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REVIEW

Thrombosis in Covid-19 and non-Covid-19 pneumonia: role of platelets

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Abstract

Platelets may be a target of bacteria and viruses, which can directly or indirectly activate them so promoting thrombosis. In accordance with this, community-acquired pneumonia (CAP) is complicated by ischemia-related vascular disease (myocardial infarction and stroke) in roughly 10% of patients while the incidence of venous thrombosis is uncertain. In CAP platelet biosynthesis of TxA2 is augmented and associated with myocardial infarction; however, a cause-effect relationship is still unclear as unclear is if platelet activation promotes thrombosis or functional changes of coronary tree such vasospasm. Retrospective studies suggested a potential role of aspirin in reducing mortality but the impact on vascular disease is still unknown. Coronavirus disease 2019 (Covid-19) is complicated by thrombosis in roughly 20% of patients with an almost equivalent localization in arterial and venous circulation. Platelet activation seems to have a pivot role in the thrombotic process in Covid-19 as consistently evidenced by its involvement in promoting Tissue Factor up-regulation via leucocyte interaction. Until now, antiplatelet treatment has been scarcely considered for the treatment of Covid-19; interventional trials, however, are in progress to explore this issue. The aim of this review is 1) to compare the type of vascular diseases complicating CAP and Covid-19 2) to assess the different role of platelets in both diseases and 3) to discuss if antiplatelet treatment is potentially useful to improve clinical outcomes.

Keywords

Antiplatelet treatment, community-acquired pneumonia, Covid-19, platelets, thrombosis

History

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Introduction

Coronavirus disease 2019 (Covid-19) is characterized by severe acute respiratory disease needing mechanical ventilation and intensive care unit (ICU) treatment. Clinical features associated with poor survival include age, sex and comorbidities, such as coronary heart disease, cardiac failure and arrhythmia, atherosclerotic risk factors and chronic obstructive pulmonary disease [1]. Among laboratory variables, elevated D-dimer and troponin levels are associated with death [2,3]. In addition to lung disease, clinical complications of Covid-19 include myocardial damage and ischemia-related vascular disease, which are associated with a hypercoagulable state (e.g. high D-dimer levels) predisposing to thrombotic-related complications and eventually death [4]. It is of note, however, that these clinical features are not exclusive of Covid-19 as they can also occur in other forms of pneumonia, such as community-acquired pneumonia (CAP), which, in fact, is also complicated by vascular diseases occurring essentially in the arterial circulation [5]. Compared with CAP, Covid-19 is complicated by a higher rate of thrombosis with an equivalent distribution in venous and arterial circulation [6,7]. Experimental and clinical studies showed platelet activation in both settings, but the platelet contribution to the thrombotic process is still under investigation. Thus, the aim of this review is 1) to compare the type of vascular diseases complicating CAP and Covid-19 2) to assess the different role of platelets in both diseases and 3) to discuss if antiplatelet treatment is potentially useful to improve clinical outcomes.

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Thrombotic Complications in CAP and Covid-19

Even if it is difficult to establish the prevalent cause of CAP, viruses such as rhinovirus and influenza or bacteria such as streptococcus pneumoniae seem to play a major role [8]. A variety of viruses may bind to platelets via several receptors, in particular Toll-like receptors (TLRs), which may elicit platelet activation and eventually favor the occurrence of thrombosis-related cardiovascular events [5]. Thus, viral single-stranded RNA viruses, such as influenza virus, have been found inside platelets where they may activate them via binding to the intracellular receptors TLR7/8 or TLR3 [9,10].

Extensive studies have been done in patients with CAP and consistently demonstrated an association between CAP and cardiovascular disease. Using CK-MB as marker of myocardial necrosis Corrales-Medina et al. were the first to show that CAP is complicated by myocardial infarction in roughly 4% of patients [11]. Using troponin as marker of myocardial necrosis we confirmed such association, which, however, appeared much higher (roughly 10%); of note, most MI complicating the clinical course of CAP were non-ST-segment elevation myocardial infarction (NSTEMI), and were not associated with chest pain [12]. Also, a large number of CAP patients (>50%) displayed elevated levels of troponin not related to EKG changes suggesting the coexistence of myocardial injury not related to coronary disease [12]. The association between CAP and cardiovascular disease was further confirmed in a large, prospective,

multicenter study conducted in >1000 patients where myocardial infarction (MI) and stroke occurred in 11% of CAP patients [13]. The clinical relevance of such complication was corroborated by an increased short- and long-term mortality risk in patients experiencing vascular events during the hospitalization [14,15].

