Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Review

NLRP3 inflammasome activation in COVID-19: an interlink between risk factors and disease severity

Saiful Amin [a], Salma Aktar [b,*], Md. Mijanur Rahman [b], Mohammed Mehadi Hassan Chowdhury [b]

[a] Chittagong Medical University, Chattogram, Bangladesh
[b] Department of Microbiology, Noakhali Science and Technology University, Sonapur, Noakhali 3814, Bangladesh

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ABSTRACT

NLRP3 inflammasome is a critical immune component that plays a crucial role in mounting innate immune responses. The deleterious effects of inflammasome activation have been correlated with the COVID-19 disease severity. In the presence of several underlying disorders, the immune components of our bodies are dysregulated, creating conditions that could adversely affect us other than providing a required level of protection. In this review, we focused on the occurrence of NLRP3 inflammasome activation in response to SARS-COV-2 infection, dysregulation of NLRP3 activation events in the presence of several comorbidities, the contribution of activated NLRP3 inflammasome to the severity of COVID-19, and available therapeutics for the treatment of such NLRP3 inflammasome related diseases based on current knowledge. The primed state of immunity in individuals with comorbidities (risk factors) could accelerate many deaths and severe COVID-19 cases via activation of NLRP3 inflammasome and the release of downstream inflammatory molecules. Therefore, a detailed understanding of the host −pathogen interaction is needed to clarify the pathophysiology and select a potential therapeutic approach.

Well-regulated cell death and inflammatory immune responses are essential to promote protection against viral infection; however, a loss of balance induced by either the host or virus may further create a condition that could impact the virus or otherwise adversely affect the host. When there is a viral infection in humans, the innate immune components of the body will mount a suitable immune response, and consequently, an antiviral environment will be created to clear the virus. Unfortunately, apart from the viral clearance, sometimes the body will face some adverse effects due to overreaction of the excessively produced mediators that can further cause severe clinical outcomes. One innate immune component is the NLR family pyrin domain containing 3 (NLRP3) inflammasome that has been considered a critical component of host immune defense against varieties of viruses [1,2]. However, dysregulation of the pathway involved with NLRP3 activation (especially upstream NF-κB pathway) involved with NLRP3 activation has been linked with the pathogenesis of several inflammatory disorders, including cryopyrin-associated periodic syndromes (CAPS), Alzheimer's disease, diabetes, gout, autoinflammatory diseases, atherosclerosis, and acute respiratory disease syndrome with massive cell death by pyroptosis [3,4]. Interestingly, this type of dysregulation also worsens and increases the severity of coronavirus disease 2019 [3].

In the context of the current COVID-19 pandemic, the severe form of the disease is mainly characterized by severe pneumonia, which may evolve to acute respiratory distress syndrome and death [5]. This severity is a consequence of multiple inflammatory responses mediated by the release of several key inflammatory molecules, including caspase-1, IL-1β, IL-18, IL-6, TNF-α, and pore-forming molecule gasdermin D (GSDMD) with a clinical outcome of alveolar damage, pulmonary fibrinolysis and injury [6–8]. On the basis of clinical data gathered from SARS-COV-2 infected patients, the most critical form of COVID-19 disease is acute respiratory disease syndrome [3,9]. The ARDS caused by COVID-19 can manifest as alveolar damage, fluid leakage into the pulmonary interstitium, remarkable disruption of gas exchange with a final consequence of hypoxia, and respiratory failure [10,11]. The major inflammatory biomarkers (IL-6, IL-8, IL-18, and TNF-α) of cytokine storms largely contribute to the exacerbation of ARDS [5].

* Corresponding author.
E-mail address: salma.mbg@nstu.edu.bd (S. Aktar).

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In this review, to delineate the interlink between NLRP3 inflammasome activation and underlying conditions of COVID-19 severity, we focused on the mechanisms of NLRP3 mediated inflammatory reactions in the progression of COVID-19, possible host and environmental risk factors that may influence the NLRP3 activation, mechanisms of each factors behind the disease progression, and several potential therapeutics or inhibitors that could be used for inflammasome mediated inflammation treatment based on currently available literature.

