AS A THROMBOINFLAMMATORY AMPLIFIER**

The platelet–neutrophil interaction may constitute a central loop of amplification of thromboinflammation in COVID-19. Platelet–neutrophil aggregates form in conditions of hemodynamic changes, acute lung injury, renal inflammation, as well as viral infection, all of which are encountered in COVID-19. It has been documented that...
activated platelets enhance NET formation\textsuperscript{114,115} giving a substrate for a thrombotic auto-amplification mechanism. The physical interaction of activated platelets with neutrophils through P-selectin induces platelet-derived HMGB1 which, in turn, triggers NET formation.\textsuperscript{116,117} Platelet aggregates, formed as a result of the NET-platelet interaction bind to neutrophils via glycoprotein Ib (GPIb) further amplifying NETosis.\textsuperscript{118} Complement seems to play multiple roles enhancing NETosis (C5a) and TF release and potentially platelet activation (C3a), which together could induce thromboinflammation.\textsuperscript{119} Interactions between NETs and activated platelets have been reported to enhance procoagulant activity in acute stroke patients with internal carotid artery occlusion.\textsuperscript{120} Activated, necrotic, and apoptotic cells through the release of DAMPs constitute an amplification mechanism that may contribute to the activation of the thromboinflammatory reaction encountered in severe COVID-19 patients. DAMP increase in COVID-19 patients may both originate from and reveal the activation of neutrophils (HMGB1, S100 proteins, cell free nucleic acids, histones [H3]), endothelium, and platelets (ATP/ADP, serotonin, VWF).

Beside this key contribution in the thrombotic outcomes, platelet chronic activation contributes to enhance inflammatory status through the release of DAMP, cytokines, and also procoagulant EV emission. IL-1 induces TxA2 in COVID-19 causing inflammation and microthrombi evidenced by the inhibitory effect of the IL-1 receptor antagonist (IL-1Ra).\textsuperscript{121} In vitro, platelet EVs promote neutrophil adhesion to endothelial cells\textsuperscript{122} and EVs released from activated platelets were shown to carry IL-1β and caspase-1 capable of promoting platelet-neutrophil aggregation.\textsuperscript{123}

8 | THERAPEUTIC OPTIONS

Numerous therapeutic approaches could limit COVID-19 consequences targeting neutrophils and neutrophil inflammation-dependent cascade, platelet activation, and aggregates to restrain thromboinflammation.

8.1 | Using the currently available pharmacopoeia

In many countries, vaccination against COVID-19 is being deployed but its development requires time and SARS-CoV-2 variants could limit its efficacy. Antiviral treatments have so far proved to be relatively ineffective. Hence, various strategies have been tested to limit consequences of severe forms, including inflammation and thrombosis, and decrease morbimortality of COVID-19.

8.1.1 | Anti-inflammatory strategies

In the Randomized Evaluation of COVID-19 Therapy (RECOVERY) trial on hospitalized patients with COVID-19, dexamethasone was associated with a lower 28-day mortality compared to usual care in severe cases requiring mechanical ventilation at inclusion.\textsuperscript{124} To date, it is the only treatment to have demonstrated a reduction of mortality rate during COVID-19. Steroids are commonly used as anti-inflammatory treatments in diseases in which the immune system is abnormally activated.\textsuperscript{125} Their anti-inflammatory effects arise from several pathways resulting in immunosuppressive actions mediated by interference with nuclear factor κB (NF-κB), the key inflammatory transcriptional regulator.\textsuperscript{125} Steroids can curb neutrophil activation at different levels. Indeed, steroids reduce the expression of L-selectin and decrease neutrophil adhesion on the endothelium;\textsuperscript{126} the oxidative stress in neutrophils by lowering superoxide release;\textsuperscript{127} and inflammation as dexamethasone reduces transcription of IL-1β, TNF-α, and IL-8 in neutrophils.\textsuperscript{128} Steroids also have an impact on platelet activation. In a study on patients with immune thrombocytopenia, the positive effect of steroids was in part assigned to a reduction of platelet activation status.\textsuperscript{129} These data may partially explain clinical effects of steroids during COVID-19. However, because the use of steroids is associated with an increased risk of venous thromboembolism,\textsuperscript{130} further studies are required to confirm their efficacy without negative impact.

