

# Aspirin as an Adjunctive Pharmacologic Therapy Option for COVID-19: Anti-Inflammatory, Antithrombotic, and Antiviral Effects All in One Agent

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**Introduction:** Pharmacologic therapy options for COVID-19 should include antiviral, anti-inflammatory, and anticoagulant agents. With the limited effectiveness, currently available virus-directed therapies may have a substantial impact on global health due to continued reports of mutant variants affecting repeated waves of COVID-19 around the world.

**Methods:** We searched articles pertaining to aspirin, COVID-19, acute lung injury and pharmacology in PubMed and provide a comprehensive appraisal of potential use of aspirin in the management of patients with COVID-19. The scope of this article is to provide an overview of the rationale and currently available clinical evidence that supports aspirin as an effective therapeutic option in COVID-19.

**Results:** Experimental and clinical evidence are available for the potential use of aspirin in patients with COVID-19.

**Discussion:** Aspirin targets the intracellular signaling pathway that is essential for viral replication, and resultant inflammatory responses, hypercoagulability, and platelet activation. With these multiple benefits, aspirin can be a credible adjunctive therapeutic option for the treatment of COVID-19. In addition, inhaled formulation with its rapid effects may enhance direct delivery to the lung, which is the key organ damaged in COVID-19 during the critical initial course of the disease, whereas the 150–325 mg/day can be used for long-term treatment to prevent thrombotic event occurrences. Being economical and widely available, aspirin can be exploited globally, particularly in underserved communities and remote areas of the world to combat the ongoing COVID-19 pandemic.

**Keywords:** COVID-19, acetyl salicylic acid, platelets, inflammation, lungs, acute respiratory syndrome

## Introduction COVID-19 and Host Response

The rapid development of coronavirus disease-19 (COVID-19) as an acute lung injury/acute respiratory distress syndrome (ALI/ARDS) progressing to death has become a daunting challenge to manage. Options such as treatment with antiviral, anti-inflammatory, and anticoagulant agents and mechanical ventilation may not be readily available in underserved communities and in remote areas of the world. Within this framework, the degree of efficacy of most strategies assessed individually is variable ranging from low to moderate.<sup>1,2</sup>

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Aspirin can influence different disease-relevant pathways during the course of COVID-19. The interplay of these distinct aspirin-mediated effects may contribute to the outcome improvement of COVID-19 patients by interfering with the viral replication as well as its anti-inflammatory and antithrombotic properties.<sup>3</sup> Currently available antiviral agents and other novel therapies that directly target the virus may exhibit limited effectiveness over time due to adaptive mutations of the viral genome.<sup>4</sup> A limitation of virus-directed therapies may have a substantial impact on global health due to continued reports of mutant variants affecting repeated waves of COVID-19 around the world, which may further undermine the efficacy of these therapies that are also expensive.<sup>4</sup> Therefore, it is important to explore economically feasible and readily accessible resilient mechanisms to target COVID-19. Such strategies will facilitate the treatment of patients with COVID-19 and viral mutants in remote parts of the world and underserved communities. The scope of this article is to provide a comprehensive description of the rationale and supporting clinical evidence of aspirin as a multimodal therapeutic option in COVID-19.

## Mechanisms of Virus Entry into the Host Cells and Inflammatory Cascade

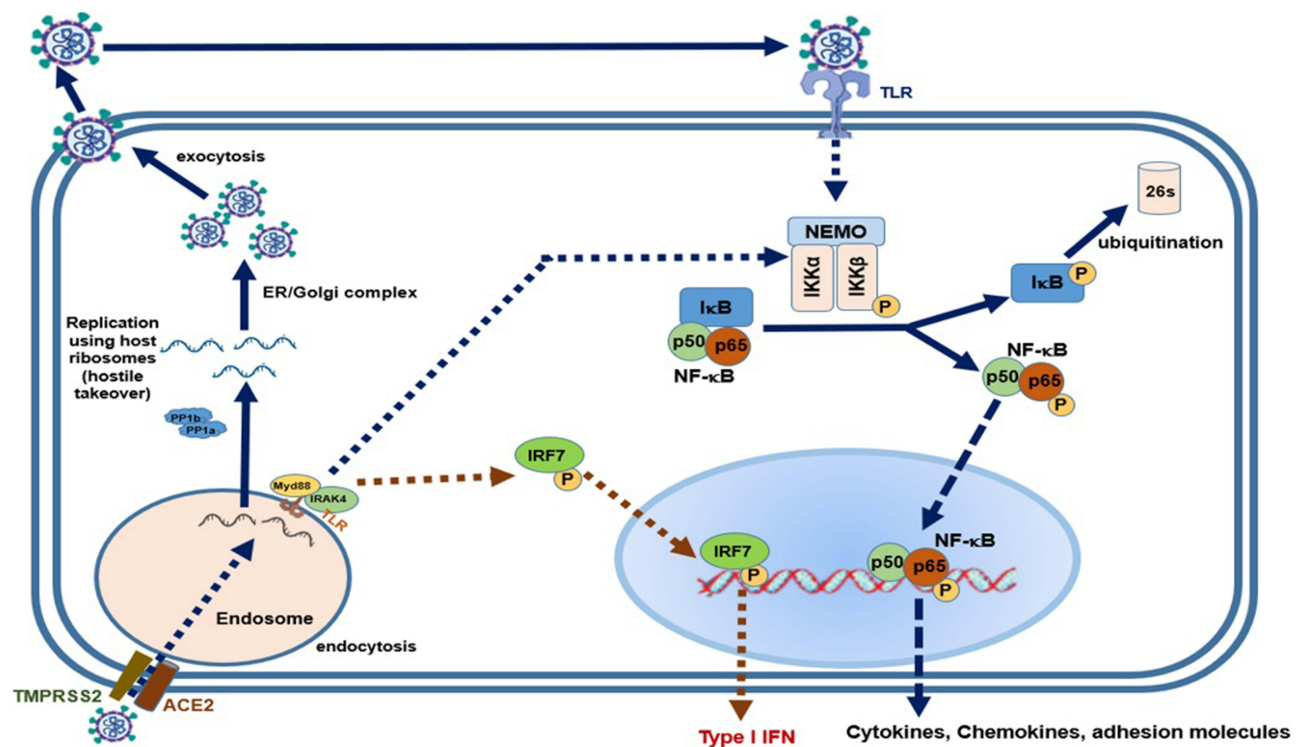
During the initial steps of SARS-CoV-2 (severe acute respiratory syndrome- coronavirus-2) infection, spike (S)-glycoprotein on the surface of the SARS-CoV-2 binds to the angiotensin-converting enzyme receptor 2 (ACE2). Transmembrane protease serine (TMPRSS) 2 is involved in S protein priming that facilitates virus entry into the host cell (endocytosis). The ACE2 receptor and TMPRSS2 are highly expressed on the surface of alveolar cells in the lower respiratory tract.<sup>5,6</sup> After viral release into the cytoplasm of the host cell, the SARS-CoV-2 viral genome produces two polyproteins (PPs). These PPs help control the host cell machinery in a “hostile takeover” resulting in their own rapid translation and replication in endosomes. Mature virions are later released by exocytosis and can bind to TLR4 on host cell membrane to trigger the activation of the intracellular inhibitor of kappa B kinase (IKK) complexes.<sup>5</sup> The viral ribonucleic acid (RNAs) bind to Toll like (TLR) receptors (TLR3 for double-stranded RNAs or TLR7/8 for single-stranded RNAs) on endosomal membrane leading to the activation of transcription of the interferon-regulatory factor (IRF) family and