1. Viral infection and NLRP3 activation

A PAMP (pathogen-associated molecular pattern) is an antigenic substance generated by pathogenic invasion of the host cell, whereas a PRR (pattern recognition receptor) is a host cell sensory molecule that can recognize the presence of that PAMP. The PRR—PAMP interaction is the very early event for awakening a cascade of cell signaling pathways against pathogen invasion. An inflammasome is a group of intracellular multimeric protein complexes that activate inflammatory substances, especially caspase-1, a major inflammatory pathway in response to multiple virus infections [12]. The NLRP3 inflammasome is an oligomeric complex comprised of the NOD-like receptor NLRP3, the adaptor is known as apoptosis-associated speck like protein (ASC), and the caspase-1 domain. It is the most extensively studied inflammasome that plays an essential role in inflammation and antiviral responses [13]. Although this inflammasome activation has enormous roles in pathogen invasion, deregulated activation of this pathway causes various inflammatory disorders.

The events associated with NLRP3 inflammasome activation upon a particular viral infection include: recognizing PAMPs (RNA, DNA, whole virus or part of it) by host cell sensory molecules such as toll-like receptors (TLRs) induce the oligomerization of NLRP3 followed by K^+ efflux. Subsequently, downstream recruitment of the apoptosis associated speck like protein (ASC), and the effector, pro-caspase-1 through pyrin domain (PYD—PYD) and caspase recruitment domain (CARD—CARD) interactions assembled the NLRP3 inflammasome. The PYCARD then recruits the pro-CASP1 zymogen via its CARD domain to form an NLRP3-PYCARD-pro-CASP1 complex; as a result, activation of pro-Caspase-1 into activated caspase-1 occur. The activated caspase-1 can cleave its substrates pro-IL-1β, pro-IL-18, and the pore-forming protein named gasdermin D (GSDMD). Furthermore, activated GSDMD can induce pore formation in the cell membrane and release the active IL-1β, IL-18, IL-6 and other inflammatory molecules that cause extensive inflammatory reactions and subsequent cell death [6]. A schematic diagram presented in Fig. 1 illustrated how NLRP3 activation occurs following a particular infection.

Several viruses from distinct families have been reported for aberrant activation of NLRP3 inflammasome that can cause severe pathological injury [13]. For example, Influenza A virus-mediated NLRP3 activation and subsequent lung injury in a Juvenile mouse model has already been reported [14]. Moreover, NLRP3 associated neuroinflammation in HIV1 infected microglia has been reported previously [15]. In addition, hepatitis C virus (HCV) infection-mediated NLRP3 inflammasome activation promoted chronic intrahepatic inflammation and liver injury [16]. cGAS mediated detection of HSV1 DNA was also reported to activate NLRP3 inflammasome [17].

2. Inflammasome activation in COVID-19

The hyperactivated immune response that starts with the innate immune system's efficient detection of the SARS-COV-2 is a crucial factor leading to unfavorable COVID-19 outcomes. Since its emergence in December 2019, COVID-19 has been characterized as a highly inflammatory disease manifested by organ failure and inflammatory cell death in severe cases [7].

An elevated level of proinflammatory cytokines, including interleukin IL-1, IL-6, and tumor necrosis factor (TNF), has been characterized as a “cytokine storm,” which may ultimately lead to alveolar damage, pulmonary fibrinolysis and injury with increased mortality [18]. The detection of LDH, IL-6, IL-1β, and IL-18 in severe cases of the disease suggest that disproportionate inflammation is the principal cause of poor clinical outcome [19,20]. The detection of these proinflammatory molecules in the plasma of severe COVID-19 patients confirmed the engagement and activation of the inflammasome [7].

A study conducted by M. Zheng et al. showed that infection of bone marrow-derived macrophages (BMDMs) from mice with strain A59 of mouse hepatitis virus (MHV) activated the NLRP3 inflammasome and induced inflammatory cell death [21]. Moreover, GU-rich ssRNA-mediated inflammasome activation by SARS-COV-2 has also been reported, though the research was not conducted with active viruses [6]. The study showed that inflammasome is activated in response to GU-rich ssRNA in a non-classical way without inducing cell death. A more detailed study conducted by A.C. Ferreira et al. reported that SARS-CoV-2 engages inflammasomes and pyroptosis in human monocytes, either by experimental or natural infection [22]. These outcomes were linked with caspase-1 activation, IL-1β production, GSDMD cleavage, and dysregulation of cytokine release. However, the study further showed that inhibiting early steps of the SARS-CoV-2 life cycle by Atazanavir (ATV) could block pyroptosis in SARS-CoV-2-infected human primary monocytes. Meanwhile, SARS-COV-2 infection of lung epithelial cells have been shown to induce caspase-8 activation, further promoting cell apoptosis upon processing of proinflammatory cytokine, especially, pro-IL-1β into the bioactive form [23]. The study further confirmed that release of IL-1β induced necroptosis pathway with inflammatory responses, thus, a dual-mode of cell death assumed to cause severe lung damage and fatal infection outcome [23].

