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REVIEW

NLRP3 inflammasome and interleukin-1 contributions to COVID-19-associated coagulopathy and immunothrombosis

Nicola Potere [1]*, Evan Garrad[2,3], Yogendra Kanthi[2], Marcello Di Nisio[1], Gilles Kaplanski[4,5], Aldo Bonaventura[6], Jean Marie Connors[7], Raffaele De Caterina [8,9,10], and Antonio Abbate [11]*

[1]Department of Medicine and Ageing Sciences, 'G. d'Annunzio' University, Via Luigi Polacchi 11, Chieti 66100, Italy; [2]Laboratory of Vascular Thrombosis and Inflammation, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA; [3]University of Missouri School of Medicine, Columbia, MO, USA; [4]Aix-Marseille Université, INSERM, INRAE, Marseille, France; [5]Division of Internal Medicine and Clinical Immunology, Assistance Publique - Hôpitaux de Marseille, Hôpital Conception, Aix-Marseille Université, Marseille, France; [6]Department of Internal Medicine, Medicina Generale 1, Medical Center, Ospedale di Circolo e Fondazione Macchi, ASST Sette Laghi, Varese, Italy; [7]Division of Hematology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [8]University Cardiology Division, Pisa University Hospital, Pisa, Italy; [9]Chair and Postgraduate School of Cardiology, University of Pisa, Pisa, Italy; [10]Fondazione Villa Serena per la Ricerca, Città Sant'Angelo, Pescara, Italy; and [11]Robert M. Berne Cardiovascular Research Center, Department of Medicine, Division of Cardiovascular Medicine, University of Virginia, 415 Lane Rd (MR5), PO Box 801394, Charlottesville, VA 22903, USA

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Abstract

Immunothrombosis—immune-mediated activation of coagulation—is protective against pathogens, but excessive immunothrombosis can result in pathological thrombosis and multiorgan damage, as in severe coronavirus disease 2019 (COVID-19). The NACHT-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome produces major proinflammatory cytokines of the interleukin (IL)-1 family, IL-1β and IL-18, and induces pyroptotic cell death. Activation of the NLRP3 inflammasome pathway also promotes immunothrombotic programs including release of neutrophil extracellular traps and tissue factor by leukocytes, and prothrombotic responses by platelets and the vascular endothelium. NLRP3 inflammasome activation occurs in patients with COVID-19 pneumonia. In preclinical models, NLRP3 inflammasome pathway blockade restrains COVID-19-like hyperinflammation and pathology. Anakinra, recombinant human IL-1 receptor antagonist, showed safety and efficacy and is approved for the treatment of hypoxaemic COVID-19 patients with early signs of hyperinflammation. The non-selective NLRP3 inhibitor colchicine reduced hospitalization and death in a subgroup of COVID-19 outpatients but is not approved for the treatment of COVID-19. Additional COVID-19 trials testing NLRP3 inflammasome pathway blockers are inconclusive or ongoing. We herein outline the contribution of immunothrombosis to COVID-19-associated coagulopathy, and review preclinical and clinical evidence suggesting an engagement of the NLRP3 inflammasome pathway in the immunothrombotic pathogenesis of COVID-19. We also summarize current efforts to target the NLRP3 inflammasome pathway in COVID-19, and discuss challenges, unmet gaps, and the therapeutic potential that inflammasome-targeted strategies may provide for inflammation-driven thrombotic disorders including COVID-19.

Keywords Coagulation • COVID-19 • Endotheliopathy • Interleukin-1 • NLRP3 inflammasome • Pyroptosis • Thrombosis

1. Introduction

Innate immunity is an essential first-line defence against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] Dysregulated innate immune responses can be however detrimental, resulting in exaggerated release of proinflammatory cytokines that exacerbate tissue damage.[1,2] Hyperinflammation in coronavirus disease 2019 (COVID-19) has been indeed linked to the development of acute respiratory distress syndrome (ARDS), thrombosis, and multiorgan injury.[2,3]

Inflammation and coagulation are closely intertwined.[4,5] In order to limit pathogen replication and spread, innate immunity triggers the coagulation system including the blood coagulation cascade, platelets, and the vascular endothelium in an evolutionarily conserved process known as immunothrombosis.[5–7] Nevertheless, excessive immunothrombosis can promote coagulopathy, leading to thrombosis and tissue ischaemia.[5–7] The clinical relevance of immunothrombosis in COVID-19 may be attested by the increased incidence of macrovascular, primarily venous, and microvascular thrombosis observed in patients with severe disease.[8–11] COVID-19 lungs exhibit diffuse alveolar damage with abundant inflammatory exudates and leukocyte infiltration next to endothelialitis, microangiopathy, and fibrin deposits.[9–11] Several lines of evidence suggest that hyperinflammation contributes to excessive activation of immunothrombosis pathways sustaining

* Corresponding author: Tel: +39 0871358255, Fax: +39 0871358887, E-mail: nic.potere@gmail.com (N.P.); Tel: +1 434 2979449, Fax: +1 434 9242828, E-mail: antonio.abbate@virginia.edu (A.A.)

the hypercoagulability, endotheliopathy, and thrombocytopathy occurring in COVID-19-associated coagulopathy (CAC).[5–8,11]

The intracellular innate immune receptor NACHT-, LRR-, and pyrin domain-containing protein 3 (NLRP3) is a regulator of inflammatory responses to sterile and non-sterile noxious stimuli including viruses.[12,13] NLRP3 inflammasome activation leads to production of biologically active interleukin (IL)-1β and IL-18, two potent, primordial proinflammatory cytokines with prothrombotic activity, and can induce pyroptosis, a proinflammatory form of lytic cell death.[12–14] Prompt and adequate activation of innate immunity is a prerequisite for adaptive immune responses, crucial to control infection, and to achieve immunogenicity following infection or vaccination.[1] Nevertheless, dysregulated inflammatory responses, in part sustained by exuberant NLRP3 inflammasome activation, could be detrimental and may favour COVID-19 progression as well as the development of CAC and COVID-19-associated thrombosis.[1–3,15,16]

2. Immunothrombosis and CAC

Immunothrombosis, considered an intravascular effector of innate immunity, refers to complex networks of cellular interactions and molecular pathways aimed at limiting pathogen survival and spread.[5,17] Conversely, thromboinflammation describes the immune-mediated activation of coagulation under sterile conditions.[5,17] These processes are sustained by the activation of immune cells that release a number of proinflammatory mediators, with crucial contributions from platelets and the endothelium, that synergistically prime the activation of blood coagulation factors.[5,17]

Distinctive features of CAC have been widely documented (*Figure 1*).[7,18–20] These include overproduction of procoagulant factors such as tissue factor (TF), factors VIII and V, fibrinogen and von Willebrand factor (VWF), in parallel with downregulation of endogenous anticoagulants (e.g. TF pathway inhibitor, protein C, and antithrombin), resulting in a hypercoagulable *milieu* capable of increased thrombin generation.[7,18–20] The increased release of proinflammatory mediators can affect leukocyte, endothelial, and platelet responses both locally (i.e. in the lungs) and systemically.[5–7] Among multiple proinflammatory cytokines, IL-1β and downstream IL-6 can induce procoagulant programmes and suppress anticoagulant mechanisms in a concentration-dependent manner[21–23] and have been implicated in the pathogenesis of CAC.[6,15,16,24]

Imbalance in fibrinolysis has also been described in CAC.[7,20] The fibrinolytic agents tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) physiologically work to prevent fibrin accumulation.[20] In severe COVID-19, increased levels of plasminogen activator inhibitor-1 and thrombin-activatable fibrinolysis inhibitor tend to outbalance tPA/uPA activity, with resultant intra-alveolar fibrin deposition.[25–27] The extremely high levels of D-dimer observed in severe COVID-19 might not only reflect fibrinolytic activation secondary to clot formation but also be indicative of the intensity of the hyperinflammatory response, as proinflammatory cytokines including IL-1β and tumour necrosis factor (TNF) can induce endothelial expression of both fibrinolytic and antifibrinolytic molecules.[20] Eventually, the net increase in intra-alveolar fibrin production can be considered the culmination of immunothrombosis since fibrin enables the entrapment of the invading virus.[5–7,20]