subsequently type I interferon and also induce NF- $\kappa$ B (nuclear factor- $\kappa$ B) activation (Figure 1). The activation of IKK results in the phosphorylation of the cytoplasmic inhibitor factor, I $\kappa$ B $\alpha$  triggering its ubiquitination and degradation by the 26S proteasome. Simultaneously, NF- $\kappa$ B (a heterodimer complex consisting of protein subunits p50 and p65) is released from I $\kappa$ B $\alpha$ , then it translocate into the nucleus and induces transcription of various genes coding for pro-inflammatory proteins such as cytokines, chemokines, adhesion molecules, acute phase proteins, and growth factors.<sup>7–9</sup>

These cytokines activate inflammatory leukocytes (neutrophils, macrophages) and trigger their infiltration into the alveolar space further boosting the generation and release of large quantities of inflammatory cytokines in a “cytokine storm”. Activated macrophages express tissue factor that activates coagulation leading to systemic hypercoagulability (Figure 2).<sup>7,10</sup> COVID-19-induced coagulopathy (CIC) is characterized by neutrophilia and elevated levels of interleukin-6, C-reactive protein, D-dimer, FVIII, and fibrinogen. The latter processes induce inflammatory cell activation and infiltration, vascular leakage, pulmonary edema, and rapid progress to ARDS, multi-organ dysfunction, and death in a substantial percentage of patients. Similarly, cytokine storm and hypercoagulability have been shown to be associated with pulmonary microthrombosis and eventually apoptosis of pulmonary cells.<sup>11</sup>

## Role of Platelets in COVID-19

In addition, multiple lines of evidence point towards an essential role of platelets during COVID-19 ALI/ARDS (7–10). Platelets play a critical role in hemostasis, thrombosis, and innate immune and inflammatory responses. Platelet-leukocyte aggregate formation and abnormal leukocyte accumulation in the lungs, hypercoagulability, and elevated levels of thromboxane A<sub>2</sub>, P-selectin and biomarkers of inflammation have been demonstrated in animal models of ALI/ARDS.<sup>12</sup> Direct binding of SARS-CoV-2 to platelets through ACE2 and TMPRSS2 expressed on platelets has been demonstrated in *in vitro* studies. Elevated platelet aggregation, glycoprotein (GP) IIb/IIIa expression at the platelet surface, P-selectin expression, and platelet granule secretion has been demonstrated in ALI/ARDS models. Subsequently, activated platelets bind to leukocytes and enhance their infiltration into the lungs to further propagate inflammation.<sup>13</sup> Subjects suffering from viral upper respiratory tract infections have been



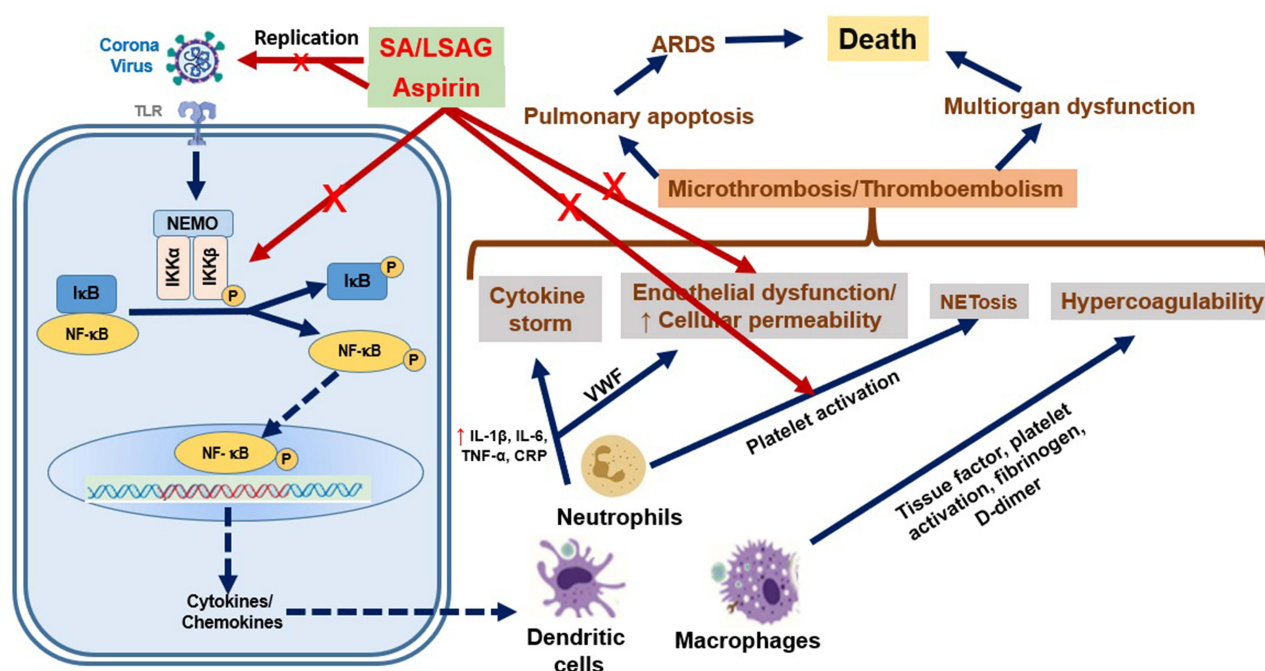
**Figure 1** SARS-CoV-2 infection and replication inside the host cell. Initially, SARS-CoV-2 enters the host cell through binding to the ACE2 receptor and the function of TMPRSS2. The virus undergoes replication using host machinery ("hostile takeover") and the mature virus is released (exocytosis) from the host cell. Inside the endosome, the viral RNA binds to the TLR and activates IRF7 that translocate into the nucleus to induce the expression of Type I interferon. SARS-CoV-2 binding to TLR also activates IKK $\beta$  and leads to NF- $\kappa$ B-mediated expression of cytokines, chemokines and adhesion molecules.