Nevertheless, studying moderate and severe cases of COVID-19 patient's sera, PBMC, and postmortem lung section, different studies reported the presence of active inflammasome-driven molecules such as caspase-1, IL-18, IL-6, and LDH [7,23]. A recent experiment showed that SARS-COV-2 infected monocytes and peripheral blood mononuclear cells (PBMC) isolated from healthy donors could induce cell death followed by inflammasome formation [7]. S. Toldo et al. reported detecting active inflammasome molecules in the lung section of fatal COVID-19 patients who had died of cardiopulmonary arrest [24]. The injured lung area expressed a significant level of ASC inflammasome specks and intense inflammasome expression in leukocytes [24].

2.1. Mechanism of inflammasome activation by SARS-CoV-2 infection

The outcome of the NLRP3 inflammasome activation is a natural host cell response to SARS-COV-2 infection that has double roles; it either protects from infection or leads to chronic inflammation [13].

Notably, SARS-COV-2 infection into the target cell line causes the release of viral antigen (ssRNA) known as PAMPs that can be recognized by endosomal TLR [6]. Ligand-induced activation in TLR (TLR8/TLR7) is then recognized by adapter protein (especially, MYD88), which recruits the IL-1 receptor-associated kinases, leading to the nuclear factor-kB-dependent transcription of numerous proinflammatory mediators, including IL-6, IL-12, IL-27, TNF, IFN-γ, and IL-1β [25]. The production of pro-IL-1β is a priming step, which should be cleaved by caspase-1 into active IL-1β.