8.1.2 | Interleukin blocking strategies

Because cytokine secretion plays a prominent role in COVID-19, several interleukin blocking strategies are being investigated. Observational studies showed potential efficacy of anti-IL6, which was associated with an improved survival in patients with severe COVID-19.\textsuperscript{131,132} A randomized, double blind, placebo-controlled trial reported that tocilizumab, a humanized monoclonal anti-IL-6 receptor antibody, had no significant effect on the risk of mechanical ventilation or death in patients with a moderate case of COVID-19.\textsuperscript{133} Nevertheless, the use of IL-6 antagonist tocilizumab and sarilumab in critically ill patients improved outcomes including survival.\textsuperscript{134} Anti-IL6 treatment was associated with reduced D-dimer levels during COVID-19 but to date, no studies have assessed the effect of immunomodulatory pharmacology specifically on thrombosis. On small sample size studies, both the blockade of IL-6 receptor with anakinra\textsuperscript{135} and IL-1β blocking monoclonal antibodies (canakinumab)\textsuperscript{136} were shown to reduce CRP and improve oxygenation associated with survival. Of note, a randomized clinical trial is ongoing to assess the efficacy of anti-IL-8 in patients with COVID-19 (NCT04347226).

All these results must be confirmed on larger cohorts and future studies should evaluate the susceptibility of developing thrombosis in patients taking these treatments.

8.1.3 | Anticoagulation

Therapeutic anticoagulation during hospital stay is associated with improved outcomes among COVID-19 patients.\textsuperscript{6,138,139} Hence, the International Society on Thrombosis and Haemostasis proposed...
to reinforced thromboprophylaxis (twice standard dose) in severe cases or in the presence of thrombotic risk factors.\textsuperscript{140} However, intensified use of anticoagulants exposes patients to bleeding risks.\textsuperscript{141} Several randomized controlled trials are investigating outcomes of COVID-19 patients with different anticoagulation including heparin and direct oral anticoagulants.\textsuperscript{142} In the majority of these trials, anticoagulant intensity is proportional to the severity of COVID-19 and thus the expected thrombotic event rates. Less intensive therapies with oral anticoagulants and prophylactic doses of low molecular weight heparin (LMWH) are used in outpatient and hospitalized patients without a severe form of COVID-19. More intensive therapies with intermediate or therapeutic doses of heparin are tested in patients hospitalized in the ICU with severe forms. Until now, disappointing results have been provided by the INSCRIPTION trial showing no benefit of intermediate versus standard-dose prophylactic anticoagulation in the reduction of thrombotic events, extracorporeal oxygenation, and mortality.\textsuperscript{143} In addition, recent results from REMAP-CAP, ACTIV-4a, and ATTACC trials showed that an initial strategy based on therapeutic dose of heparin improved survival of non-critically ill patients and reduced the use of cardiovascular or respiratory support compared to usual-care thromboprophylaxis\textsuperscript{144} but did not improve outcomes in critically ill patients.\textsuperscript{142}