**Abbreviations:** SARS-CoV-2, severe acute respiratory syndrome-coronavirus-2; ACE2, angiotensin converting enzyme receptor 2; TMPRSS2, transmembrane protease serine; PPs, polyproteins; RNA, ribonucleic acid; TLR, toll-like receptor; IKK $\beta$ , IKK $\beta$  kinase complex; NF- $\kappa$ B, nuclear factor- $\kappa$ B; NEMO, NF- $\kappa$ B essential modulator; IRF7, interferon regulatory transcription factor 7; ER, endoplasmic reticulum; TMPRSS2, transmembrane serine protease 2.

shown to exhibit elevated platelet P-selectin expression and heightened platelet reactivity.<sup>14</sup> It has been hypothesized that the thrombo-inflammatory state has an important influence on the outcomes of patients with cardiovascular disease and human immunodeficiency virus infection during and after percutaneous coronary intervention.<sup>15</sup>

Megakaryocytes are precursors of platelets. Following their release from bone marrow, platelets circulate in large numbers through the lungs, where they are dynamically released. A recent study using direct imaging of lung microcirculation in mice suggested that the lungs can contribute up to 50% of total platelet release.<sup>16</sup> Since the lungs are severely affected during COVID-19, platelet generation/function may also be severely affected. In the presence of endothelial dysfunction, elevated levels of von Willebrand Factor (VWF) expression in the lung, elevated systemic levels of fibrinogen, and activated platelets likely play a significant role in pulmonary thromboembolic event occurrences.<sup>17–19</sup> Activated platelets stimulate neutrophils to produce neutrophil extracellular traps (NETs). NETs consist of deoxyribose nucleic acid (DNA) fibers, histones,

and antimicrobial proteins that help to entrap and degrade invading bacteria and viruses (NETosis) as part of the innate immune response. However, platelet-induced NETosis can be more lethal resulting in tissue damage, hypercoagulability, and thrombosis.<sup>20</sup> In a prospective cohort study in patients with COVID-19, elevated levels of platelet–neutrophil aggregates, plasma myeloperoxidase (MPO)-DNA complexes, platelet factor 4, and Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted (RANTES) have been demonstrated. Circulating NET levels correlated with the severity of COVID-19.<sup>20</sup> Furthermore, in autopsies, the presence of NET-containing microthrombi with platelet–neutrophil infiltration in lungs again highlights the critical role of platelets in COVID-19.<sup>20</sup> Post-mortem examination of patients with COVID-19 found a nine times higher prevalence of microvascular platelet-fibrin clots in the pulmonary vasculature than in patients with influenza virus infection.<sup>19</sup> In vivo evaluation of the sublingual microcirculation in patients with severe COVID-19 requiring mechanical ventilation revealed microvascular thrombi in



**Figure 2** Host cell response to SARS-CoV-2 infection and role of aspirin in SARS-CoV-2 infection. SARS-CoV-2 induced cytokine storm, endothelial dysfunction, NETosis and hypercoagulability result in microthrombosis/thromboembolism in lungs as well as heart and kidney leading to multiorgan dysfunction, ARDS and ultimately death in a substantial percentage of patients. Aspirin/SA/LSAG can attenuate the viral replication and inhibit NF-κB activation and subsequent expression of cytokines and chemokines. In addition, ASA exhibits anti-inflammatory and antiplatelet effects, and attenuates NETosis, endothelial dysfunction and hypercoagulability.

**Abbreviations:** ASA, acetyl salicylic acid; SA, salicylic acid; LASAG, D, L-lysine-acetylsalicylate glycine; NF-κB, nuclear factor-κB; IL, interleukin; TNF-α, tumor necrosis factor-α; CRP, C-reactive protein; MCP-1, macrophage chemoattractant protein-1; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; NETs, neutrophil extracellular traps; ARDS, acute respiratory distress syndrome.

85% of the cases.<sup>20</sup> Finally, the presence of microthrombi in the heart, kidneys, and liver in patients with COVID-19 may indicate widespread thrombotic microangiopathy resulting in multiorgan dysfunction.<sup>21–23</sup>

## Potential Role of Aspirin in COVID-19

The totality of evidence consistently indicates that the resilience mechanism to target during COVID-19 for both prevention and treatment should involve effective and simultaneous attenuation of viral infection and replication, cytokine release, endothelial dysfunction, coagulation, fibrinolysis, and importantly, platelet function. Therefore, aspirin – an inexpensive, widely available, safe, and time-tested agent with anti-inflammatory, antithrombotic, and antiviral properties – can be a credible adjunctive therapeutic option for the treatment of COVID-19.<sup>24</sup> Most importantly, instead of targeting the virus directly, aspirin targets the intracellular signaling pathway of the host cell that is essential for viral replication, and resultant inflammatory responses, hypercoagulability, and platelet activation that follow infection. Thus, aspirin would be predicted to be effective even in the presence of mutant virus forms. The role of aspirin may

be particularly relevant in situations where more expensive therapeutic options are not readily available.