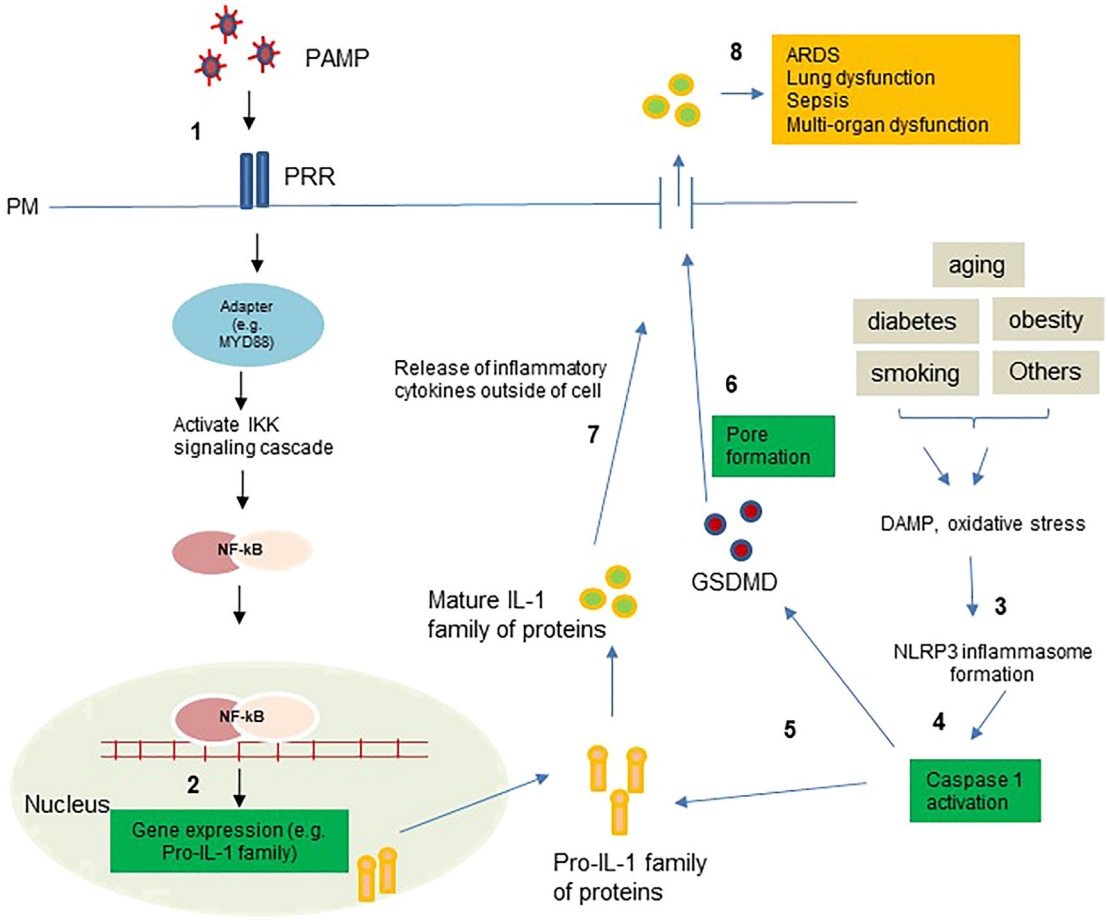

Fig. 1. Proposed model of NLRP3 inflammasome activation in COVID-19 inflammation. The first signal of the process begins, followed by the release of viral antigen (PAMPs) and subsequent recognition of susceptible PRR [3]. This ligand-induced conformational change in toll-like receptor (TLR) then recruits IL-1 receptor-associated kinases and resulting in transcription of several genes associated with inflammation followed by NF-kB pathway activation (2). The second signal comes from the cytosolic K^+ efflux resulted from oxidative stress that causes recruitment and oligomerization of NLRP3 components as NLRP3-PYCARD-pro-CASP1 complex (3). Self-activation of pro-CASP1 through proteolytic cleavage into active CASP1 is the main event (4) that further catalyzes the maturation of IL-1 related cytokine and GSDMD (5). The active GSDMD then induce pore formation into the cytoplasmic membrane (6); thus the release of inflammatory cytokines outside of cellular environment occur (7) that causes different clinical outcome with massive cell deaths (8). PM, plasma membrane; PAMP, pathogen associated molecular pattern; DAMP, damage associated molecular pattern; PRR, pattern recognition receptor; TLR, toll like receptor; CASP1, caspase 1; GSDMD, gasdermin D.

The second signal comes from cytosolic K^+ efflux, which formed from viral or environmental damage molecules induced oxidative stress.

Several reports have been published on how SARS-COV-2 triggers NLRP3 inflammasome activation, what proteins are linked with this activation, and further steps. SARS-COV-2 encoded ORF3a [26,27], ORF-3b [26,28], envelope protein E [29] and nucleocapsid protein, N [30] has been reported to activate the inflammasome formation.

2.1.1. Illustration of NLRP3 induced pathological changes in COVID-19

In a more elaborate sense of view, from infection until clinical outcome, the NLRP3 inflammasome proceeds through a diverse of signaling cascades to exert its pathological effects in COVID-19 cases [9]. The signaling events linked to SARS-COV-2 induced NLRP3 formation can be implicated as-

Viroporins induce ion-transport activity: The E protein of coronavirus, also called viroporin due to the formation of pores in viral infected cells [31]. The protein can gather in membranes upon formation of multimeric protein-lipid pores to induce ion transport [32]. Besides, studies showed that the protein can stimulate Ca^{2++} leakage from ERGIC/Golgi membrane by forming a Ca^{2++} permeable ion channel, thus the resulting ion imbalance may augment the pathogenesis [29,32]. Since, Ca^{2++} Efflux is a crucial stage in NLRP3 activation, this E protein induced ion channel activity may play a major role in SARS-COV infected cell to proceed through NLRP3 associated immune reactions. However, there is no direct evidence of such E protein activity in SARS-COV-2, yet, the protein has a conserved structural binding motif with SARS-COV-1 [33].

In addition to E protein, another viroporin, ORF3a has been reported to induce ion channel activity of K^+, a vital step of NLRP3 inflammasome formation through activation of caspase-1 zymogen [34]. Recently, I. Y. Chen et al. showed that ion channel activity of ORF3a is sufficient to induce NLRP3 activation in lipopolysaccharide-primed macrophages by promoting IL-1β secretion [35]. Moreover, another study reported that the protein can activate the NLRP3 inflammasome upon inducing TNF receptor-associated factor 3 (TRAF3)-mediated ubiquitination of apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) [27]. Though the study reported that ORF3a was indeed capable of inducing both pro−IL-1β gene transcription and IL-1β protein secretion, it also showed that this activity was independent of its ion channel activity [27].