8.1.4 | Inhibitors of platelet function

A retrospective analysis has recently emphasized that, among COVID-19-positive veterans, pre-diagnosis aspirin prescription was associated with a 2-fold decrease in 14-day and 30-day overall mortality.\textsuperscript{142} Although the results of ACTCOVID19 (NCT04324463) and RECOVERY (NCT04381936) prospective studies are still awaited, these results support the use of aspirin for primary prevention. The P2Y12 ADP receptor inhibitors constitute the most important treatment to fight arterial thrombosis. Rationally, P2Y12 receptor inhibitors have been shown to display anti-inflammatory effects in many conditions including acute coronary syndrome or septic shock.\textsuperscript{145} The results of the RECOVERY (NCT04381936) and ACTIV-4 (NCT04505774) clinical trials aiming at evaluating the benefit of P2Y12 treatment in COVID-19 are particularly awaited. In the context of COVID-19, ticagrelor, a reversible oral P2Y12 inhibitor, may be of particular interest. In the Study of Platelet Inhibition and Patient Outcomes (PLATO), ticagrelor was associated with a superior benefit compared to clopidogrel to prevent fatal thrombosis in patients with acute coronary syndrome.\textsuperscript{146} Compared to clopidogrel, ticagrelor inhibited in a larger extent platelet and leukocyte EV emission.\textsuperscript{147} Finally, ticagrelor was shown to reduce mortality secondary to bacterial lung infection and sepsis in the PLATO study.\textsuperscript{148}

These effects can be attributable, at least in part, to ENT (equilibrative nucleoside transporter)-1 inhibition, which increases local concentration of adenosine.\textsuperscript{149} In turn, adenosine, through A2A receptor activation, limits platelet aggregation and secretion, experts anti-inflammatory effects, and prevents the formation of platelet-neutrophils aggregates. Importantly, adenosine has recently been reported to inhibit emission of NETs in antiphospholipid syndrome.\textsuperscript{150} Altogether these data strengthen the rationale of using ticagrelor in COVID-19 patients. Of importance, the Targeting Platelet-Leukocyte Aggregates in Pneumonia with Ticagrelor (XANTHIPPE) trial demonstrated the clinical benefit of ticagrelor in patients with pneumonia with a significant reduction of NET release, platelet-leukocyte interactions, and IL-6 levels.\textsuperscript{151}

The considerations that increasing adenosine might benefit COVID-19 patients can be transposed to the use of dipyridamole,\textsuperscript{152} which also improves adenosine receptor signaling by its inhibition of nucleoside reuptake. This antithrombotic drug also has an anti-inflammatory and antiviral activity by increasing the synthesis of type I IFN.\textsuperscript{153} A previous study reported beneficial effects of dipyridamole during COVID-19 including inhibition of SARS-CoV-2 replication in vitro, reduction of D-dimer elevation, and improvement of platelets count.\textsuperscript{154}

8.2 | Potential experimental strategies

In addition to efficient vaccines, fast accumulation in the knowledge of COVID-19 pathophysiology allows envisioning new, unconventional—but rational—therapeutic options. Although not discussed in the present review, early interference with SARS-CoV-2 entry in host cell using soluble or EV-bound ACE\textsuperscript{2} or TMPRSS2\textsuperscript{157} seems to give promising results in vitro. Considering the central role of platelets and neutrophils, strategies that aim at reducing their activation to dampen thromboinflammation may be valuable to decrease disease-associated morbidity. Different pathways could be targeted to achieve this goal, from the early cytokine production to DAMP release and other amplification mechanisms such as NETosis (non-exhaustively listed in Table 2).

Neutrophil extracellular traps, as discussed above, play a central role in the thrombotic events associated with COVID-19. Barnes et al.\textsuperscript{23} discussed strategies to target NETosis. Beside inhibition of inflammatory mediators known to be strong inducers of neutrophils such as IL1β, these encompass NE inhibitors, which have undergone phase I, but also several experimental strategies such as DNaseI to dissolve NETs, and PAD4 inhibitors to block their release. Colchicine, which prevents neutrophil recruitment, has been tested in the COLCORONA study and showed significant benefit in terms of reducing hospitalization.\textsuperscript{158} Finally, several larger trials in progress are expected (NCT04326790, NCT04328480, NCT04322565, NCT04322682).\textsuperscript{223}