As far as venous thrombosis is concerned, literature data are equivocal. Retrospective studies reported a consistent association between venous thrombosis, defined as deep venous thrombosis (DVT) or pulmonary embolism (PE), and CAP [12,16–18]; such association was recently reinforced by Mei et al. [19] reporting a rate of venous thrombosis in 360 CAP patients even higher (>3% during hospitalization) than that detected in Covid-19 ones. However, lack of information regarding diagnostic work-up and clinical presentation (symptomatic versus asymptomatic) makes difficult the correct analysis of the results. In contrast with these reports, a prospective investigation of CAP patients showed absence of symptomatic venous thrombosis in CAP during the intrahospital stay [13,20]; it should be underscored, however, that the cohort included patients hospitalized in internal medical wards and none of them requested intensive care unit (ICU); thereby, the relationship between CAP and venous thrombosis needs to be further investigated.

Taken together these data indicated that CAP is complicated by ischemia in the coronary and cerebral circulation [13], but it is unclear if thrombosis plays a major role. The fact that in the majority of cases coronary ischemia is characterized by type II MI suggests that mechanisms other than thrombosis such as coronary vasospasm may have a pivotal role and a mismatch between oxygen demand and supply could be a key factor precipitating coronary ischemia.

Early analysis of thrombotic complications of Covid-19 outlined venous thrombosis as the more prevalent thrombotic complication. Mackman et al. analyzed 15 studies from different countries and showed that venous thrombo-embolism was the most frequently reported vascular complication with a rate, however, very large, i.e. from 0.9% to 69%. This large variability may depend upon several factors including small sample size and overall inclusion of patients with different degrees of severity, being, for instance, ICU patients more prone to experience venous thromboembolism (VTE) [21]. Conversely, the rate of arterial thrombosis was scarcely represented with an incidence of 2.8–3.8% [22]. This apparent discrepancy between venous and arterial thrombosis was disproved by a study in a small series of 73 patients, of whom 17 (23%) experienced an almost similar distribution of venous and arterial thrombosis [7]. This finding was confirmed and extended in a larger prospective study including 3,334 patients, who experienced 533 (16%) thrombotic events during the hospitalization; 207 (6.2% were venous; 3.2% PE and 3.9% DVT) and 365 (11%) were arterial (8.9% MI, 1.6% stroke and 1% thromboembolism) [6]. Among the laboratory variables elevate D-dimer was more frequently detectable in ICU patients and independently associated with mortality whatever was the site of thrombosis [6].

It seems, therefore, that CAP and Covid-19 display different rate and features of vascular disease, being CAP prevalently complicated by arterial disease while venous and arterial thromboses are consolidated features of Covid-19; the exact role of thrombosis in ischemia-related vascular disease of CAP should be further investigated. The mechanism accounting for thrombosis must be further elucidated but it is conceivable that in the case of venous thrombosis infection may affect the classic Virchow's triad, i.e. endothelial dysfunction, hypercoagulability and venous stasis; thus, the last guidelines enlisted infective diseases as confirmed risk factors for PE/DVT [21].

Platelet Activation in CAP

Based on the association between CAP and ischemia-related arterial disease, experimental studies have been addressed to explore the role of platelets in the pathogenesis of MI and stroke. Thus, markers of in vivo platelet activation such as plasma *P*-selection and CD40 Ligand (CD40L) were found elevated in CAP compared to controls, particularly in CAP patients with MI [15]. These changes were suggested to be dependent on platelet activation as depicted by over-biosynthesis of Thromboxane $(Tx)B_2$; of note, multiple regression analysis showed that serum TxB_2 was independently associated with MI [12]. Enhanced ex vivo platelet aggregation and thrombocytosis have been also detected in patients with CAP, further supporting the interplay between platelets and infections [23].

Inhibition of platelet activation and its impact with clinical outcome have been explored in a retrospective study including patients with sepsis/CAP, where aspirin use was associated with lower mortality [24]. A similar effect, even if more modest, was confirmed in a recent meta-analysis of ten cohort studies including roughly 700,000 patients with sepsis, revealing that aspirin may reduce admission to ICU or mortality [25]. Taken together, these findings indicate that CAP is associated with platelet activation but the extent to which antiplatelet treatment is able to lower MI and stroke remain to be ascertained.