Endotheliopathy and thrombocytopathy are other major features of CAC.[6,7,28] SARS-CoV-2 has the ability to directly infect the vascular endothelium decreasing its physiological antithrombotic properties. Activated endothelial cells can also release proinflammatory cytokines (e.g. IL-1α, IL-1β, IL-6, and TNF) acting in an autocrine and paracrine fashion to systemically induce a prothrombotic endothelial phenotype.[6,7,28] Increased endothelial expression of TF and VWF facilitate the assembly of coagulation modules and recruitment of platelets.[29,30] Studies have shown that increases in circulating VWF in COVID-19 patients are associated with consumption in ADAMTS13 (disintegrin and metalloproteinase with a thrombospondin Type 1 motif, member 13), resulting in the generation of ultralarge VWF multimers, enhanced platelet–endothelial interactions and thrombotic microangiopathy.[30,31] Increased procoagulant platelets and platelet–monocyte aggregates were associated with worse COVID-19 prognosis.[32,33] Plasma from COVID-19 patients was found to induce activation of naïve platelets. Platelets isolated from severely ill COVID-19 patients induced TF expression by monocytes from healthy donors, which was suppressed by inhibiting platelet P-selectin and integrin αIIb/β3, thus suggesting that altered platelet–endothelial and platelet–monocyte interactions contribute to CAC.[32] Further experiments revealed that TF produced by SARS-CoV-2-infected cells potently activates platelets isolated from healthy subjects.[34] This phenomenon required coagulation factors X, VII, and II and involved thrombin-mediated activation of platelet protease-activated receptors.[34] Platelets from COVID-19 patients also exhibit procoagulant phenotypic and functional profiles, including constitutive expression of P-selectin, tendency to form aggregates with leukocytes, and enhanced release of procoagulant extracellular vesicles, proinflammatory cytokines, chemokines, and growth factors.[33,35,36]

Activated platelets also contribute to the generation of neutrophil extracellular traps (NETs).[5,7,28,37] NETs are extruded web-like structures composed of a DNA backbone, histones, and proteolytic enzymes with antibacterial properties such as neutrophil elastase, myeloperoxidase, and cathepsin G.[37] NETs promote thrombosis through multiple mechanisms including activation of blood coagulation factors XII and II, inhibition of anticoagulant pathways (e.g. TF pathway inhibitor, and antithrombin), and recruitment of platelets and leukocytes, and by providing a scaffold, resistant to fibrinolysis, for thrombus stability and enlargement.[37] Increased circulating NETs-derived products including cell-free DNA, myeloperoxidase, and citrullinated histone H3 were detected in COVID-19 patients who developed thrombosis.[38] Histopathology of COVID-19 organs also demonstrated thrombi enriched with abundant NETs and neutrophil–platelet aggregates.[39–41] In addition, sera and neutrophils isolated from patients with severe COVID-19 displayed high basal NETs levels, and incubation of healthy neutrophils with COVID-19 plasma potently triggered NETosis.[38–40,42]

Taken together, these and other observations suggest that hyperinflammatory responses occurring in severe COVID-19 can trigger excessive activation of immunothrombosis pathways contributing to endotheliopathy, thrombocytopathy, and hypercoagulability, eventually resulting in microvascular and macrovascular thrombosis.[5–7]

3. The NLRP3 inflammasome pathway

NOD-like receptors (NLRs) and Toll-like receptors (TLRs) are sets of phylogenetically conserved receptors, termed pattern recognition receptors (PRRs), that mediate innate immunity by binding pathogen-associated molecular-patterns (PAMPs) and damage-associated molecular-patterns (DAMPs).[43] NLRs include the intracellular protein NLRP3.[43] Upon stimulation, NLRP3, along with the apoptosis-associated speck-like protein containing a CARD (ASC) adaptor protein and caspase-1, form the NLRP3 inflammasome.[44,45] This macromolecular effector complex produces active cytokines of the IL-1 family including IL-1β and IL-18 and can induce pyroptosis.[12–14] NLRP3 is the most studied inflammasome; however, other inflammasomes (e.g. NLRP1 and AIM2) have been characterized and reviewed elsewhere.[13]

The sequence of events leading to assembly and activation of the NLRP3 inflammasome is finely regulated at multiple levels. In most cells, NLRP3 inflammasome activation may require two distinct signals:[46] priming, which leads to the production of inflammasome components and substrates, and triggering, which culminates with inflammasome activation (see Supplementary material online, *Figure S1*).[12,13] Priming is promoted by a wide array of PAMPs and DAMPs that activate PRRs such as TLRs or other receptors, resulting in the translocation of nuclear factor-κB (NF-κB) into the nucleus, promoting the transcription of inflammasome components and substrates.[12,13] The second trigger of inflammasome assembly can be sourced by several stimuli including ATP-mediated activation of the purinoreceptor P2X7, reactive oxygen species (ROS) as well as mitochondrial and lysosomal destabilization.[12,13] Altogether, these changes reduce intracellular potassium, which is detected by NLRP3 that oligomerizes to form the

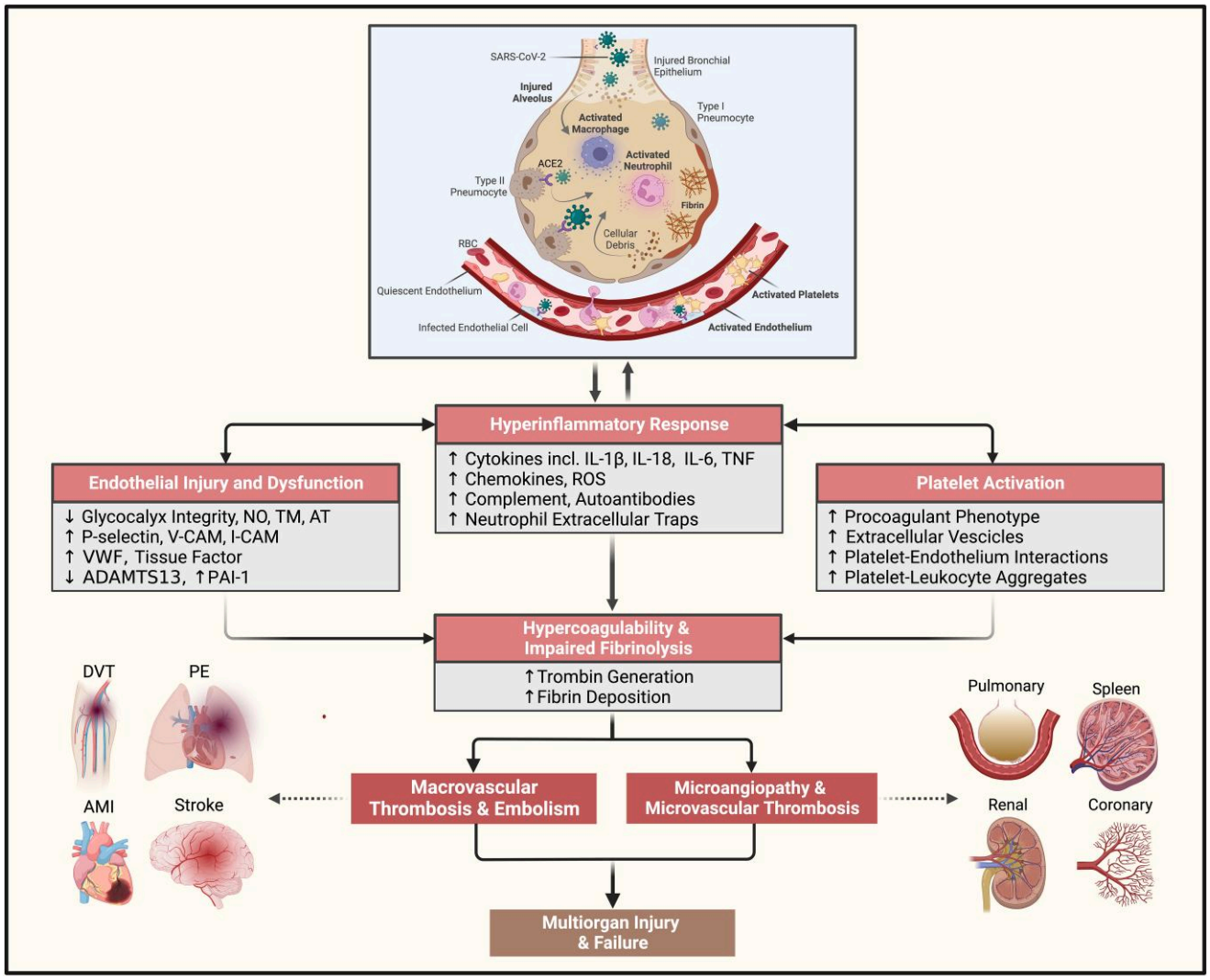

Figure 1 Overview of the pathogenetic mechanisms implicated in COVID-19-associated coagulopathy. Abbreviations: ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin Type 1 motifs, member 13; ACE2, angiotensin-converting enzyme 2; AMI, acute myocardial infarction; AT, antithrombin; DVT, deep vein thrombosis; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; NO, nitric oxide; PAI-1, plasminogen activator inhibitor-1; PE, pulmonary embolism; ROS, reactive oxygen species; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TM, thrombomodulin; TNF, tumour necrosis factor; VCAM-1, vascular cell adhesion molecule 1; VWF, von Willebrand factor. Figure created with BioRender.com.