Two major underpinnings for using aspirin in COVID-19 include the effect of aspirin on the inhibition of cyclooxygenase (COX)-1 and COX-2 enzymes by acetylation and the effect on NF-κB by salicylic acid.<sup>25</sup> The acetyl group of aspirin binds to the serine residue (Ser529) in platelet COX-1 irreversibly and noncompetitively for the lifespan of platelets, and thereby inhibits the eventual generation of an important platelet agonist, thromboxane (Tx)A<sub>2</sub>, and platelet activation. An antithrombotic effect of aspirin has been primarily attributed to the inhibition of the platelet COX-1 enzyme that occurs at low doses (75–81mg/day). In vitro studies indicate that the inhibitory potency of aspirin against COX-1 and COX-2 is similar in molar terms.<sup>26,27</sup> However, in vivo higher molar concentrations of aspirin are required because of the significant protein (COX) turnover rate of nucleated cells as opposed to the apparently missing turnover of the anucleated platelets. Thus, within the short half-life of aspirin in the circulation, amounting to 20–30 min, enough new enzyme protein might have been synthesized that escapes the acetylation by aspirin. COX-1 inhibition results in the



direct inhibition of prostaglandin intermediate endoperoxide generation in the platelet that is upstream from the generation of  $\text{TxA}_2$  by thromboxane synthase.<sup>28</sup> These inhibitory properties are reflected in reduced excretion of the stable thromboxane metabolite, urine 11-dehydro thromboxane  $\text{B}_2$  (u11-dh  $\text{TxB}_2$ ). Thus, u11-dh  $\text{TxB}_2$  represents the whole-body COX inhibitory response induced by aspirin.<sup>29–31</sup> The independent relation of urine u11-dh  $\text{TxB}_2$  to adverse outcomes in patients with cardiovascular disease and diabetes treated with aspirin has been clearly demonstrated in major clinical trials.<sup>32–34</sup>

At higher doses, aspirin also inhibits the COX-2 enzyme that is expressed during inflammatory conditions by acetylating the homologous Ser516 residue.<sup>26,27</sup> The latter effect results in the inhibition of pro-inflammatory prostaglandin  $\text{E}_2$  ( $\text{PGE}_2$ ) generation. The inhibition of COX-2 by aspirin also results in the inhibition of prostaglandin intermediate endoperoxide synthesis that participates in  $\text{TxA}_2$  generation through transcellular biosynthesis.<sup>25</sup> Acetylated COX-2 can convert arachidonic acid to 15-epoxy-lipoxin  $\text{A}_4$ , also known as aspirin-triggered lipoxin (ATL). Lipoxins and aspirin via ATL inhibit leukocyte-endothelial interactions by stimulating nitric oxide release, decrease vascular permeability, and attenuate endothelial dysfunction by improving oxygen defense.<sup>35–38</sup> In addition, the aspirin-acetylated COX-2 enzyme generates aspirin-triggered resolving  $\text{D}_1$  (AT-RvD1) molecule.<sup>39</sup> In an acid-initiated murine lung injury model, administration of AT-RvD1 significantly decreased bronchoalveolar lavage fluid neutrophils, platelet-neutrophil interactions, the release of cytokines and p-selectin, and nuclear translocation of NF- $\kappa\text{B}$ -phosphorylated p65 as determined by immunohistochemistry of lung sections.<sup>40</sup>

Aspirin and salicylic acid at 1–5 mM concentrations have been shown to inhibit NF- $\kappa\text{B}$  activation and NF- $\kappa\text{B}$  induced inflammatory cytokine generation.<sup>7,41</sup> This involves transcriptional activation of differentially regulated transcription factors, including NF $\kappa\text{B}$ , and is apparently due to nonselective kinase inhibition.<sup>42</sup> It has been demonstrated that aspirin and salicylic acid inhibit IKK activity by blocking ATP binding to IKK- $\beta$ .<sup>41–43</sup> Aspirin has been shown to attenuate thrombin generation and factor XIII activation in a microvascular injury model as well as thrombin-induced venous thromboembolism via inhibition of thromboxane action.<sup>44–46</sup> Aspirin can also enhance fibrin clot permeability and clot lysis by acetylating lysine residues in fibrin at high doses.<sup>47,48</sup> In addition,

aspirin non-specifically acetylates a variety of proteins and nucleic acids at micromolar concentrations.<sup>49–51</sup>

In an in vitro experiment with phorbol 12-myristate 13-acetate (PMA)- or tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )-activated human neutrophils, 5 mM aspirin was associated with a significant reduction in NET release. This reduction was correlated with a significant reduction in the phosphorylation of the NF- $\kappa\text{B}$  p65 subunit indicating aspirin can attenuate NETs release from neutrophils by inhibiting NF- $\kappa\text{B}$ .<sup>52</sup> Aspirin was shown to inhibit reactive oxygen species generation, neutrophil infiltration, macrophage generation, and lung edema in a hyperoxia-induced lung injury model in NF- $\kappa\text{B}$  -luciferase transgenic mice.<sup>53</sup>

With respect to direct antiviral effects of aspirin, in vitro and in vivo studies shown that aspirin can effectively block influenza virus infection. This antiviral effect has been attributed to the inhibition of viral replication and propagation through NF- $\kappa\text{B}$  inhibition.<sup>54,55</sup> Antiviral effect of the D, L-lysine-acetylsalicylate glycine (LASAG), an aerosolized formulation of aspirin, is discussed later.

## Experimental Evidences

Animal studies have shown that aspirin can prevent ARDS by decreasing neutrophil activation and recruitment in the lung, TNF- $\alpha$  expression in pulmonary intravascular macrophages, plasma  $\text{TxB}_2$  levels, and platelet aggregation in the lungs.<sup>56–58</sup> Finally, a meta-analysis of studies in animal models of ARDS revealed that aspirin administration was associated with improved oxygenation, diminished lung edema and inflammation, and increased survival in some studies.<sup>56</sup> All these data indicate that aspirin has the potential to attenuate COVID-19 induced excessive immune activation, cytokine storm, hypercoagulability, and multi-organ damage and thus, favorably affects clinical outcomes.