The exact trigger of platelet activation in CAP is still unclear. As CAP is prevalently due to influenza A virus, a direct platelet interaction between influenza virus and platelets could be a putative mechanism (see below). Alternatively, platelet activation may be triggered by bacteria if CAP is not of viral origin; thus, Gram-positive and Gram-negative bacteria may directly bind to platelets via a bacterial surface protein or indirectly via a plasma-bridging molecule [5]. The interaction between bacteria and platelets is functionally relevant as it is associated with platelet activation or formation of platelet-neutrophil complexes [5]; however, its specific impact in the thrombotic process of CAP is still to be determined. It is of interest, in this context, the interplay between the lipopolysaccharides (LPS) of Gram-negative bacteria and platelets, which results in over-production of platelet eicosanoids [26]. Of note, LPS per se are unable to activate platelets when used at concentrations detected in patients with CAP but amplify the response of platelets to common agonists [26]. Interestingly, elevated levels of LPS are elevated in CAP but it still unclear if this is related to lung infection or gut permeability-relate LPS translocation from gut to systemic circulation [27].

Platelet Activation and Thrombosis in Covid-19

Platelet function has been more extensively studied in Covid-19 compared to CAP. A reduced platelet count, approximately 100,000 /μL, was found in 5–18% of Covid-19 population, occurring prevalently in case of severe disease and, in some cases, was associated with poor survival [28]. Analysis of ex vivo platelet aggregation (PA) and assessment of platelet interaction with leucocytes are summarized in Table I and are suggestive of platelet over-activation. Thus, Manne et al. [30]. reported enhanced PA, platelet TxB_2 biosynthesis and platelet spreading on fibrinogen and collagen compared to controls; this behavior was more evident in patients admitted to ICU versus non-ICU and suggested to depend on enhanced phosphorylation

Table I. In vitro evidences of Covid-19 influence on platelet activation.

References	Patients cohort	Platelet aggregation	Markers of platelet activation	Platelet-leukocyte aggregates	Cells from HS incubated with plasma from COVID-19 patients or Spike protein	Platelet down-stream signaling
Manne et al. [30]	41 Covid-19 17 HS	↑ in response to low-dose of: Thr, Coll and 2MeSADP ↔ in response to high-dose of agonists **More evidence in ICU patients**	↑ *P*-selectin expression ↑ PDGF levels ↑ TxB2 **More evidence in ICU patients**	↑ Platelet-neutrophil, platelet-monocyte and platelet–T-cell aggregates		Activation of MAPK-signaling: ↑ ERK1/2, p38, and eIF4E phosphorylation ↑PLA2 phosphorylation
Zaid et al. [32]	115 Covid-19	↑ in response to Thr	↑sCD40L ↑ TxB2			↑PKC-δ phosphorylation
Hottz et al. [29]	41 Covid-19 11 controls		↑ *p*-selectin and CD63 expression ↑ TxB2 **More evidence in ICU patients**	↑ Platelet-monocyte aggregates ↑ Monocyte TF expression **More evidence in ICU patients**	HS platelets + Covid-19-derived pasma: ↑ *P*-selectin-mediated platelet activation	
Zhang et al. [32]	422 Covid-19 201 HS	↑ in response to: Thr, Coll and ADP after incubation with Covid-19	↑ *P*-selectin and integrin αIIbβ3 expression **More evidence in ICU patients**	↑Platelet-Leukocytes	Spike protein dose-dependently: ↑ Platelet aggregation, ↑ PAC-1 binding, ↑CD62P expression ↑ Leukocyte–platelet aggregates.	↑ ACE2 degradation Activation of MAPK-signaling: ↑ ERK1/2, p38, and JNK phosphorylation
Skendros et al. [39].	25 Covid-19 10 HS			↑Platelet-Leukocytes	HS Neutrophils + Covid-19-derived PRP: ↑TF mRNA ↑NETs formation ↑ Complement activation	

Abbreviation list: 2-Methylthio-Adenosine-5′-Diphosphate (2MeSADP); Angiotensin Converting Enzyme 2 (ACE2); Collagen (Coll); Coronavirus Disease 2019 (COVID-19); Eukaryotic Translation Initiation Factor 4E (eIF4E); Extracellular Signal-regulated Kinases (ERK1/2); Healthy Subjects (HS); Intensive Care Unit (ICU); Mitogen-activated Protein Kinase (MAPK); Mitogen-activated Protein Kinase (MAPK); Neutrophil Extracellular Traps (NETs); *P*-selectin Glycoprotein Ligand-1 (PSGL-1); Phospholipase A2 (PLA2); Platelet Derived Growthg Factor (PDGF), Platelet Rich Plasma (PRP); Protein Kinase C (PKC-δ); Messenger RNA (mRNA);Severe Acute Respiratory Syndrome Coronavirus 19 (SARS-CoV2); Thrombin (Thr); Thromboxane B2 (TxB2); Tissue Factor (TF).