Extracellular nucleotides (ATP/ADP) are considered as early DAMPs and the rationales to interfere with their signaling in COVID-19 patients are multiple. Extracellular ADP and ATP promote platelet aggregation but also inflammation through purinergic (P2) receptor activation.\textsuperscript{159} Blockade of platelet P2Y12 receptor, especially with ticagrelor (see section 8.1.4 above) may constitute an advantageous strategy to reduce thrombosis. Beyond, P2X7 ATP receptor has been proposed as a valuable
target.\textsuperscript{160} P2X7 receptor is involved in inflammasome complex activation and IL1\textbeta release.\textsuperscript{161} Considering the presumed role of IL1\textbeta-NLRP3 inflammasome axes in COVID-19 pathophysiology\textsuperscript{162} and its proven role in cardiovascular diseases,\textsuperscript{163} blocking the P2X7 receptor with specific antagonists may help to reduce the early runaway inflammatory reaction.

Considering the above-mentioned deleterious effect of ATP and ADP, targeting extracellular nucleotide metabolizing enzymes (ectonucleotidases) may be of particular interest. Ectonucleotidases from the ENTPDase/CD39 family (i.e., enzymes that hydrolyze ATP and ADP) constitute important checkpoints. Through hydrolyzing pro-aggregatory and pro-inflammatory nucleotides into adenosine, these enzymes play a role at the interface between vascular thrombosis and inflammation.\textsuperscript{164} CD39 (1) limits thrombosis by reducing platelet P2 receptor activation, (2) inhibits cytokine secretion (IL-8, IL-1\textbeta) by lowering leukocyte P2 receptor activation, and (3) may decrease the release of TF-bearing EVs by preventing P2X7 activation.\textsuperscript{165} Moreover, following their sequential hydrolysis by CD39 and CD73 (ecto 5\textsuperscript{-}-nucleotidase) adenylc nucleotide hydrolysis leads to the accumulation of adenosine.

Adenosine exerts an inhibitory/protective effect on platelet aggregation through a feedback loop involving A2B receptors. Interestingly, a recent work evidenced a strong inhibition of neutrophil NETosis by adenosine.\textsuperscript{150} Altogether, considering the inhibition of aggregation, NETosis, and the wide anti-inflammatory effect of adenosine,\textsuperscript{166} increasing its bioavailability through nucleotide hydrolysis or the use of synthetic agonists of adenosine receptors provides an excellent rationale in the context of COVID-19 vasculopathy.\textsuperscript{167}

9 | CONCLUSIONS

In COVID-19, immune dysregulation characterized by abnormal INF\gamma drives a strong increase in neutrophil count. In a sustained pro-inflammatory context, neutrophil terminal activation releases huge amounts of proteolytic enzymes and NETs. In parallel, ED and pro-inflammatory molecules contribute to strong platelet activation, favoring NETosis and massive TF\textsuperscript{+} EVs shedding, leading to thrombosis and inflammation propagation. The thromboinflammatory vicious circle involving neutrophils, platelets, and EVs constitutes a privileged therapeutic target to reduce mortality in severe cases of COVID-19. Clinical trials should validate the efficacy of specific strategies aimed at blocking cytokines or platelet activation, and in the latter case, which antiplatelet agent would be the best. A strong rationale exists to target NETosis and EV emission. Further studies are required to validate in particular the inhibition of NETosis as a new strategy and to delineate the role of EVs in endothelial dysfunction and thrombopathy associated with COVID-19.

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CONFLICTS OF INTEREST
The authors have no conflicts to declare.

AUTHOR CONTRIBUTION
AC, GK, and AT wrote the manuscript; OM, JF, and LJM corrected and edited the manuscript.

ORCID
Antoine Caillon https://orcid.org/0000-0003-0803-569X
Antonin Trimaille https://orcid.org/0000-0001-9279-4220
Gilles Kauffenstein https://orcid.org/0000-0003-4994-8305

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