## Benefit of Aspirin in Patients with ARDS

Some observational studies suggested the benefit of prior aspirin use in reducing the risk associated with ALI/ARDS.<sup>60–64</sup> Importantly, in a recent multivariate analysis of seven studies with 6764 at-risk patients, prior aspirin use compared to no aspirin use was associated with a significantly lower incidence of ARDS (OR, 0.78; 95% CI=0.64–96;  $p = 0.018$ ), but not hospital mortality (OR=0.88; 95% CI= 0.73–1.07;  $p = 0.20$ ).<sup>65</sup> In a study of 839 severely injured blunt trauma patients at risk for

multiple organ failure, prehospital antiplatelet therapy (16% of the patients on aspirin) was associated with a decreased risk of lung dysfunction and multiple-organ failure.<sup>66</sup> Concurrent use of statin and aspirin, but not aspirin alone, was shown to reduce the risk of ARDS in a cohort of 575 critically ill hospitalized patients.<sup>67</sup> In a selected cohort of 1149 critically ill hospitalized patients, a lower prevalence of ARDS was observed in patients who were already on aspirin therapy (27% vs 34%), and the effect persisted in a multivariate analysis (odds ratio [OR] = 0.66; 95% confidence interval [CI] = 0.46–94).<sup>68</sup> In a propensity-adjusted analysis involving 6823 patients, aspirin use prior to sepsis onset was associated with a 7% reduction in mortality ( $p=0.005$ ) (Table 1).<sup>69</sup>

Aspirin administration for prior 7 days significantly augmented the plasma pro-inflammatory cytokine levels, but not anti-inflammatory cytokine levels in healthy subjects who were administered with *Escherichia coli* endotoxin. There was no additional anti-inflammatory effect with aspirin plus a P2Y<sub>12</sub> receptor inhibitor.<sup>70</sup>

## Preliminary Data Demonstrating the Benefit of Aspirin in Patients with COVID-19

### Pharmacodynamic Studies

In a prospective observational study of hospitalized patients with COVID-19 ( $n = 120$ ), those who were on aspirin therapy had lower u11-dh TxB<sub>2</sub> levels than patients not on aspirin ( $3760 \pm 2295$  versus  $13,125 \pm 11,474$  pg/mg creatinine,  $p = 0.003$ ). An inadequate therapeutic aspirin response based on  $>1520$  pg u11-dh TxB<sub>2</sub>/mg creatinine cut-off was observed in 91% of the patients with COVID-19 on 81 mg daily aspirin and 50% of the patients with COVID-19 on  $\geq 162$  mg daily aspirin.<sup>71</sup> The frequency of thromboinflammation as indicated by  $>4200$  pg u11-dh TxB<sub>2</sub>/mg creatinine was 81% in patients with COVID-19 not on aspirin, 55% in patients on 81 mg daily aspirin, and 25% in patients on  $\geq 162$  mg daily aspirin. Moreover, only 17% of the patients had u11-dh TxB<sub>2</sub> values lower than the cut-off value ( $<1520$  pg/mg creatinine) for aspirin therapeutic response.<sup>72</sup>

### Clinical Studies

In an observational cohort study of adult patients with COVID-19, aspirin use at least 7 days before hospitalization or within 24 hours of hospitalization ( $n = 98$ ) compared to non-aspirin use ( $n = 314$ ) was associated with lower rates of mechanical ventilation (36% vs 48%,) and

intensive care unit (ICU) admission (39% vs 51%).<sup>73</sup> In a multivariate analysis, aspirin use remained significantly associated with decreased risk of mechanical ventilation (adjusted hazard ratio [HR], 0.56; 95% CI, 0.37 to 0.85;  $p = 0.007$ ), ICU admission (adjusted HR, 0.57; 95% CI, 0.38 to 0.85;  $p = 0.005$ ), and in-hospital mortality (adjusted HR, 0.53; 95% CI, 0.31 to 0.90;  $p = 0.02$ ). There were no differences in overt thrombosis or major bleeding between groups.<sup>73</sup> This study provided the initial clinical evidence supporting aspirin use in patients with COVID-19. Most of these patients were on 81 mg per day low-dose aspirin therapy. However, in the presence of a highly elevated inflammatory response, endothelial dysfunction, and hypercoagulability, low-dose aspirin therapy may not be adequate to produce the strong pharmacodynamic effects needed that can be translated into improved clinical outcomes.

In a study of American veterans with COVID-19, pre-existing aspirin prescription was associated with a significant decrease in overall mortality at 14- days ( $n=35,370$ ) (OR=0.38, 95% CI=0.33–0.45) and at 30-days ( $n=32,836$ ) (OR=0.38; 95% CI=0.33–0.45) compared to patients not treated with aspirin.<sup>74</sup> Similarly, in a propensity score-matched observational study of COVID-19 patients ( $n = 638$ ), in-hospital aspirin compared to no antiplatelet therapy was associated with a significantly lower cumulative incidence of in-hospital death (HR, 0.52, 95% CI).<sup>75</sup> In another retrospective population-based cross-sectional investigation (COVID-19-positive patients,  $n = 682$  and COVID-19-negative patients,  $n = 9815$ ), aspirin users had a lower rate of COVID-19 as compared to aspirin non-users (OR, 0.71; 95% CI, 0.52 to 0.99;  $p = 0.04$ ), and a shorter clinical duration of COVID-19 ( $19.8 \pm 7.8$  vs  $21.9 \pm 7.9$  days,  $p = 0.045$ ).<sup>76</sup>

Similarly, other studies also have demonstrated the reduced mortality with prior and in-hospital use of aspirin.<sup>77,78</sup> However, there were also reports of an absence or an elevated risk of mortality in COVID-19 patients.<sup>79–81</sup> The reason for the latter absence of mortality benefits with aspirin use is not clearly understood at this time. Finally, prior use of 75–325 mg daily aspirin was associated with reduced mortality (RR 0.46; 95% CI = 0.35, 0.610,  $p<0.001$ ) and in-hospital mortality (RR=0.39; 95% CI=0.16–0.96;  $p<0.001$ ) (Table 2).<sup>82</sup>

Currently, multiple studies are exploring aspirin therapy alone or as an adjunctive agent in COVID-19 (NCT04808895, NCT04363840, NCT04365309, NCT04324463, NCT04498273, NCT04466670, NCT04368377,