inflammasome.[12,13] NLRP3 oligomers engage ASC into filaments enabling recruitment and subsequent autoactivation of caspase-1. Caspase-1 then cleaves pro-IL-1β and pro-IL-18 into mature IL-1β and IL-18.[12–14] Once released extracellularly, pro-IL-1β can also be processed by proteolytic enzymes into its mature form, independently of caspase-1.[14,47] Conversely, pro-IL-1α lacks a caspase-1 processing site and is already active in its precursor form but can be cleaved into IL-1α by several enzymes that can augment its biological activity.[48] When released upon necrotic cell death, IL-1α can serve as an 'alarmin' and induce IL-1β[49,50] or function upon cell–cell contact when anchored to the cell membrane.[51]

Caspase-1 also cleaves gasdermin D (GSDMD) to produce N-terminal fragments that migrate to the cell membrane forming pores that enable extracellular release of IL-1β and IL-18.[12–14] Activation of caspase-1 and GSDMD can also induce pyroptosis.[12–14] Unlike other forms of programmed cell death, pyroptosis is characterized by cell swelling and rupture, with release of intracellular proinflammatory content. Pyroptosis can also occur through non-canonical inflammasome pathways involving caspase-4/caspase-5 in humans and caspase-11 in mice.[52]

Once released, IL-1β and IL-18 exert pleiotropic effects by binding, respectively, to the IL-1 receptor Type I (IL-1RI) and IL-18 receptor α (IL-18Rα) on target cells, leading to NF-κB activation and subsequent transcription of a wide array of proinflammatory genes.[14,53] IL-1α also binds to IL-1RI.[14,48] Endogenously occurring receptor inhibitors, namely, IL-1 receptor antagonist (IL-1Ra) and IL-18 binding protein (IL-18BP), can antagonize IL-1RI and IL-18Rα, respectively.[14,53–55] Several other regulatory mechanisms that modulate NLRP3 inflammasome activation and IL-1 signaling are detailed elsewhere.[12–14,43,48,52,53,56]

4. NLRP3 inflammasome activation and hyperinflammation in COVID-19

Marked elevations in IL-6 and C-reactive protein (CRP), inflammatory biomarkers downstream of IL-1β widely used for cardiovascular risk stratification,[57] have been observed in severely ill patients with COVID-19 and

predict adverse outcomes.[58,59] Increased levels of IL-1β were found in monocytes and sera from COVID-19 patients, however, they did not strictly correlate with disease severity.[60–64] This could be related, at least in part, to the extremely short half-life of IL-1β and is also observed in other IL-1β-driven diseases.[15,65] Because of its longer half-life, IL-1Ra, induced by IL-1β, may serve as a proxy of IL-1β activity.[14,65,66] Serum IL-1Ra levels correlate with COVID-19 severity.[63,67,68] Accordingly, analyses of bronchoalveolar lavages, reflecting the pulmonary microenvironment, detected marked IL-1β upregulation.[69,70] Increased IL-1β expression was also demonstrated in the lungs of patients with fatal COVID-19.[71,72] Unlike IL-1β, production of IL-18 strictly depends on inflammasome activation.[14,53] Plasma IL-18 concentrations correlate with COVID-19 severity and predict mortality.[63,67,68,73]

Multiple studies provided direct evidence of NLRP3 inflammasome activation in patients with COVID-19.[73–79] NLRP3 inflammasome genetic variants have been associated with worse disease severity,[80] and single-cell transcriptome analysis of airway fluids from COVID-19 patients revealed elevated expression of inflammasome-related genes.[81] Peripheral blood monocytes from COVID-19 patients exhibit distinctive features of inflammasome activation.[73–75,79,82] Further experiments suggested that monocytes were most responsive to NLRP3 inflammasome stimulation *in vitro*, while selected subsets of neutrophils and granulocytes had defects in inflammasome activation, presumably reflecting immune exhaustion during advanced stages of COVID-19.[78] Importantly, this inflammasome signature was shown to correlate with COVID-19 severity and predict adverse clinical evolution.[78,83] Consistent with these observations, overexpression of inflammasome pathway components has been demonstrated in lung tissues from COVID-19 patients, primarily registering in leukocytes and, to a lesser extent, in pneumocytes and vascular endothelial cells.[73,76,77,79]

Cellular and animal experiments indicate that distinct SARS-CoV-2 constituents, including the spike protein, may trigger NLRP3 inflammasome activation in monocytes and macrophages, amplifying the inflammatory response and contributing to COVID-19 pathogenesis.[64,79,84–88] The viral entry receptor angiotensin-converting enzyme 2 (ACE2), transmembrane protease serine 2 (TMPRSS2), and Fcγ receptors were shown to mediate NLRP3 inflammasome activation following SARS-CoV-2 exposure, or, alternatively, SARS-CoV-2 can directly interact with the inflammasome intracellularly.[85,86,89] One report suggested that SARS-CoV-2 RNA could induce monocyte IL-1β release through caspase-1/caspase-8, potassium efflux, and autophagy, independently from GSDMD and pyroptosis.[86] In monocytes, the nucleocapsid protein of SARS-CoV-2 was found to directly bind NLRP3 monomers, facilitating inflammasome assembly and activation.[89] Injection of SARS-CoV-2 nucleocapsid protein into mice markedly increased IL-1β and IL-6 plasma levels, resulting in aggravated lung injury and worse survival.[89] Murine overexpression of viroporin ORF3a also induced robust NLRP3 activation, followed by GDSMD cleavage and IL-1β release.[90] SARS-CoV-2 glycoproteins elicited similar effects in cultured human macrophages, which were suppressed in NLRP3-/GSDMD-knockout cells.[91] Conversely, other studies found that monocyte exposure to either SARS-CoV-2 envelope or nucleocapsid proteins could exert inhibitory effects on the inflammasome.[92,93] Since *in vitro* exposure to distinct SARS-CoV-2 constituents might elicit different cellular responses compared to exposure to the viable virus and that endotoxin contamination could not be excluded, additional studies using *in vivo* infection models are warranted. The effects of distinct SARS-CoV-2 variants on NLRP3 inflammasome activation and their clinical implications also remain to be elucidated.

Assembly of the NLRP3 inflammasome after SARS-CoV-2 exposure was also reported in epithelial and endothelial cells as well microglial and hematopoietic stem cells, suggesting that multiple cell lineages besides leukocytes can sustain hyperinflammation following SARS-CoV-2 infection, potentially contributing to the extra-pulmonary manifestations of COVID-19.[77,85,90,94] Persistent inflammasome pathway activation is found in monocytes and macrophages derived from convalescent COVID-19 patients after several weeks from acute infection and in subjects recovering from mild disease.[64,83] Additional studies are however required to evaluate the contribution of the NLRP3 inflammasome pathway to the pathogenesis of long COVID-19.