Receptor binding accumulation of angiotensin II Ang (II) by S protein: It is well known that spike protein of SARS-COV-2 can bind with ACE2 which belongs to membrane bound carboxypeptidase with an activity of Ang II degradation to generate angiotensin 1–7 [36]. The virus can contact with ACE2 via its S1 subunit of S protein with a subsequent fusion by S2 upon TMPRSS2 cleavage [37]. This increased receptor-ligand contact under enhanced viral replication causes an accumulation of Ang (II) due to reduce rate of its degradation, since the interaction of Ang (II) with ACE2 is attenuated. This accumulated Ang (II) can induce NLRP3 activity and results in ALI and related syndromes [38].

Complement cascade activation by N proteins: The importance of complement in COVID-19 emerged after finding a noticeable deposition of complement proteins C5b-9, C4d and MASP2 in the microvasculature of two organ systems from COVID-19 patients with severe respiratory failure [30]. An earlier study conducted by E. Asgari et al. showed that the engagement of C3a receptor by the C3a can induce ATP efflux which can promote the secretion of IL-1 family of cytokines in an extracellular signal-regulated kinase 1/2 (ERK1/2)-dependent fashion [39]. SARS-COV-2 encoded N protein has been reported to actively participate in such complement cascade mediated NLRP3 activation by mannan-binding lectin (MBL)-dependent manner [30,40]. The membrane attack complex (MAC), which is a self-polymerized terminal components of the complement cascade, can form transmembrane pores in target cell membrane [41]. As a result, increased ion accumulation can occur that further promote mitochondrial deterioration with a resulting NLRP3 dysregulation [42].

2.1.2. Potential biomarkers of NLRP3 inflammasome activation in COVID-19

The major molecules regulated by NLRP3 inflammasome that are constantly detected in COVID-19 cases can be listed as potential biomarkers of nlrp3 activation, so that the diagnosis and clinical treatment will be more effective and rapid. A recent review conducted by S. M. Vora et al. demonstrated the correlation of major immune components with COVID-19 at different stages of the disease that are either directly or indirectly influenced by NLRP3 inflammasome activation [43]. NLRP3 inflammasome mediated major biomarkers in COVID-19 cases are summarized below:

a) Detection of IL-1β can be an excellent biomarker for severe COVID-19 [7,22], but it is difficult to measure in some instances due to short half-life [44]. Alternatively, as a surrogate of IL-1β, IL-1R A is a frequently use marker as it can be consistently seen in severe COVID-19 cases [22].
b) Besides IL-1β, rising level of other cytokines and chemokines especially IL-6, TNF-α, IL-18 as well as other factors such as C-reactive protein (CRP) in severe patient's sera are particular indicators of inflammasome activation [45].
c) An elevated level of LDH in serum is a strong predictor of severe COVID-19 which is frequently released from damaged cells due to NLRP3 inflammasome activation [46].
d) Meanwhile, identification of inflammasome specks in blood monocytes and lung section of severe COVID-19 patients can be a direct evidence of inflammasome formation together with pyroptosis and cell death. Most notable specks displayed as NLRP3, ASC, caspase-1 and cleaved GSDMD (amino terminal) form monocytes and lung tissue section [47].
e) As secondary bacterial infection can be occurred in SARS-COV-2 infected lung, the release of Lipopolysaccharide (LPS) into blood plasma from bacterial cell wall is quite common. Such LPS can activate both NLRP3 and AIM2 inflammasome [48].

2.2. Major risk factors associated with inflammasome activation in COVID-19

In this scenario, we listed some possible risk factors and their mechanisms that may contribute to NLRP3-dependent inflammation associated with COVID-19 in Table 1.

2.2.1. Age

Multiple biological changes in the immune system are a natural process of aging associated with age-related disorders and susceptibility to natural infection. Older aged people have a reduced number of lymphoid and non-lymphoid tissues that causes degeneration of primary lymphoid organs with lower production of naive T and B lymphocytes, resulting in reduced migration to secondary lymphoid organs and sites of antigen encounter. Several studies suggested that older people suffering from COVID-19 may develop a pulmonary disease resembling pulmonary fibrosis [8,49]. An earlier study conducted by S. Delgado et al. showed that oral administration of Bleomycin in C57BL/6 mice (a drug that induces ROS formation that leads to NLRP3 activation) resulted in a greater rate of death followed by collagen deposition in aged mice than younger one, provide a link that age is a significant risk factor for NLPR3 inflammasome mediated death in COVID-19 patients [50].