**Table I** Studies of Aspirin Therapy in Patients with Acute Respiratory Distress Syndrome

	Study	Aspirin or Other Antiplatelet Therapy	Outcomes	Comments
Panka BA et al. Systematic review and meta-analysis. 2017 <sup>59</sup>	15 Lung injury model/preclinical studies in mice, sheep and dogs	10–125mg aspirin/kg	Beneficial effect of antiplatelet drug on ARDS reported in 13 studies- improved oxygenation, diminished lung edema, inflammation, and in some an increased survival	Aspirin-triggered lipoxin reduced inflammation and attenuated edema; AT-RvD1- and COX-dependent inhibitory effects were also suggested.
	Meta-analysis of 3 clinical studies in patients with ARDS (n=5155)	Prior aspirin use	Aspirin use was associated with lower incidence of ARDs (OR=0.59; 95% CI = 0.36–0.98)	A potential beneficial role for aspirin in ARDS prevention and treatment
Liang H et al. Systematic review and meta-analysis. 2020 <sup>65</sup>	7 Studies in patients with ARDS (n=6764)	Prior aspirin use	Aspirin use was associated with lower incidence of ARDs in at-risk patients (OR = 0.78; 95% CI = 0.64–0.96; p = 0.018) and was not associated with in-hospital mortality (OR= 0.88; 95% CI= 0.73–1.07; p = 0.204)	A potential beneficial role for aspirin in ARDS prevention.
Harr JN et al. Multicenter study. 2013 <sup>66</sup>	Severely injured blunt trauma patients at risk for multiple organ failure (n=839)	15% of the patients on APT of which 66% on aspirin	Transfused patients on APT had significantly lower ORs of lung dysfunction and multiple organ failure vs patients not on APT at time of injury (interaction PRBC × APT, p = 0.01 for lung dysfunction, p = 0.03 for MOF).	A potential beneficial role for aspirin in blunt trauma patients at risk for MOF.
O'Neal HR et al. Cross-sectional analysis. 2011 <sup>67</sup>	Critically ill patients (n=575)	In total, 24% patients on aspirin and 26% on statins before hospitalization; 50% of statin treated patients on aspirin	Pre-hospital aspirin plus statin use (50% of statin users) associated with lowest rate of rates of ALI/ARDS, severe sepsis, and hospital mortality.	Prior aspirin use may provide additional benefit in the presence of statins in ALI/ARS patients
Chen W et al, Propensity-adjusted analysis. 2015 <sup>68</sup>	Critically ill patients admitted to ICU (n=1149)	25% of the patients on prior aspirin	Prior aspirin use was associated with lower prevalence of ARDS (27% vs 34% no prior aspirin, p=0.034), and independently associated with a decreased risk of ARDs (OR= 0.66; 95% CI= 0.46–0.94) in the entire cohort and in a subgroup of 725 patients with sepsis (OR= 0.60; 95% CI= 0.41–0.90).	-
Trauer J et al. Propensity analysis. 2017 <sup>69</sup>	6823 Patients with sepsis from 11 studies	Prior aspirin use	Prior aspirin use was associated with 7% reduction in mortality (p=0.0023)	-

**Abbreviations:** APT, antiplatelet therapy; PRBC, packed red blood cells; OD, odds ratio; CI, confidence interval; ALI/ARDS, acute lung injury/acute respiratory distress syndrome; MOF, multiorgan failure; ICU, intensive care unit; COX, cyclooxygenase; AT-RvD1, aspirin-triggered resolvin D1.

NCT04410328, NCT04381936, NCT04333407), but none of these studies are focused on high-dose aspirin (325 mg per day) in COVID-19.

In the RECOVERY Trial, a multinational, randomized trial hospitalized COVID-19 patients who were treated with 150mg aspirin (n=7351) as compared to usual care

**Table 2** Studies of Aspirin Therapy in Patients with Coronavirus-19

	Study	Aspirin or Other Antiplatelet Therapy	Outcomes	Comments
Chow JH et al Retrospective, observational study. 2020 <sup>73</sup>	Hospitalized COVID-19 patients (n=412)	24% of the patients on aspirin	Prior aspirin therapy independently associated with less mechanical ventilation (adjusted HR = 0.56, 95% CI, 0.37–0.85, p = 0.007), ICU admission (adjusted HR, 0.57, 95% CI, 0.38–0.85, p = 0.005), and mortality (adjusted HR, 0.53, 95% CI, 0.31–0.90, p = 0.02).	Prior aspirin use may improve outcomes in hospitalized COVID-19 patients
Osborne TF et al. Veterans health administration study. 2020 <sup>74</sup>	Patients with COVID-19 (n=68,156)	19% of the patients on aspirin	Prior aspirin prescription was associated with a decrease in overall mortality at 14-days (OR=0.38; 95% CI 0.32–0.46) and at 30-days (OR=0.38, 95% CI=0.33–0.45)	Prior aspirin reduces mortality in COVID-19 patients
Meizlish M et al. Propensity score-matched analysis. 2021 <sup>75</sup>	Hospitalized adult COVID-19 patients (n=2785)	In-hospital aspirin = 1956; Propensity matched cohort=638	In-hospital aspirin vs no antiplatelet therapy independently associated with lower incidence of in-hospital death (HR=0.52; 95% CI = 0.336–0.812; p=0.001)	In-hospital aspirin reduces death
Merzon E et al. Retrospective population-based cross-sectional study. 2021 <sup>76</sup>	COVID-19-positive (n=662) and -negative patients (n=9815)	Prior aspirin, COVID-positive group = 11% (n=73) vs -negative group= 16% (n=589) (p=0.001).	Aspirin use was associated with lower likelihood of COVID-19 infection, as compared to nonusers (adjusted OR 0.71 (95% CI, 0.52 to 0.99; P = 0.041). Aspirin use was associated with lower likelihood of COVID-19 infection, as compared to nonusers (adjusted OR 0.71 (95% CI, 0.52 to 0.99; P = 0.041). Aspirin use was associated with lower likelihood of COVID-19 infection, as compared to nonusers (adjusted OR 0.71 (95% CI, 0.52 to 0.99; P = 0.041). Aspirin use independently associated with lower likelihood of COVID-19 infection (adjusted OR =0.71; 95% CI=0.51–0.99, p=0.04) and shorter disease duration (19.8±7.8 vs 21.9±7.9, p=0.045).	Prior aspirin was associated lower likelihood of COVID-19 infection and disease duration.
Liu Q et al. Propensity score-matched analysis. 2021 <sup>77</sup>	Hospitalized adult COVID-19 patients (n=232)	Hospitalized COVID-19 patients on aspirin (n=28) vs not on aspirin (n=204).	Aspirin therapy was associated with lower risk of 30-day (HR=0.19, 95% CI = 0.05–0.78), p=0.021) and 60-day mortality (HR=0.25, 0.07–0.87, p=0.03)	In-hospital aspirin therapy reduces death
Haji Aghajani M, Prospective study. 2021 <sup>78</sup>	Hospitalized COVID-19 patients (n=991)	Patients with in-hospital aspirin therapy (n=336) and without aspirin therapy (n=655)	In-hospital aspirin therapy was independently associated with reduced in-hospital mortality (HR=0.74; 95% CI=0.560–0.994; p=0.046)	In-hospital aspirin therapy reduces death