of PLA_2 [30]; in accordance with this, Zaid et al. reported enhanced platelet activation in both severe and non-severe Covid-19 [31]. Using other assays of ex vivo platelet activation, Hottz et al. [29] and Zhang et al. reported platelet surface overexpression of CD62P(*P*-selectin) and CD63, markers of platelet alpha and dense granule secretion respectively, and overactivation of glycoprotein(Gp) IIb/IIIa [32]; these changes appeared to be more marked in patients with severe disease. The relationship between Covid-19 and platelet activation was corroborated by other experiments, where platelets incubated with plasma from patients with Covid-19 or Spike protein displayed increased PA, over-expression of *P*-selectin and CD63, activation of GpIIb/IIIa, enhanced platelet spreading on fibrinogen and platelet-related thrombus overgrowth [32].

Platelet interaction with leucocyte has been another aspect of thrombogenesis which has been investigated in Covid-19. Thus, platelet-leucocyte interaction contributes to thrombosis via leucocyte migration, secretion, extrusion of neutrophil extra-cellular traps (NETs) and eventually up-regulation of Tissue Factor (TF), which acts as cofactor of the coagulation protease FVII/VIIa and to convert FX to FXa [33]. Increased platelet-leucocyte aggregates have been reported in patients with Covid-19 resulting in over-expression of monocyte TF compared to Covid-19 monocytes alone or incubated with platelets from healthy donors [29,30,32]. These findings were corroborated by experiments where TF production by platelet-monocytes aggregates was inhibited by an anti-*P*-selectin neutralizing antibody or abcximab, an antibody against platelet Gp IIb/IIIA commonly used in patients with acute coronary syndrome [29]. Of note, aspirin or clopidrogel did not change the rate of platelet-monocyte aggregates or platelet-induced monocyte TF formation [29].

These data, however, should be wisely considered as among the blood cells monocytes seem to be the only one expressing TF [34]. The negative impact of leucocyte activation in the thrombotic process of patients with Covid-19 has been strengthened by autoptic studies that revealed a high prevalence of microthrombi in alveoli and pulmonary capillaries of patients died from Covid-19, where a massive presence of leucocytes was detected [35–37]. As mentioned above, the interaction between platelets and leucocytes may lead to the formation of NETs, which comprise of DNA and histones and are released upon neutrophil stimulation by patterns recognition receptors or chemokines; such release requires the formation of reactive oxidant species (ROS) and calcium mobilization which activate protein arginine deaminase 4(PAD4) to deaminate arginine residues on histones [38]. Besides antimicrobial activity, NETs possess procoagulant activity by expressing TF; autoptic studies in patients with Covid-19 demonstrated the presence of platelets, neutrophil and NETS in the lung and in structures consistent with blood vessels, suggesting that platelet-neutrophil interaction may lead to NETs formation and eventually thrombosis [35–37]. In accordance with this, neutrophils from patients with Covid-

19 are more prone to produce NETs and over-express TF; a significant correlation between circulating NETs and thrombin-antithrombin complexes was detected in the blood of patients with Covid-19 [39]. Furthermore, in vitro experiments showed that platelet-rich plasma from subjects with Covid-19 co-incubated with control neutrophils increased the levels of TF mRNA and generated NETs expressing TF [39]. The thrombogenicity of platelet-neutrophil interaction was blocked by an inhibitor of thrombin and C5a complement factor suggesting a complex network of immune-thrombogenesis where platelets and leucocytes may be activated by complement and thrombin [39]. Further evidence in favor of this hypothesis was the presence of elevated blood levels of PF4 and RANTES, two platelet factors that trigger NETosis, in the blood of Covid-19 patients [40]; however, we cannot exclude that NETs formation can be triggered by Covid-19 independently from platelet-leucocyte interaction as exposure of leukocytes to Covid-19 antigen directly activated leukocytes to release NETs via interaction with ACE2 [32]. Furthermore, we cannot exclude that other leucocytes such as eosinophils may also contribute to the thrombotic process. Thus, eosinophils participate to the thrombotic process by interacting with platelets, giving formation of eosinophil extracellular traps, which are detectable in human as well as experimental thrombosis in animals [41]. The above reported mechanisms are summarized in Figure 1.