Table 1
List of risk factors associated with NLRP3-mediated COVID-19 inflammation.

Risk factors	Underlying health conditions contribute to NLRP3 activation	References
Aging	1. DAMP (mtDNA, mtROS release by mitochondrial deterioration)	[51,52]
	2. Telomere dysfunction	[53]
	3. Reduced B cell maturation	[49]
	4. Increase infiltration of inflammatory cells	[49]
Diabetes	1. DAMPs (release by increased glyco-lipotoxicity and oxidative stress)	[112]
	2. Delayed IFN response	[61]
	3. Decreased T cell count	[19]
	4. Infiltration of inflammatory cells due to increased vascular permeability	[62]
Obesity	1. DAMPs (ceramides and cholesterol crystal mediated ROS formed from impaired adipocytes)	[64]
	2. An elevated level of ACE2 expression due to increased mass of adipocytes	[66]
	3. Exacerbated lipolysis due to hyperplasia induce mitochondrial damage	[65]
	4. Aberrant ionic flux due to increased levels of cathepsin B and Ca^{2+} from lysosomal damage	[64]
Smoking	1. Increase ACE2 expression induced by nicotine	[85]
	2. An elevated level of inflammatory cytokine secretion due to increased ACE2 expression	[35]
	3. Weaker states of the lungs	[78]
Gender	1. Hormonal difference	[91]
	2. X-chromosome based sensor expression	[92]
	3. Immune responses variations	[88]

The dysregulation of the innate immune system with aging accompanied by tissue inflammation is a common feature of the aged immune system [8]. The salient reasons for such compromised immunity include several naturally accompanied reasons are- 1) deterioration of mitochondrial performance with diminished metabolisms results in increased production of mtROS and mtDNA that can act as DAMP to induce cellular signaling pathways including NLRP3 inflammasome [51,52]; 2) gradual shortening and consequent dysfunction of telomere is a common outcome in aging which causes reduced host response to immunity, stress, inflammation, and damage [53]; and 3) The ACE2 enzyme plays a critical anti-inflammatory role in the renin-angiotensin-aldosterone system (RAAS) signaling pathway by catalyzing the proinflammatory angiotensin-2 to anti-inflammatory angiotensin 1–7 [54]. The age-related expression of lower ACE2 may compromise the anti-inflammatory response and predispose older individuals to exaggerated inflammatory responses [55].

However, the findings of several recent studies suggested that age is an extreme risk factor for severe COVID-19 disease. The case fatality ratio (CFR) of COVID-19 reported in China showed to increases with age, such as 0.4% in patients of ≤40 years and 8% in 70 years, while the ratio increased up to 14.8% in 80 years or older; with the overall CFR of approximately 2.8% worldwide, and particularly 2.7% in the US as of October 19, 2020 [56]. In Italy, the severity of case fatality rate was more such as 0.4% in patients aged ≤40, and 20% in ≥80 years with an overall CFR of 7.2% [57], whereas in the USA, CFR was documented as 3.3% in ≤40 and 26% in 80 years of age [3]. Furthermore, the CDC also reported significantly higher hospitalizations, ICU admissions, and deaths secondary to COVID-19 among older adults than younger adults.

2.2.2. Diabetes

Although people with diabetes are not likely to have a higher chance of getting COVID-19, the risk of developing more severe complications is quite familiar with increased mortality associated with cytokine storm, metabolic, and immune derangement [58]. A Cohort study conducted by J. M. Gregory et al. showed that type 1 or 2 diabetes could independently worsen the COVID-19 disease severity with 3–4 fold higher than people without diabetes, though the data were entirely collected from only hospitalized patients [59]. NLRP3 inflammasome activation is a more likely event behind this adverse outcome. In addition to chronic hyperglycemia and vascular disease, social determinants of health and decreased use of diabetes technology have been correlated significantly with COVID-19 severity. However, NLRP3 inflammasome activation in diabetic individual may lead to — 1) elevated products of the intermediate metabolism (e.g., urate, cholesterol crystals, extracellular ATP, certain fatty acids and islet amyloid peptides) produced by glycolipotoxicity and oxidative stress resulting in hyperactivation of inflammasome and caspase-1 which in turn increases the release of IL-1β and IL-18 [60]; 2) decrease of $CD4^+$ and $CD8^+$ T cell count linked with poor prognosis such as an experiment in mouse model showed that the depletion of both cells led to an increase of neutrophils and macrophages in the lesions of interstitial pneumonitis [19]; 3) delay of IFN-γ response due to depleted $CD4^+$ and $CD8^+$ with the accumulation of inflammatory monocytes and macrophages are thought to be a principle cause of lethal pneumonia which had been proved by a mouse model [61]; 4) elevated level of proinflammatory memory B cells may cause the early maturation of antibody response due to low grade inflammation [62]; and 5) thicker alveolar epithelial and endothelial capillary basal laminae in diabetic patients may affect alveolar gas exchange and pulmonary function [58].