Besides direct SARS-CoV-2 cytotoxicity, indirect viral effects and host-related mechanisms may sustain NLRP3 inflammasome activation in COVID-19.[15,16] Lysis of virus-infected cells induces massive generation of DAMPs including ATP, ROS, complement, and a plethora of proinflammatory mediators. Among these, IL-1α released from damaged epithelial and endothelial cells is a robust inducer of inflammatory responses by adjacent and remote cells.[95,96] Such paracrine and endocrine mechanisms can therefore contribute to the systemic, inflammatory manifestations of COVID-19 (*Figure 2*).[15,16] This notion might be supported by the fact that supernatants collected from SARS-CoV-2-infected epithelial cells triggered intense IL-1β production by naïve macrophages.[97] In addition, patients with comorbidities such as diabetes, obesity, or heart disease, characterized by basal NLRP3 inflammasome activation, tend to be more prone to experience hyperinflammatory responses and adverse outcomes, suggesting that pre-existing inflammation may potentially prime a favourable substrate for inflammasome hyperactivation when infection occurs.[15,16]

Abrogation of the NLRP3 inflammasome pathway suppresses immune hyperactivation and mitigates COVID-19 pathology.[82–84,88–90,98] The selective NLRP3 blocker MCC950 inhibited spontaneous and lipopolysaccharide-induced secretion of IL-1β and IL-18 by monocytes isolated from COVID-19 patients. Similar effects were observed in monocytes isolated from COVID-19 patients receiving anakinra, a recombinant human IL-1Ra, blocking the activity of both IL-1α and IL-1β.[82] In a ACE2 humanized mouse model of SARS-CoV-2 infection, genetic deletion or pharmacological inhibition of the NLRP3 inflammasome reduced proinflammatory cytokine release, cell death, and viral load, alleviating lung injury.[98] In another study, Sefik *et al.* demonstrated that macrophage NLRP3 inflammasome activation is necessary for COVID-19-like immunopathology.[88] The same group also showed that selective NLRP3 and caspase-1 inhibition reverses chronic lung injury but associates with higher viral titres.[88] In addition, the NLRP3 inflammasome was shown to mediate the innate-adaptive immune crosstalk during SARS-CoV-2 infection and following mRNA vaccination.[64,87] These observations collectively suggests that adequate NLRP3 inflammasome pathway activation aids in containing infection and achieving immune memory and vaccine immunogenicity, whereas excessive activation can sustain hyperinflammation and immunopathology. Additional research is necessary to clarify the precise triggers together with virus- and host-related factors regulating NLRP3 inflammasome pathway activation and to establish the exact contribution of distinct inflammasome pathway components to disease pathogenesis.[15,16]

5. NLRP3 inflammasome pathway activation as a potential pathogenetic mechanism contributing to immunothrombosis and CAC

The association between infection, inflammation, and thrombosis has long been established.[4,5,99] In immunothrombosis, innate immune cells—primarily macrophages and neutrophils—interact with platelets and the endothelium to synergistically activate the coagulation system.[4,5,17,99] Thrombosis is frequent in severe COVID-19 and affects prognosis.[8–11,100,101] Increased levels of CRP at hospitalization independently associate with in-hospital venous thromboembolism, critical illness, and mortality.[59,102] Higher pre-discharge CRP also predicts the occurrence of post-discharge deep vein thrombosis,[103] suggesting that both acute and residually heightened inflammation may promote CAC, augmenting thrombotic risk in the short and longer terms.[104,105]

The prothrombotic effects of inflammasome-derived IL-1β and IL-18 have been widely recognized.[106] IL-1β and IL-18 released by damaged cells increase vascular permeability and induce recruitment and transmigration

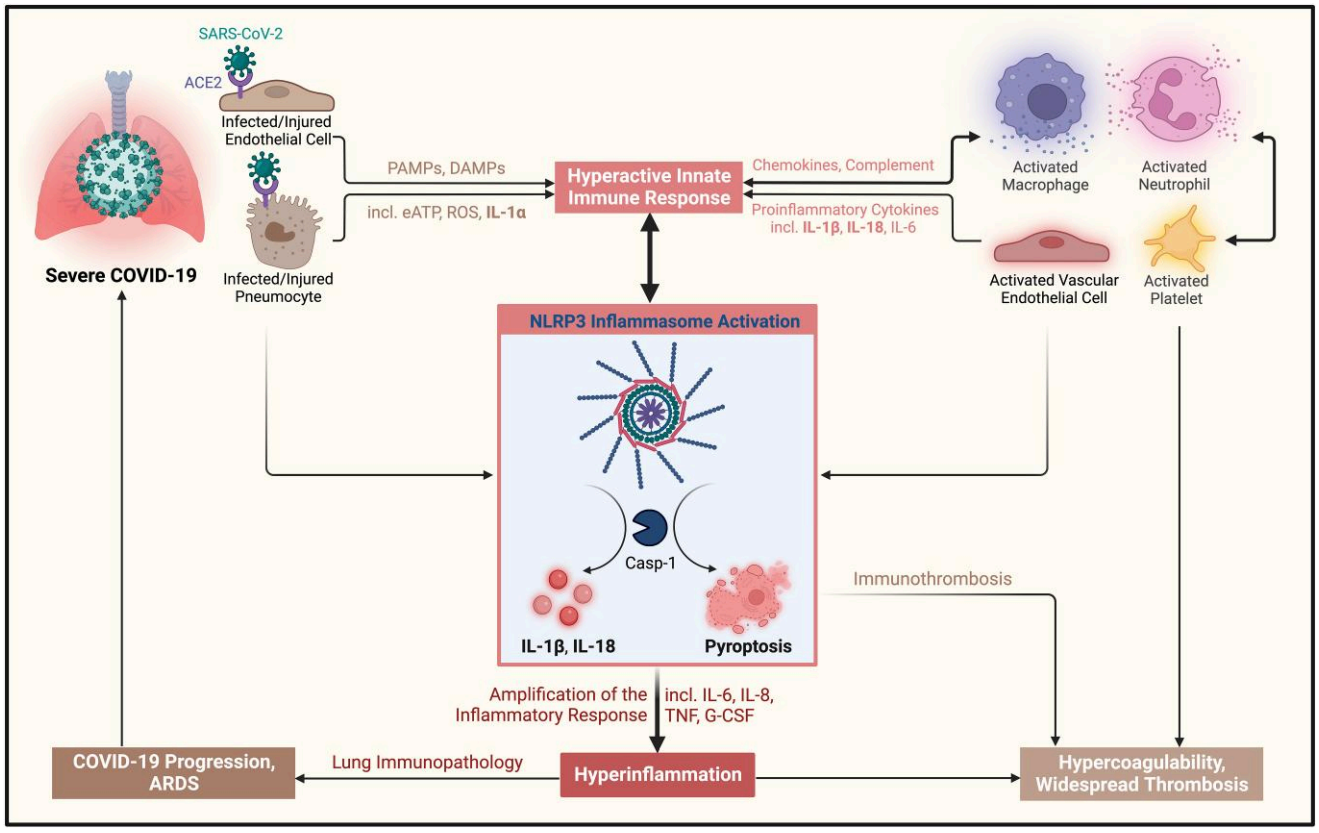

Figure 2 NLRP3 inflammasome pathway activation in COVID-19-associated hyperinflammation and lung immunopathology. Abbreviations: ACE2, angiotensin-converting enzyme 2; ARDS, acute respiratory distress syndrome; Casp-1, caspase-1; DAMPs, damage-associated molecular patterns; G-CSF, granulocyte colony-stimulating factor; IL, interleukin; eATP, extracellular ATP; PAMPs, pathogen-associated molecular-patterns; ROS, reactive oxygen species; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. Figure created with BioRender.com.

of macrophages and neutrophils at the site of injury. In turn, IL-1β and IL-18 produced by leukocytes can activate the endothelium and enhance platelet recruitment, activation, and aggregation.[106] In a feed-forward loop, activated endothelial cells and platelets can further secrete proinflammatory IL-1 cytokines sustaining immunothrombosis programmes.[106] Although inflammasome activation and IL-1β are established players in arterial thrombosis,[5,107] their roles in venous thrombosis under sterile- and pathogen-mediated inflammatory environments have only recently begun to be elucidated.[99]

In macrophages, NLRP3 inflammasome activation and subsequent caspase-1 cleavage induces abundant release of highly procoagulant microvesicles containing TF (*Figure 3*).[108] In a murine endotoxaemia model, inflammasome activation triggered, through caspase-1/caspase-11 and GSDMD, monocyte pyroptosis with subsequent TF release and widespread thrombosis.[109] GSDMD membrane pore formation also favours intracellular calcium entry, which promotes externalization of phosphatidylserine and enhances TF procoagulant activity.[110] Activation of the inflammasome, caspase-1/-11 and GSDMD might therefore represent IL-1-independent mechanisms sustaining immunothrombosis. This concept might be supported by the extreme levels of TF-rich extracellular vesicles found in the plasma of COVID-19 patients, which positively correlated with the intensity of the inflammatory response and mortality.[29,111,112] A role for caspase-11 and its human homolog caspase-4 in promoting immunothrombosis in COVID-19 has been identified. Compared to GSDMD-deficient and wide-type mice, SARS-CoV-2-infected mice lacking caspase-11 exhibited restrained lung neutrophil recruitment and expression of IL-1β and IL-6 and were protected from vascular damage including thickening, obliteration, angiogenesis, and neovascularization.[113] Additionally, caspase-11 deficiency drastically reduced pulmonary expression of VWF, while preserving Kruppel-like factor 2, an endothelial transcription factor that maintains vascular integrity.[113] Mice deficient in caspase-11 also exhibited more pronounced defects in neutrophil movement and function and suppressed NETosis.[113]