While retrospective and prospective studies consistently demonstrated a close association between clotting activation and poor outcomes including thrombotic event and death in Covid-19, data regarding the impact of platelet activation on clinical outcomes are scarce. Barrett et al. [42] reported data on several markers of in vivo platelet activation in 100 patients affected by Covid-19, who experienced thrombosis or death in 34 cases; according with previous reports thrombotic events were almost equally distributed between arterial and venous circulation (eight VTE, five MI, one VTE and MI). Among the platelet biomarkers, soluble CD40L, *P*-selectin and serum TxB2 were independently associated to thrombosis or death. When considering thrombosis separately, only TxB_2 was independently associated with thrombosis; the association between TxB_2 and both clinical outcomes persisted also after excluding patients on aspirin treatment. Even if interesting, these data should be considered preliminary and warrant further analysis to elucidate the relationship between platelet activation and thrombosis in Covid-19 patients. Figure 1 synthetizes the above reported interactions between Covid-19 and platelets.

Mechanism of Disease

That influenza virus may directly activate platelets has been demonstrated by Koupenova et al., who found influenza A virus particles within platelets and a virus-mediated platelet aggregation and C3 release-dependent neutrophil-DNA release and aggregation via Toll-like receptor 7 (TLR7) [43]. These findings demonstrated that platelet-leucocyte cross-talk is an important defense mechanism against virus influenza A but may be potentially dangerous for its implication with thrombotic process [43].

These data were reinforced by experiments in animal model of mice infected with the H1N1 viruses. These studies evidenced that antiplatelet drugs such as the COX1 inhibitor aspirin or the antagonist of the P_2Y_{12} platelet receptor clopidogrel were able to inhibit virus induced platelet activation [44,45].

Recent studies provided further support to this report as Zhao et al. and Choudhury et al. [46,47] showed that Covid-19 uses TLR4 to activate cells implicated in the thrombotic process such

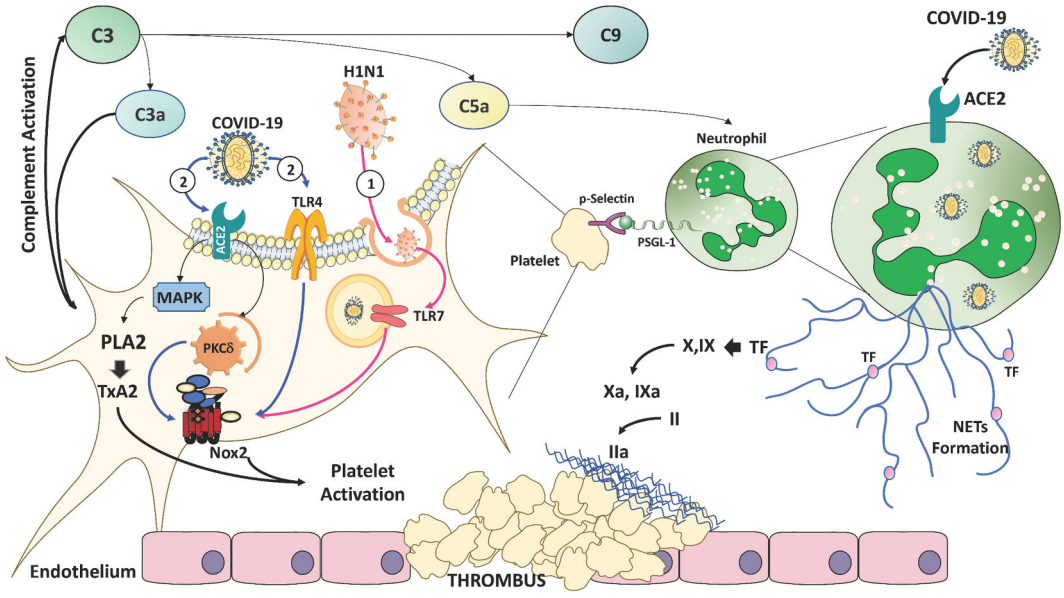

Figure 1. Hypothetic mechanisms of platelet activation in Covid-19 and CAP patients.

In CAP patients Influenza A virus may activate platelets via TLR7-mediated Nox2 activation (1). Coronavirus 19 can entry in platelets by ACE2 binding or by platelet endocytosis so activating TLR4 (2). Intracellular signaling would lead to *P*-selectin surface expression and complement C3 release from α-granules. *P*-selectin mediates platelet-leukocyte interactions, primarily with neutrophils. C3 enhances neutrophil DNA release (C3-mediated NETosis) and ensures capture and removal of virus. Moreover, the signaling downstream of the ACE2 receptor (2) would induce the activation of platelet MAPK and PKCδ, which are responsible, in turn, for platelet activation by TxB2 biosynthesis and Nox2 activation, respectively. (Covid-19 = Coronavirus 19, CAP = community-acquired pneumonia, ACE2 = angiotensin converting enzyme 2, Ang II = angiotensin converting enzyme II; MAPK = mitogen-activated protein kinase; NETs = neutrophil Extracellular Traps; PLA2 = phospholipase A2; PSGL-1 = *p*-selectin glycoprotein ligand-1; TLR7 = Toll-Like Receptor 7; TLR4 = Toll-Like Receptor 4; TxA2 = Thromboxane A2).