(Continued)



Table 2 (Continued).

	Study	Aspirin or Other Antiplatelet Therapy	Outcomes	Comments
Sahai A et al. Propensity-matched analyses. 2021 <sup>79</sup>	Hospitalized COVID-19 patients treated with and without aspirin (n=1994)	Patients on aspirin = 285, not on aspirin = 1709, 248 propensity-matched patients	There were no differences in the incidence of MI (2.0% vs 0.81%) VTE (4.0% vs 1.6%) and mortality (OR=0.85; 95% CI=0.51–1.41; $p = 0.52$ )	No effect of aspirin therapy on thrombotic events and mortality
Yuan S et al. Prospective study. 2021 <sup>80</sup>	Hospitalized COVID-19 patients with coronary artery disease (n=183)	Patients with prior aspirin (n=52) vs without aspirin (n=131).	No difference in in-hospital mortality between patients with aspirin and without aspirin (21.2% vs 22.1%, $P = 0.885$ ).	No influence of prior aspirin use on in-hospital mortality in COVID-19 patients with coronary artery disease.
Reese JT et al. Retrospective, multi-center observational study 2021 <sup>81</sup>	Aspirin use in the osteoarthritis cohort	COVID-19 patients with osteoarthritis (n=2266)	Aspirin use was associated with increased mortality (OR=3.25, 95% CI=2.76–3.83)	Aspirin therapy was associated with elevated mortality
Martha JW et al. Systematic review and meta-analysis. 2021 <sup>82</sup>		Six studies with pre-hospital 75–325 mg daily aspirin use in COVID-19 patients (n=13,993)	Prior aspirin use was associated with reduced mortality (RR=0.46; 95% CI = 0.35, 0.610, $p<0.001$ ) and in-hospital mortality (RR=0.39; 95% CI=0.16–0.96; $p<0.001$ ).	Prior-aspirin use was associated with total and in-hospital mortality.

(n=7541) had a similar rate of death in 28 days (17% in each group, rate ratio = 0.96; 95% CI=0.89–1.04; 41  $p=0.35$ ) and there was no reduction in the risk of progression to the composite endpoint of invasive mechanical ventilation or death in patients not on invasive mechanical ventilation at baseline. However, 150 mg aspirin therapy was associated with a 0.6% absolute reduction in thromboembolic events and a 0.6% increase in major bleeding. Interestingly, aspirin therapy was associated with non-significant shorter duration of hospitalization (median 8 vs 9 days), a significantly higher percentage of patients discharged from hospital alive within 28 days (75% vs 74%; rate ratio 1.06; 95% CI 1.02–1.10;  $p=0.0062$ ).<sup>81</sup> In the absence of strong evidence, the treating physician should be cautious about recommending optimal dose aspirin in COVID-19 patients.

There are some caveats in this study. First, 7351 patients were randomized to the aspirin group, where 90% of those received at least one dose in the intention to treat (ITT) analysis. Thus, about 10% of the patients in the aspirin group did not receive any aspirin treatment, while 3% of the non-aspirin group did. Moreover, 12.5% mortality reduction was hypothesized, and according to

this estimation, the aspirin subgroup was closed as “sufficient” patients had been recruited. Clearly, the difference at day 28 is small, but it might separate at later time points. A total observation period of 6 months was announced for several types of drug treatment, unfortunately not for the aspirin group, and also there is no discussion on on-treatment analysis. Secondly, patients were all on high-dose unfractionated heparin/low-molecular-weight heparin (UFH/LMWH) and are at markedly reduced thrombin levels, although one has to consider that the half-life of UFH is only 2 hours and its effect is highly variable.<sup>81</sup> Importantly, in the absence of any pharmacodynamic assessment of aspirin, it is not clear whether 150mg daily aspirin dose is sufficient to inhibit thromboxane synthesis in the presence of hypercoagulability, very high levels of inflammation, and endothelial dysfunction. An easy tool to test this would be the measurement of u11-dh-TxB<sub>2</sub>. Moreover, up to 3g daily oral aspirin dose in adults and up to 2g daily in the elderly (>65 years) are approved medications for the treatment of flu-like symptoms in Germany. Interestingly, the dose issue is not mentioned as a possible explanation for a less-than-expected (12.5%) reduced mortality by the authors. Therefore, adequately

controlled and appropriately sized prospective randomized study is needed to support the results of the RECOVERY trial.

Taken together, the study does not sufficiently answer the question of whether 150 mg aspirin might be beneficial in the treatment of SARS-CoV-2 or not. However, it tends to suggest that aspirin on the background of anticoagulants might add some therapeutic benefit as seen here from the reduced in-hospital stay. The results again indicate that a higher dose (325 mg during hospital stay) may be more associated with more efficient antithrombotic and anti-inflammatory effects accompanied by improved clinical outcomes. But the potential increase in bleeding may be a concern. Therefore, the administration of inhaled aspirin may be an effective option during the early period of hospitalization since the latter strategy may be associated with lesser bleeding. Finally, since hospitalized patients in the RECOVERY trial are treated with “usual” care with adequate antiplatelet and anti-inflammatory agents in addition to mechanical ventilation, a 325mg aspirin may be an effective option, particularly in underserved communities and remote areas of the world where immediate in-hospital treatment and other drugs are readily available.