2.2.3. Obesity

Obesity is a complex body condition with excessive body fats that increase mortality due to a significant contributing factor for developing various severe health disorders such as diabetes, cardiovascular disease, hypertension, dyslipidemia, musculoskeletal conditions such as osteoarthritis, and other diseases [63]. The release of adipokines and cytokines due to low-grade inflammation in obese individuals may contribute to NLRP3 inflammasomes activation [64], and this condition can be exacerbated in the presence of particular viral infections such as SARS-COV-2 [64].

Obese people are more prone to inflammasome activation due to several reasons, such as — 1) hyperplasia or hypertrophy causes an impairment in the function of adipocytes that could further induce the formation of ceramides and cholesterol crystals with the activation of tissue-resident macrophages, which itself triggers the formation of ROS [64] and subsequent transcription of NLRP3 and downstream genes IL-1β, IL-18 and other cytokines [65]; 2) due to increased AT mass, an obese person has elevated expression of ACE2, thus, more likely to have increase SARS-COV-2 infection [66]; and 3) due to chronic low-grade inflammation in obese individuals, the innate immune system might be already in a primed state which could promote a hyperinflammatory response [64].

A systemic review and meta-analysis (combining 22 studies) conducted by Y. Chu et al. showed that the severity of COVID-19 was much higher in people with greater body mass index (BMI) than those with lower BMI [67]. Another meta-analysis with a similar approach identified 22 studies and showed that obesity significantly increased the risk of death and critical complications of H1N1 infection [68]. Meanwhile, a retrospective Chinese case–control study of young people with COVID-19 reported that obesity is the most vital factor contributing to their death [69]. A similar study in France proposed a higher frequency of obesity among intensive care unit (ICU) patients with SARS-CoV-2-related pneumonia [70]. In addition, Peng et al. showed that non-survivors in COVID-19 are more likely to have high BMI than survivors [71]. Though several studies conducted in Italy failed to show obesity as a pre-existing factor associated with death in COVID-19 patients [57], the high mortality prompted the notion that obesity has a strong correlation in COVID-19 progression with ARDS [4,49,64,67].

2.2.4. Smoking

Since smoking directly affects the immune system, it has been considered a significant risk factor for many respiratory infections; therefore, hypothetically, it could also contribute to the severe conditions in COVID-19. Due to its adverse impact on health and subsequent outcomes with multiple severe life-threatening health disorders including lung cancer, COPD, asthma, pneumonia, multiple viral and bacterial infections caused nearly 8 million deaths per year, making smoking a significant burden of this decade [72].

Several earlier studies demonstrated the role of NLRP3 inflammasome in the lung inflammation of cigarette smokers with increase expression of different NLRP3 mediated components and caspase-1 and release of inflammatory cytokines IL-18 and IL-1β [73–75]. A study conducted by L. Chen et al. showed an upregulation of NLRP3 and caspase-1 in human bronchial epithelial (HBE) cell line at transcriptional and translational level following exposure to cigarette smokes at different time points [73]. The study further confirmed the cigarette-smoke-induced NLRP3 activation in the HBE cell line using a transwell migration assay. A similar study reported by G. Yi et al. found an enhancement of the expression of multiple genes associated with inflammation including IL-1β in the lung of COPD patients [74]. According these previous reports and ongoing COVID-19 disease severity and

complications, we can predict a link of smoking in NLRP3 activation under SARS-COV-2 primed states, though no direct links have been revealed yet in this regard [76].

Several studies on COVID-19 disease severity showed that infection rate and the subsequent worsening situations are much higher in people who smoke than non-smokers. The higher requirements of ventilators and ICU supports to death also reported with the smoker [77,78]. A systemic review and meta-analysis (10 studies) of A. Emami et al. showed an overall smoking history of 7.63% of 76,993 hospitalized patients who were infected with SARS-COV-2, while another analysis suggested a prevalence of 6.5% (out of 5906) [79]. Moreover, several studies concluded that the risk of COVID-19 disease severity and progressive illness is greater or doubled among smokers compare to non-smokers [80–82]. Conversely, some other studies could not find any association of smoking in the severity and progression of the disease [83,84].