NETs centrally contribute to the immunothrombotic pathogenesis of microvascular and macrovascular thrombosis.[5,7,37–39,99] Activation of the NLRP3 inflammasome pathway in neutrophils can induce, through GSDMD, the release of NETs.[114] In turn, NETs sustain inflammasome activation and production of IL-1β and IL-18.[114–117] NLRP3 inflammasome assembly was demonstrated in neutrophils derived from peripheral blood and tracheal aspirates of subjects with severe COVID-19.[118] NLRP3 inflammasome mostly assembled in neutrophils with intact polylobulated nuclei, correlating with histone H3 citrullination and localizing with the microtubule organizing centre long before NETosis, which suggests an upstream role for the inflammasome in NETs formation during severe COVID-19.[118] In another study, blood neutrophils from COVID-19 patients displayed elevated expression of active caspase-1/caspase-4 and GSDMD, which accumulated in the plasma membrane and NETs, with higher expression detected in severely versus moderately ill patients.[81] A positive relationship between GSDMD and NETs levels was also found in the sera of these patients.[81] Importantly, pharmacological inhibition of caspases or GSDMD abrogated NETs release by SARS-CoV-2-infected neutrophils.[81] Notably, abrogation of the NLRP3 inflammasome pathway was previously shown to drastically reduce NETosis and thrombogenesis in murine vein thrombosis models.[119,120] IL-1α also contributes to enhance

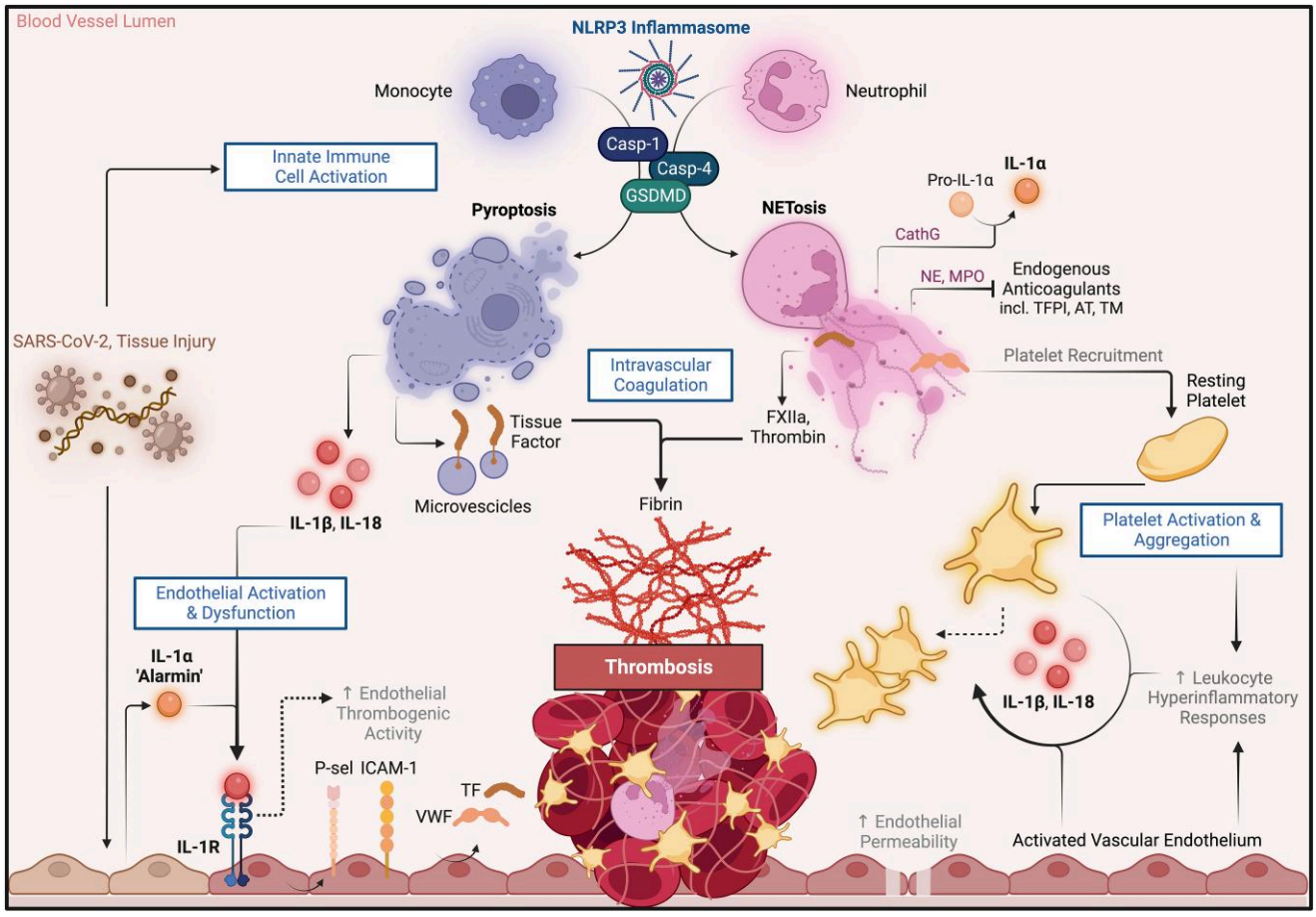

Figure 3 Proposed role of NLRP3 inflammasome pathway activation in COVID-19-associated coagulopathy and immunothrombosis. Abbreviations: AT, antithrombin; FXIIa, factor XIIa; CathG, cathepsin G; GSDMD, gasdermin D; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; IL-1R, interleukin-1 receptor; MPO, myeloperoxidase; NE, neutrophil elastase; NET, neutrophil extracellular trap; P-sel, P-selectin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TF, tissue factor; TFPI, tissue factor pathway inhibitor; TM, thrombomodulin; VWF, von Willebrand factor. Figure created with BioRender.com.

NETs-induced endothelial activation and thrombogenicity by inducing the expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and TF, which is abolished in the presence of antibodies against IL-1α or IL-1Ra.[121] Interestingly, cathepsin G released with NETs potentiated the prothrombotic endothelial effects of IL-1α by cleaving the pro-IL-1α precursor into its mature form.[121]

Endothelial cells and platelets represent, with innate immune cells, key cellular drivers of immunothrombosis.[28,99] These cells also constitute major compartments for NLRP3 assembly and critical targets for IL-1 activity.[122–125] Activation of the NLRP3 inflammasome was shown to induce, through TLR4 and Bruton's tyrosine kinase, platelet activation, and aggregation, leading to thrombus formation.[126,127] Platelet–monocyte interactions engage reciprocal activation loops feeding immunothrombosis in COVID-19.[32,33] Monocytes from patients with severe COVID-19 displayed enhanced platelet binding and IL-1β secretion in response to P-selectin and fibrinogen.[33] Platelets also potentiate monocyte immunothrombotic responses including cytokines release and TF expression during SARS-CoV-2 infection.[33] In particular, platelet adhesion was proposed by Hottz *et al.* as an upstream event inducing proinflammatory cytokine secretion and TF expression, while TF sustains immunothrombosis by triggering additional IL-1β and TNF release.[33]

Endothelial NLRP3 inflammasome activation and proinflammatory IL-1 cytokines are also able to promote vascular permeability and dysfunction.[128] Exposure of healthy human endothelial cells of different anatomical origins to exosomes isolated from COVID-19 patients induces robust increases in mRNA expression of NLRP3, caspase-1 and IL-1β, with activated caspase-1 being detected in the culture medium, suggesting that endothelial NLRP3 inflammasome activation contributes to the systemic endotheliopathy characteristic of CAC.[129] Proinflammatory IL-1 cytokines reportedly enhance the endothelial expression of adhesion molecules (e.g. P-selectin, ICAM-1, and VCAM-1) and procoagulant factors (e.g. VWF and TF) and reduce anticoagulant molecules (e.g. protein C and thrombomodulin).[21,22] In turn, TF and fibrin produced during thrombogenesis can work to potentiate IL-1-driven endothelial activation (*Figure 3*).[130,131] Importantly, interrupting this vicious circuit by abrogation of the NLRP3 inflammasome pathway or IL-1 signalling significantly reduces thrombus formation and propagation in preclinical models of venous thrombosis.[119,120,132–136]