The RECOVERY (Randomized Evaluation of COVID-19 Therapy) study is evaluating the impact of aspirin on all-cause mortality among hospitalized patients with COVID-19 and is the largest adaptive platform randomized clinical trial for COVID-19 with 20,000 participants (NCT043819). The RECOVERY II (Randomized Evaluation of COVID-19 Therapy II) trial is now being planned to test the effectiveness of low-dose aspirin as an anti-inflammatory and antithrombotic treatment in COVID-19 patients (NCT04381936).

## Aerosolized Aspirin for the Acute Treatment of COVID-19

In the above-mentioned studies, a conventional 81–325 mg daily aspirin dose was largely used. Systemic concentrations of aspirin and salicylic acid obtained by conventional oral aspirin doses mentioned above may not reach the airway and alveolus at a level needed for meaningful antiviral effects. The concentrations of aspirin needed for sufficient deposition in the lungs to attenuate rapid viral replication and thrombotic risk in the presence of cytokine storm, hypercoagulability and microthrombosis in patients with COVID-19 likely will not be reached following 81–325 mg daily oral dose. Moreover, high oral aspirin doses can be limited by salicylate

uncoupling of oxidative phosphorylation at anti-inflammatory doses.<sup>83</sup> Aspirin may also cause respiratory irritation at high levels due to acidic properties. The LASAG can be administered directly as an aerosol and has been evaluated in clinical studies of chronic obstructive pulmonary disease (COPD) and influenza.<sup>84,85</sup> D, L-lysine improves the stability and tolerability of inhaled aspirin, whereas glycine further increases stability.<sup>86</sup> LASAG dissociates readily after topical administration and the pharmacodynamic properties of LASAG are similar to aspirin.<sup>88</sup>

LASAG was effective at inhibiting coronavirus replication in an in vitro cell system infected with either a low (human corona virus (HCoV)-229E) or high pathogenic strain (Middle East Respiratory Syndrome (MERS)-CoV).<sup>87</sup> Virus-induced NF- $\kappa$ B activity was attenuated early in the viral replication cycle by LASAG in highly pathogenic avian influenza virus strains leading to reduced viral titers, decreased viral protein accumulation, viral ribonucleic acid (RNA) synthesis, and impaired formation of viral replication transcription complexes.<sup>88</sup>

Nebulized LASAG three times a day to achieve a total daily alveolar dose of 133.5 mg in 24 patients afflicted with severe influenza was found to be associated with faster alleviation of symptoms versus controls.<sup>85</sup> LASAG is currently available and approved for intravenous administration in Germany. Up to 750 mg twice-daily LASAG administration has been shown to be well tolerated and to decrease inflammation markers when inhaled by patients with chronic obstructive pulmonary disease.<sup>85</sup>

An inhaled nanoparticle aspirin formulation that can be administered as a dry powder inhaler to enhance the speed of platelet inhibition is under development.<sup>88</sup> In a recent pilot, Phase 1, open-label, single dose-escalation study, the inhalation formulation of aspirin compared to chewing and swallowing soluble aspirin formulation is associated with earlier drug exposure (2–4 minutes versus 30 minutes, respectively) and earlier greater inhibition of arachidonic acid-induced platelet inhibition (2 minutes versus 40 minutes, respectively).<sup>89</sup> Treatment with the above two inhaled formulations of aspirin may attenuate serious symptoms in COVID-19 during initial acute conditions.

With respect to safety concerns, high-dose aspirin as an adjunctive therapy with other agents has been associated with elevated risk for bleeding particularly gastrointestinal bleeding. COVID-19 is characterized by hypercoagulability and prothrombotic nature, the risk bleeding may not be a major concern. The risk bleeding may be lower with inhaled aspirin formulation.

## Conclusions

Aspirin exerts plural effects that position it as an ideal pharmacologic agent to treat COVID-19. These effects include anti-inflammation, antithrombosis, and antiviral properties that are mediated through two key pathways: the inhibition of IKK kinase and the inhibition of COX. Other non-canonical pathways such as acetylation of endothelial nitric oxide synthase (eNOS) NOS, endothelial protection and upregulation of heme oxygenase-1 that are involved in protection from oxygen radicals may also play a role in modulating the inflammatory and prothrombotic responses observed in COVID-19. Optimal dose of aspirin to treat COVID-19 is critically dependent on the disease state. In patients who are hospitalized for COVID-19, readily available high-dose aspirin (325 mg/day) in addition to other standard medications may attenuate high inflammation and thrombotic risk. Aspirin effects may be enhanced by aerosol delivery to the lung that is the key organ damaged in COVID-19. The inhaled formulation with rapid effects may be an effective strategy during the critical initial course of the disease, but studies are ongoing with these formulations. For long-term treatment in post-COVID-19 scenario, 325 mg per day can be used to prevent recurrent thrombotic event occurrences. Further clinical studies are required to support this suggestion. However, low dose (75–162mg/day) is not optimal to attenuate the high inflammation and platelet function as has been demonstrated in recent pharmacodynamic studies. Therefore, the low-dose aspirin may not be associated with significant clinical benefits. Elevated bleeding risk associated with higher dose of aspirin may be a major limitation. COVID-19 is a hypercoagulable disease with lower risk for bleeding, the net outcomes may favor towards attenuating thrombotic risk. In the recent prospective ROCOVERY trial, aspirin use was associated with an elevated bleeding risk. There are many limitations to this study discussed earlier and the study results have not been published in a peer reviewed journal yet.

Being a widely available, inexpensive drug with multiple proven benefits, aspirin can be exploited globally, particularly in underserved communities and remote areas of the world, to combat the ongoing COVID-19 pandemic. Furthermore, by being uninfluenced by viral mutagenesis in its unique mechanisms of action, aspirin holds the promise of being a strong ally in the fight against our new enemy and other viruses that will certainly follow.

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All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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