as monocytes and leucocytes. Covid-19 binding to TLRs may have deleterious effect for its implication in thrombotic process via up-regulation of Nox2, the most important cellular producer of reactive oxidant species (ROS), resulting in endosomal hydrogen peroxide generation [26,48]. Nox2 is a key enzyme of the innate immune system, which, intriguingly, is also localized in endothelial cells and platelets [49,50]. Investigation of patients with chronic granulomatous disease, a rare disease characterized by hereditary deficiency of Nox2, demonstrated that Nox2 is a powerful vasoconstrictive molecule likely via inactivation of nitric oxide(NO) [51] and favors platelet activation via production of F2-isoprostanes and inactivating NO [50]; recent studies confirmed a role for Nox2-mediated platelet activation in an experimental model of thrombosis [52]. Analysis of circulating levels of soluble Nox2, which is a marker of Nox2 activation by blood cells including platelet and leucocytes [50], revealed that Nox2 activation occurs not only in CAP but also in Covid-19 [53,54].

In the case of Covid-19, however, an alternative mechanism may be proposed for explaining platelet activation. Thus, the virus entry into the cells occurs by its binding to angiotensin converting enzyme 2(ACE2) upon its Spike protein cleavage by a serine protease, i.e. TMPRSS2 [32]. Covid-19 RNA has been detected in platelets from patients with severe and non-severe Covid-19 but it is still unclear if this occurs via the Spike protein-ACE2 axis (Figure 1) as not all agree that platelets actually express ACE2. Thus, while Zaid et al. [31] and Manne et al. [30] failed to detect platelet ACE2 in samples from healthy subjects or patients with Covid-19, while Zhang et al. reported that platelets express ACE2 and TMPRSS2 and that the interplay between Covid-19 or its Spike protein with ACE2 results in platelet activation [32]. In support of this, platelet pre-treatment with ACE2 protein or anti-Spike protein inhibited Covid-19-potentiated platelet aggregation, reversed Covid-19 and Spike protein-induced PAC-1 binding and CD62P expression and prevented Covid-19 or Spike protein-accelerated platelet spreading on fibrinogen [38]. The Spike protein-Ace2 axis may have detrimental effect on platelet activation. Thus, spike protein binding and entry into human cells results in surface ACE2 expression downregulation and loss of function and is accompanied by angiotensin II (AngII) up-regulation as ACE2 degrades Ang II to Ang 1–7 [55]. The ensuing elevated serum levels of Ang II [56,57] could have potentially deleterious effects as Ang II is implicated in artery dysfunction via Nox2-mediated ROS overproduction [58] and play a direct role in platelet activation [59,60] Of note, Nox2 is more activated in patients with Covid-19 versus controls, in severe versus non severe disease and in patients experiencing thrombotic events [53].

In alternative to this mechanism RNA viruses could activate platelets when antibodies opsonize viral particles and interact with platelet FcyRIIA [43]; however, they would be expected to work at later stage of the disease, thereby their potential impact on platelet activation and thrombosis in patients with Covid-19 needs to be elucidated (Figure 1).

Finally, platelet activation can occur via overproduction of inflammatory pro-aggregating cytokines such as, for example, TNF alpha [61], which, in fact is elevated in patients affected by Covid-19 [62]. The fact that, plasma from patients with Covid-19 incubated with normal platelets enhances the platelet response to agonists or the interaction between platelets and leucocytes could support this hypothesis (Table I).

Down-stream signaling eliciting platelet activation consistently showed a role of MAPK pathway up-regulation, which is relevant for platelet activation via phospholipase A_2 activation and eventually TxA_2 formation (Figure 1). Also, Zaid et al. reported enhanced phosphorylation of the PKC delta, which is a key regulator of PA via Ca mobilization, in thrombin-stimulated platelets from patients with severe and non-severe Covid-19 [31].

In this context, it is worthwhile to underline the important role played by PKC in the platelet formation of ROS via activation of Nox2, reinforcing the potential role of oxidative stress as an important mechanism accounting for platelet activation in Covid-19.