6. NLRP3 inflammasome pathway blockade in COVID-19

The NLRP3 inflammasome and IL-1 cytokines play a central role in the pathogenesis of several rheumatologic and cardiovascular

diseases.[65,107,137–139] Agents targeting IL-1 are clinically available for the treatment of multiple inflammatory disorders.[65,138,139] Early in the pandemic, IL-1 blockers were repurposed for COVID-19. Initial data from observational studies suggested that anakinra and the anti-IL-1β monoclonal antibody canakinumab could blunt hyperinflammation associated with COVID-19, possibly improving outcomes.[140–142]

Trials with anakinra yielded overall favourable yet heterogeneous results (*Table 1*).[143–147] Findings from studies including the REMAP-CAP multiplatform trial showed that anakinra was safe and well tolerated but did not provide additional benefit over usual care in hospitalized patients with severe-to-critical COVID-19.[143–145] In the SAVE trial, addition of anakinra to usual care reduced progression to severe respiratory failure at Day 14 and mortality at Day 30 in patients with moderate-to-severe COVID-19 and elevated soluble urokinase plasminogen activator receptor (suPAR), an early indicator of hyperinflammation and predictor of COVID-19 progression.[146] A larger suPAR-guided trial with anakinra (SAVE-MORE) later showed that anakinra significantly shortened hospital stay and improved 28-day clinical status and mortality compared to placebo in COVID-19 patients without invasive mechanical ventilation.[147]

Canakinumab was also tested in COVID-19.[148–150] In the proof-of-concept Three C study enrolling 45 moderate-to-critically ill hospitalized COVID-19 patients with myocardial injury and elevated CRP, canakinumab was safe but did not significantly reduce time to clinical improvement.[148] In the CanCovDia trial, the effects of canakinumab versus placebo with regard to a composite of length of survival, ventilation, intensive care unit stay, and hospitalization were evaluated among 116 hospitalized COVID-19 patients with Type 2 diabetes and body mass index >25 kg/m^2.[149] In this study, addition of canakinumab to standard-of-care did not result in a statistically significant improvement in the primary outcome, while reducing systemic inflammation and the number of glucose-lowering drugs.[149] In the CAN-COVID trial that randomized 454 severely ill COVID-19 patients to receive canakinumab or placebo, canakinumab did not reduce progression to invasive mechanical ventilation at Day 29.[150] However, canakinumab was associated with a significant reduction of the composite of death, invasive mechanical ventilation, or use of other immunomodulators as rescue therapy, thus suggesting a biological efficacy.[150]

Colchicine, currently employed for the treatment of several inflammatory disorders including gout and pericarditis, is an alkaloid extracted from the *Colchicum autumnale*.[151] Originally only thought to block microtubule polymerization and leukocyte chemotaxis, colchicine possesses numerous anti-inflammatory effects including inhibition of NLRP3 inflammasome assembly and activation at clinically relevant concentrations, being regarded to as a non-selective inflammasome inhibitor.[151–154] The colchicine arm in the RECOVERY trial was interrupted prematurely due to futility.[155] In the ECLA PHRI COLCOVID trial, colchicine did not significantly decrease mechanical ventilation or 28-day mortality in severely ill COVID-19 patients.[156] Conversely, in the GRECCO study, colchicine improved time to clinical deterioration in patients with moderate-to-severe COVID-19.[157] In another small, placebo-controlled trial including moderately-to-severely ill subjects, colchicine shortened the duration of oxygen therapy and hospital stay.[158] The ColCORONA trial that enrolled 4488 outpatients with COVID-19, diagnosed by either polymerase chain reaction testing or clinical criteria, at risk for progression, clinical improvement did not reach statistical significance possibly due to the low number of events.[159] Although not meeting the study primary endpoint, in a prespecified analysis considering only patients with laboratory-confirmed COVID-19 ($n = 4159$), colchicine significantly reduced the composite of death and hospital admission compared to placebo.[159]

Multiple selective NLRP3 inflammasome blockers have been developed and tested in preclinical and early phase clinical studies, showing potential for efficacy across a wide range of inflammatory disorders.[137–139,160–162] Preclinical studies indicated that selective NLRP3 inflammasome inhibition mitigates COVID-19-like immune overactivation and pathology, suggesting that such therapeutic approach could be promising in humans.[82–84,88–90,98] In a recently completed phase 2a trial enrolling 143 hospitalized COVID-19 patients with impaired respiratory function, the selective NLRP3 inhibitor DFV890 did not reduce the primary endpoint of APACHE II score at Day 14 (or on day-of-discharge, whichever came first) as compared to standard-of-care alone.[163] DFV890 was however well tolerated with no safety concerns and associated with faster viral clearance, improved mechanical ventilation-free survival, and fewer fatal events at Day 28.[163] Additional trials testing NLRP3 inflammasome pathway inhibitors in COVID-19 are ongoing.

7. Targeting the NLRP3 inflammasome pathway in COVID-19: challenges, unmet gaps, and future perspectives

Most trials to date indicate that targeting the NLRP3 inflammasome pathway can be safe and well tolerated in patients with COVID-19.[143–150,156–159,163] Mixed results on the efficacy of these interventions have been however obtained, with some studies showing little or no clinical benefits.[143–145,150,155] As most trials were conceived during the initial stages of the pandemic, it is possible that the fewer-than-expected number of events made some of these studies underpowered.[164] Yet, the heterogenous concomitant use of drugs later proven to be effective including antivirals, glucocorticoids, IL-6 receptor blockers, and low-molecular-weight heparins, as either background or rescue therapies, may have blurred potential signals for clinical efficacy.[164,165] Notwithstanding these limitations, some trials provided positive results.[146,147,157–159] The European Medicines Agency recommended anakinra for selected hypoxaemic COVID-19 patients with elevated suPAR levels,[166] and the US Food and Drug Administration recently authorized its emergency use.[167] Colchicine prevented hospitalization and death in a selected subgroup of ambulatory patients with laboratory-confirmed, mild-to-moderate COVID-19 and slowed clinical deterioration in some patients with moderate-to-severe COVID-19.[157–159] Taken together, these data may prompt important considerations when addressing hyperinflammation in COVID-19. For instance, anti-inflammatory therapies could be detrimental if administered too early in the course of disease, as they may favour viral replication and reduce immune competence and memory. By contrast, delayed immunomodulation may be ineffective when massive cytokine release and organ injury already occurred. The inflammasome and IL-1 cytokines occupy an apical role in the inflammatory cascade (*Figure 4*).[57,138] In critical disease, where immune overactivation is likely sustained by several proinflammatory cytokines, targeting a single, upstream inflammatory pathway could be insufficient to blunt hyperinflammation and improve clinical outcomes. This might partially explain the benefits of glucocorticoids (e.g. dexamethasone), IL-6 receptor inhibitors (e.g. tocilizumab), and Janus kinase (JAK) inhibitors (e.g. baricitinib) with broader anti-inflammatory effects[165] and those of early NLRP3 inflammasome pathway blockade, which were not observed in critically ill patients. Identifying the optimal therapeutic window and refining strategies to select patient subgroups most likely responsive to NLRP3 inflammasome pathway inhibition remain major challenges warranting further investigation.

In recent years, seminal studies including the CANTOS, COLCOT, and LoDoCo2 trials have demonstrated antithrombotic effects by targeting the NLRP3/IL-1β axis with canakinumab or colchicine.[168–170] Notably, these agents provided significant antithrombotic protection on top of conventional antithrombotic drugs, without increasing the risk of bleeding.[168–170] COVID-19 trials testing inflammasome pathway blockade primarily focused on assessing inflammatory biomarkers and respiratory function. Although organ failure might be used as an indirect indicator of microvascular thrombosis, macrovascular thromboembolic events were not uniformly reported (mostly as safety outcomes) in these trials, with lack of systematic use of diagnostic strategies to detect thrombosis and standardized thromboprophylaxis regimens. In the GRECCO trial, colchicine was associated with reduced D-dimer.[157] A secondary analysis of the REMAP-CAP trial found that anakinra reduced major thromboembolic events comprising myocardial infarction, pulmonary embolism, ischaemic stroke, and systemic arterial embolism.[143] However, the overall low number of thromboembolic events recorded across the above-mentioned

Table 1 Main clinical trials targeting the NLRP3 inflammasome pathway in patients with COVID-19

Trial	Sample size	Study design				Intervention	Main inclusion criteria	Main findings	NCT/ PMID number
		R	DB	PC	M				
Anakinra									
CORIMUNO-ANA-1 (Cohort Multiple Randomized Controlled Trials Open-Label of Immune Modulatory Drugs and Other Treatments in COVID-19 Patients-Anakinra trial)	114	X			X	Anakinra 400 mg/day IV on Days 1–3, followed by 200 mg on day 4 and 100 mg on day 5	Hospitalized patients with severe COVID-19 with CRP ≥25 mg/L (59 received anakinra)	Anakinra did not significantly reduce MV or death at Day 4 and Day 14; no significant increase in AEs; trial interrupted prematurely	04341584
COV-AID (Treatment of COVID-19 Patients with Anti-interleukin Drugs)	342	X			X	Anakinra 100 mg daily SC for 28 days or until discharge	Hospitalized patients with severe COVID-19 and laboratory signs of cytokine release syndrome (112 received anakinra)	Anakinra did not shorten time to clinical improvement; SAEs events similar across study arms	04330638
REMAP-CAP (Randomized Embedded Multifactorial Adaptive Platform for Community-Acquired Pneumonia)	2216	X			X	Anakinra 300 mg IV on Day 1, followed by 400 mg/day for 14 days or until free from IMV or discharge from ICU	Hospitalized patients with critical COVID-19 (378 received anakinra)	Anakinra did not reduce median organ support-free days or mortality	02735707
SAVE (suPAR-Guided Anakinra Treatment for Management of Severe Respiratory Failure by COVID-19)	260				X	Anakinra 100 mg/day SC for 10 days	Hospitalized patients with moderate-to-severe COVID-19 with suPAR ≥6 ng/mL (130 received anakinra)	Anakinra significantly reduced progression to severe respiratory failure at Day 14 and mortality at Day 30	04357366
SAVE-MORE (suPAR-Guided Anakinra Treatment for Management of Severe Respiratory Failure by COVID-19)	594	X	X	X	X	Anakinra 100 mg/day SC for 10 days	Hospitalized patients with moderate-to-severe COVID-19 with suPAR ≥6 ng/mL (405 received anakinra)	Anakinra significantly shortened hospital stay and improved clinical status and survival at Day 28; fewer AEs in the anakinra arm	04680949
Canakinumab									
Three C (Canakinumab in COVID-19 Cardiac Injury)	45	X	X	X		Canakinumab 600 mg or 300 mg as single dose IV on Day 1	Hospitalized patients with moderate-to-critical COVID-19, elevated troponin and CRP >50 mg/L (15 received canakinumab 600 mg, 14 canakinumab 300 mg)	Canakinumab did not shorten time to clinical improvement at Day 14; AEs similar between groups	04365153
CanCovDia (Canakinumab in Patients with COVID-19 and Type 2 Diabetes)	116	X	X	X	X	Canakinumab (body weight adapted dose of 450–750 mg) as single dose IV on Day 1	Hospitalized patients with Type 2 diabetes and a BMI >25 kg/m^2 (58 received canakinumab)	Canakinumab did reduce the primary composite outcome (unmatched win-ratio approach based on length of survival, ventilation, ICU stay, and hospitalization at Day 29); reduced number of anti-diabetes drugs, IL-6, and hs-CRP; SAEs similar between two arms	04510493
CAN-COVID (Study of Efficacy and Safety of Canakinumab Treatment for CRS in Participants with	448	X	X	X	X	Canakinumab 450, 600, or 750 mg (depending on body	Hospitalized patients with severe COVID-19 and CRP ≥20 mg/L or ferritin	Canakinumab did not increase survival without IMV at Day 29 but was associated with improvement in the	04362813

Continued

Table 1 *Continued*

Trial	Sample size	Study design R	DB	PC	M	Intervention	Main inclusion criteria	Main findings	NCT/ PMID number
COVID-19-Induced Pneumonia)						weight) as a single IV dose on Day 1	≥600 μg/L (225 received canakinumab)	composite of death, IMV or use rescue therapy with tocilizumab or anakinra; fewer SAEs in canakinumab arm	
Colchicine									
RECOVERY (Randomised Evaluation of COVID-19 Therapy)	11,340	X			X	Colchicine 1 mg PO at randomization and 0.5 mg after 12 h, followed by 1 mg/ day for 10 days or until discharge	Hospitalized patients with moderate-to-severe COVID-19 (5610 received colchicine)	Colchicine did not reduce 28-day mortality, progression to IMV or death, or time to discharge	04381936
ECLA PHRI COLCOVID (Effects of Colchicine on Moderate/High-risk Hospitalized COVID-19 Patients)	1279	X			X	Colchicine 1.5 mg PO at randomization and 0.5 mg within 2 hours, followed by 1 mg/day for 14 days or until discharge	Hospitalized patients with moderate-to-severe COVID-19 (640 received colchicine)	Colchicine did not significantly reduce MV or 28-day mortality; diarrhoea more frequent with colchicine	04328480
COL-COVID (Trial to Study the Benefit of Colchicine in Patients with COVID-19)	102	X				Colchicine 1.5 mg PO within 48 h from hospitalization, followed by 1 mg/ day for 1 week and 0.5 mg/day for 28 days	Hospitalized patients with moderate-to-severe COVID-19 (52 received colchicine)	Colchicine did not improve clinical status at Days 14 and 28 but associated with a lower risk of clinical deterioration after adjustment for risk factors and concomitant therapies	04350320
COLORIT (COLchicine versus Ruxolitinib and Secukinumab in Open-Label Prospective Randomized Trial in Patients with COVID-19)	43	X				Colchicine 1 mg/day PO for 1–3 days, followed by 0.5 mg/ day for 14 days	Hospitalized patients with moderate-to-severe COVID-19 (21 received colchicine)	Colchicine improved clinical status at Day 12 or at hospital discharge and associated with reduced CPR	33734043
Effects of colchicine for moderate to severe COVID-19: a randomized, double-blinded, placebo-controlled clinical trial	74	X	X	X		Colchicine 1.5 mg/day PO for 5 days, followed by 1 mg/ day for 5 days	Hospitalized patients with moderate-to-severe COVID-19 (36 received colchicine)	Colchicine shortened duration of oxygen therapy at Day 7 and hospital stay, and associated with reduced CPR; diarrhoea more frequent with colchicine	33542047
GRECCO (The Greek Study in the Effects of Colchicine in Covid-19 Complications Prevention)	105	X			X	Colchicine 1.5 mg PO and 0.5 mg after 1 h, following by 1 mg/ day until hospital discharge or up to 21 days	Hospitalized patients with moderate-to-severe COVID-19 (55 received colchicine)	Colchicine improved time to clinical deterioration; no significant differences in CRP and troponin between the two study arms; AEs similar, except for diarrhoea (more frequent with colchicine)	04326790
ColCORONA (Colchicine Coronavirus SARS-CoV2 Trial)	4488	X	X	X	X	Colchicine 1 mg/day PO for the first 3 days, followed by 0.5 mg/day for 27 days	Outpatients with mild-to-moderate COVID-19 with high risk for progression (2235 received colchicine)	Colchicine reduced the composite of death or hospital admission a subgroup of patients with laboratory-confirmed COVID-19; diarrhoea more frequent in the colchicine arm, SAEs, and pneumonia less frequent	04322682

Continued

Table 1 *Continued*

Trial	Sample size	Study design R	DB	PC	M	Intervention	Main inclusion criteria	Main findings	NCT/ PMID number
Selective NLRP3 Inhibitor Study of Efficacy and Safety of DV890 in Patients with COVID-19 Pneumonia	143	X			X	DFV890 50 mg PO twice daily for 14 days	Hospitalized patients with COVID-19 pneumonia, impaired respiratory function, APACHE II score ≥10, CRP ≥20 mg/L, and/or ferritin ≥600 μ/L (71 received DFV890)	DFV890 not superior to SoC alone in reducing the primary endpoint (APACHE II score at Day 14), but associated with faster viral clearance, improved clinical status, reduced mechanical ventilation-free survival, and death at Day 28	04382053

Note: COVID-19 severity refers to the World Health Organization severity classification (https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-2). The individual trial-specific criteria for disease severity classification as well as detailed descriptions of study inclusion/exclusion criteria, design, and findings can be found on https://clinicaltrials.gov/and in relative publications. Abbreviations: AEs, adverse events; CRP, C-reactive protein; DB, double-blind; IMV, invasive mechanical ventilation; IV, intravenously; M, multicentre; MV, mechanical ventilation; PC, placebo-controlled; PO, per os; R, randomized; SAEs, severe adverse events; SC, subcutaneously, SoC, standard-of-care.