Therapeutic Implications

While the effects of aspirin on clinical outcomes of CAP patients have been investigated providing preliminary results on its potentially usefulness to lower mortality, data regarding the efficacy of antiplatelet drugs in Covid-19 are scarce. Chow et al. [63] studied 420 Covid-19 patients, 314 (76.3%) aspirin-free and 98 (23.7%) on aspirin within 24 hours of admission or 7 days prior to admission. After adjusting for eight confounding variables, aspirin use was independently associated with decreased risk of mechanical ventilation (−46%), ICU admission (−43%) and in-hospital mortality (−47%) while no differences in major bleeding thrombosis were detected between aspirin users and non-users [63]. The small sample study and the retrospective nature of the study does not allow definite conclusions.

While we are not aware of clinical trials investigating the effect of anticoagulants (AC) to prevent vascular disease in CAP, AC have been given as prophylactic or intermediate-full dosage in Covid-19; low-molecular weight heparin (LMWH) was the anticoagulant more extensively used. The impact of AC on clinical outcomes of Covid-19 seems to favor the use of AC but there are several still open issues regarding the dosage and, overall, the clinical setting, for instance ICU versus non ICU, where one of the two dosages should be preferred. A recent consensus from the American Society of Hematology recommend the use of prophylactic dose of low-molecular weight heparin whatever is the clinical presentation while the ISTH suggests the use of higher dosage in case of critical illness [64,65]. However, high dosage (intermediate-full anticoagulation) is associated with enhanced bleeding risk, which is even more impressive if an antiplatelet drug such as aspirin is combined [66]; thus, alternative approaches other than classic antiplatelet drugs would be, therefore, of interest. We have previously reported a dose-dependent decrease of ADP-induced PA, platelet TxB_2 biosynthesis, cPLA2 phosphorylation and arachidonic acid release in platelets from healthy subjects added with concentrations of glucocorticoids, which are usually detected in patients treated with this drug's category in CAP patients [67]. Furthermore, we showed lower levels of urinary 11-dehydro-TxB2, a marker of systemic COX1 activation, in glucocorticoids-treated CAP patients compared to untreated ones, suggesting that glucocorticoids could represent a novel therapeutic approach to reduce platelet activation and thrombosis [67]; of note, in 758 CAP patients, of whom 241 (32%) were treated with systemic corticosteroids (methylprednisolone, betamethasone, or prednisone), corticosteroid use was associated with a lower incidence of MI [68]. It would be, therefore, interesting to know if glucocorticoids lower the thrombotic risk in Covid-19 but, unfortunately, this information is lacking in a recent study reporting that glucocorticoids improved survival in Covid-19 [69].

TLRs may be another target to inhibit platelet function in Covid-19. Thus, over-expression of TLR4 has been detected in the coronary thrombi of patients with MI [70] and, in an experimental model of thrombosis, infusion of an inhibitor of TLR4 was associated with reduced thrombus growth and

soluble levels of *P*-selectin [70]. There are no data, however, as to whether inhibition of TLR7 would reduce platelet activation in vivo; further study is necessary to explore this issue.

Inhibition of Nox2 may be another intriguing therapeutic perspective as Nox2 has a prominent role in platelet ROS formation and platelet activation [71]. Further study is, therefore, warranted to assess if Nox2 inhibition is a useful therapeutic approach to reduce platelet activation and eventually the thrombotic risk associated to Covid-19.

Albumin, which comprises antiplatelet and anticoagulant activity, may be another option as previous study reported that its infusion lowers platelet activation in human [72]. Of note, patients with Covid-19 have reduced serum levels of albumin, with values < 35 g /L overall in patients with severe disease; interestingly, a significant association between serum albumin, elevated D-dimer and increased risk of thrombotic events have been reported [7]; this finding is consistent with previous studies reporting that serum albumin < 35 g/L is associated with an increased risk of arterial and venous thrombosis [73]. Finally, ACE2 administration or inhibition of Spike protein may represent an interesting therapeutic approach as experimental study demonstrated that both treatments have a negative effect on thrombus growth [33].

Other therapeutic options already known to inhibit platelet function and potentially useful in Covid-19 include thrombin/FXa/vitamin K antagonists, PAR1/4, vWF or collagen receptor inhibitors. In this context some trials are in progress with antiplatelet/anticoagulant drugs in Covid-19, including P2Y12 inhibitor, tirofiban, aspirin and fondaparinux (ClinicalTrials.gov Identifier: NCT04445623, NCT04409834, NCT04368377). All the potential above reported therapeutic options to lower platelet activation in Covid-19 are summarized in Figure 2.

Conclusions

Experimental and clinical studies consistently showed that platelets are activated in CAP and Covid-19 and could play a role in precipitating the vascular diseases which complicate the clinical course of both settings. The mechanism of platelet activation may include different and multiple pathways, which appear more complex in the case of Covid-19, where the virus could bind to the cells using several entry mechanisms such as ACE2-AngII axis and/or TLRs (see Figure 1); if confirmed, inhibition of these two pathways may be a tool to develop novel antiplatelet strategies. It cannot be excluded that these potential multiple entry mechanisms by Covid-19 account for the elevated response to injury and eventually thrombotic risk; in this context, it is of interest that CAP and Covid-19 do not share similar features of vascular disease, being Covid-19 more frequently complicated by both venous and arterial thromboses while vascular disease is less frequent in CAP and localized prevalently in the arterial circulation. These findings may have impact in the antithrombotic strategy as antiplatelet drugs such as aspirin could be a first choice for CAP treatment unless the disease severity entails alternative treatment; in the case of Covid-19 the typology of vascular disease indicate AC as first choice while the contemporary use of AC is still debated also in view of the potential bleeding risk. Experimental data are, however, in support of the fact platelets contribute to the thrombotic process and, considering the still elevated residual risk of thrombosis and death despite AC treatment, combination of antiplatelet treatment may be an important option to improve clinical outcome in Covid-19. The aforementioned ongoing trials with antiplatelet drugs will provide useful information regarding the potential efficacy of this treatment in Covid-19.

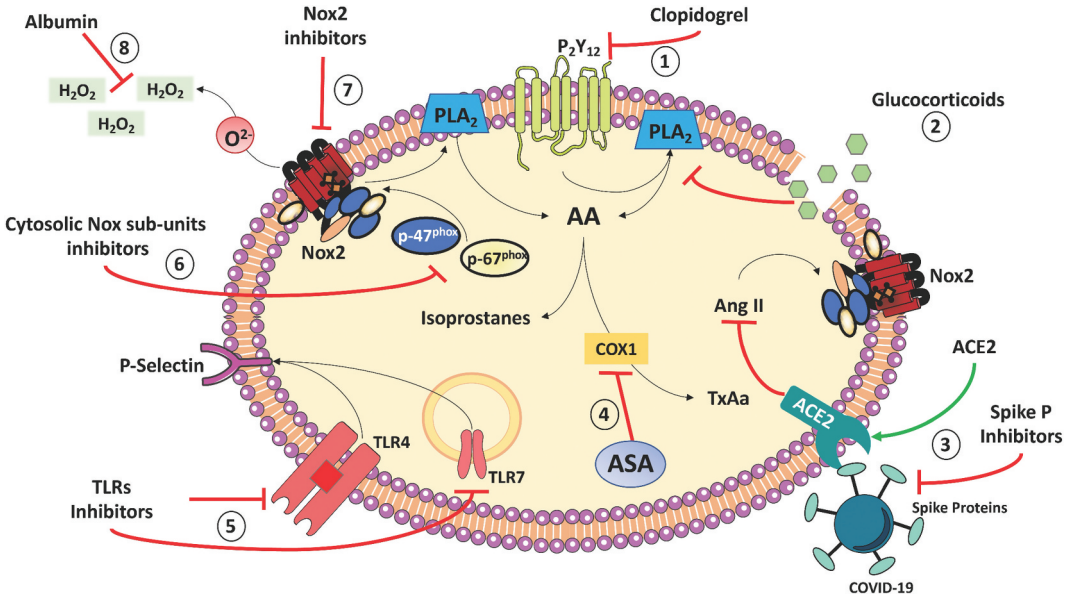

Figure 2. Therapeutic strategy: antiplatelet drugs.

Therapeutic approaches to inhibit platelet activation in Covid-19 patients: 1) P2Y12 receptor blockers, Clopidogrel; 2) Glucocorticoids; 3) ACE2 agonists or Spike Protein(P) Inhibitors; 4) Aspirin; 5) inhibition of TLRs; 6) Nox2 inhibition and 7) cytosolic Nox sub-units inhibition; 8) Albumin supplementation. Green line: activation pathway. Red lines: inhibition pathway. (ASA = aspirin; AA = arachidonic acid; Ang II = angiotensin II; ACE2 = Angiotensin converting enzyme 2; COX1 = cyclooxygenase-1; COVID-19 = Coronavirus Disease 2019; H_2O_2, hydrogen peroxide; PLA_2 = phospholipases A2; TLRs = Toll Like Receptors).

Author contributions

Francesco Violi: Conceptualization, writing and supervision; Vittoria Cammisotto: Investigation and original draft preparation. Pasquale Pignatelli: Investigation and Writing.

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