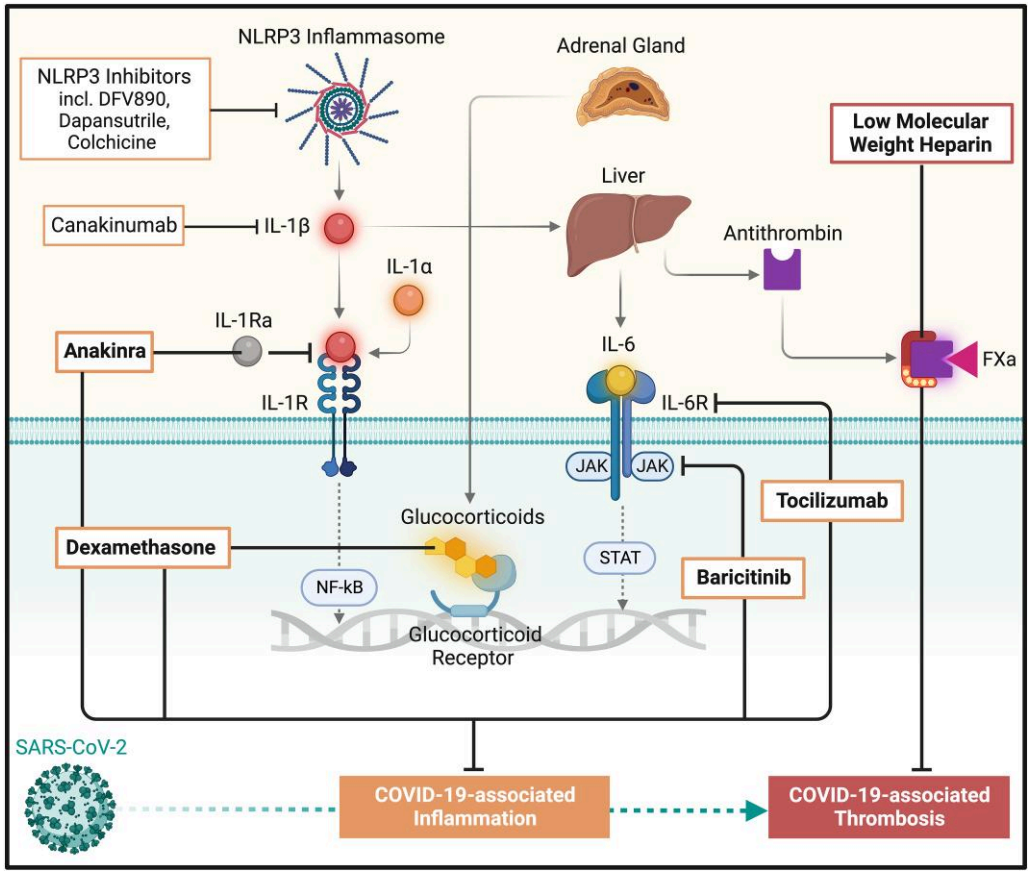

Figure 4 Pharmacological agents addressing COVID-19-associated inflammation and thrombosis. Abbreviations: FXa, factor Xa; IL, interleukin; IL-1R, interleukin-1 receptor; IL-6R, interleukin-6 receptor; IL-1Ra, interleukin-1 receptor antagonist; JAK, Janus kinase; NF-κB, nuclear factor-kappa B; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; STAT, signal transducer and activator of transcription protein. Figure created with BioRender.com.

trials—generally much lower than in COVID-19 anticoagulation trials[171]—may not have allowed to capture clinically significant antithrombotic signals. Adequately designed studies are therefore needed to specifically assess the effects of NLRP3 inflammasome pathway inhibition on CAC and COVID-19-associated thrombosis.

Activation of the NLRP3 inflammasome pathway can exert prothrombotic effects through both IL-1-dependent and IL-1-independent mechanisms.[15] It is therefore important to consider that anti-IL-1 agents may not completely address inflammasome-mediated, IL-1-independent immunothrombotic responses. Additionally, individual IL-1 blockers may exert distinct clinical effects owing to their different mechanism of action (e.g. IL-1α/IL-1β inhibition with anakinra versus IL-1β inhibition with canakinumab) as well as to their different pharmacokinetic and pharmacodynamic properties.[138,139] It is also possible to consider that selective NLRP3 inhibitors might target the NLRP3 inflammasome more effectively than non-selective NLRP3 inhibitors and other immunomodulatory agents, with potential clinical implications on immunothrombosis and CAC.[83,84] Future investigations should also address whether NLRP3 inflammasome inhibitors and IL-1 blockers could be used in synergy with other anti-inflammatory and anticoagulant drugs, potentially maximizing their benefits on inflammation and thrombosis without compromising safety. Importantly, additional studies are needed to evaluate the clinical usefulness of immunomodulatory agents including NLRP3 inflammasome pathway blockers with regard to vaccination, the evolving landscape of SARS-CoV-2 variants, and long COVID-19.

8. Concluding remarks

Inflammation and thrombosis are key features of severe COVID-19. Activation of the NLRP3 inflammasome pathway occurs in COVID-19 patients and is implicated in the inflammatory pathogenesis of the disease. The NLRP3 inflammasome pathway may also promote the hyperactivation of immunothrombosis programmes including generation of NETs and TF, as well as prothrombotic endothelial and platelet responses. Clinical trials testing the IL-1 inhibitors anakinra and canakinumab, the non-selective NLRP3 inhibitor colchicine, and the selective NLRP3 inhibitor DFV890 in COVID-19 patients showed reassuring results in terms of safety and tolerability and overall a signal for efficacy. Blockade of IL-6 signalling, downstream of IL-1, also reduced mortality in severe COVID-19 pneumonia. Anakinra and tocilizumab are now approved for the treatment of hypoxaemic COVID-19 patients with hyperinflammation. Further investigation is warranted to better characterize the exact mechanisms driving NLRP3 inflammasome pathway activation and its effects on CAC and COVID-19-associated thrombosis. The possibility that targeting the NLRP3 inflammasome pathway may simultaneously address inflammation and thrombosis might offer novel therapeutic perspectives for multiple inflammation-driven thrombotic disorders including COVID-19.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

Author contributions

N.P., Y.K., M.D.N., E.G., G.K., A.B., J.M.C., R.D.C., and A.A.

Conflict of interest: N.P. has received a training fellowship from the International Society on Thrombosis and Haemostasis and research funding from the International Network of VENous Thromboembolism Clinical Research Networks (INVENT), outside of the present work. Y.K. is an inventor on a patent application (US20180369278A1) by the University of Michigan on the use of biogases in vascular disease. M.D.N. reports personal fees as an invited speaker from Bayer, Daiichi Sankyo, and Viatris, personal fees for advisory board membership from LEO Pharma and Pfizer, and institutional funding from LEO Pharma. G.K. has received honorary fees from Swedish Orphan Biovitrum, Chugai-Roche, and Amgen. A.B. received a travel grant from Kiniksa Pharmaceuticals Ltd to attend the 2019 AHA Scientific Sessions and honoraria from Effetti s.r.l. (Milan, Italy) to collaborate on the medical website http://www.inflammology.org, outside the present work. J.M.C. has received personal fees for scientific advisory boards and consulting from Abbott, Anthos, Alnylam, Bristol Myers Squibb, Five Prime Therapeutics, Pfizer, Takeda, and research funding from CSL Behring, outside of the submitted work. R.D.C. has received personal fees from Boehringer-Ingelheim, Bayer, BMS-Pfizer, Daiichi Sankyo, Novartis, Roche, Sanofi, Amgen, Milestone, Menarini, AstraZeneca, and Guidotti, outside the submitted work. A.A. has received research grant funding and has served as a paid scientific advisor to Implicit Biosciences, Kiniksa, Lilly, Merck, Novartis, Novo Nordisk, Olatec, R-Pharm, Serpin Pharma, and Swedish Orphan Biovitrum, outside of the submitted work. E.G. has nothing to disclose.

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