The risk of COVID-19 neurological infection for nicotine exposers are more remarkable due to several reasons such as — 1) nicotine can induce the expression of ACE2 receptors in the respiratory tract of smokers that predispose that person to be significantly infected with the virus [85]; 2) highly ACE2 expressing cell infected by SARS-COV-2 induce the release of inflammatory cytokines due to active virus replication that can lead to pyroptosis [20], and (3) the immune system of a smoker is more tolerant and less reactive than patients who have never smoked and whose immune system could more readily trigger a cytokine thought to be related to the high death rate associated with COVID-19 [78].

2.2.5. Gender

Gender or sex difference is considered an essential contributing factor in many diseases' occurrence, severity, and outcome. It also seems to act as a risk factor to the newly emerge COVID-19. Several published reports showed that males are more susceptible to SARS-COV-2 infection. Hospital admission and severity were more frequent in males than females [86]. A study conducted on a large group of health care providers showed that males are more susceptible to the virus infection and subsequent disease severity [87]. Another study conducted by T. Takahashi et al. found that male patients had higher plasma levels of innate immune cytokines such as IL-8 and IL-18, whereas female patients had more robust T cell activation during SARS-CoV-2 infection; and worse disease outcomes found in male patients but not in female patients [88]. However, the study further concludes that higher levels of innate immune cytokines were also associated with the worsening of COVID-19 disease in female patients.

Nevertheless, Asian men are more prone to get COVID-19 disease than women due to the prevalence of ACE2 expression in men, thus implies another proof of sex-related COVID-19 disease severity and preference [89]. Moreover, X. Yang et al. showed that more males (67%) were diagnosed as critically ill than women in hospitalized COVID-19 patients [90]. The preference of such gender-based difference in males may be because of several reasons such as — 1) hormonal differences between males and females-since it has been seen that estrogens level appear to upregulate ACE-2 in younger children and adult women, this upregulation can lead to a higher level of ACE2, explain the milder COVID-19 in women and children [91]; 2) the differentiation in sex chromosome is thought to be another reason in this aspect because of the expression of toll-like receptor-7 (TLR7) on the X chromosomes, a sensor responsible for the initiation of several early cell-signaling pathways following viral infection [92]; and 3) different immune response between male and female in producing abundant neutralizing autoantibodies against IFN-1 in men than women with severe COVID-19 [88]. Moreover, other social factors, lifestyles and different exposures may play roles in such gender-based COVID-19 severity.

3. Potential drugs for NLRP3 mediated inflammation

In addition to ARDS and acute lung injury (ALI), activation of the inflammasome and subsequent cytokine storm have likely occurred in severe COVID-19 patients, with the consequence of death in most cases. Therefore, the NLRP3 inflammasome appeared as a potential drug target in treating the disease or at least lowering the inflammatory reactions. Since NLRP3 activation can be quickly confirmed by identifying some common molecules from the serum of COVID-19 patients, such as the IL-1 family of cytokines, especially IL-1β, IL-18, IL-6, TNF-α, and in extreme cases GSDMD (if cell undergoes lysis). Consequently, it is likely to choose a drug that targets these cytokines, especially upstream of the enzymatic target, to reduce the subsequent reactions. Nonetheless, there is an urgent need for effective therapy (novel agents or repurposed drugs, natural products, and biological agents) against the SARS-CoV-2 to reduce the mortality of COVID-19.

Oridonin, a naturally synthesized product from *Rabdosia rubescens* plant, is a known anti-inflammatory drug that has recently been proposed as a therapeutics targeting NLRP3 inflammasome [93]. Bay 11-7082, another synthetic vinyl sulfone compound, has been shown to exert its effects via inhibiting the NLRP3 inflammasome [93]. Moreover, parthenolide and Bay 11-7082 could hamper the NLRP3 inflammasome and inflammatory NF-κB pathways, reduce lung inflammation and improve survival in SARS-CoV-2 infected animals [94]. Curcumin, an ingredient of turmeric, has been shown to suppress the inflammation of COVID-19 without any side effects [95]. The study further reviewed that curcumin significantly suppresses IL-1β and TNF-α expression at both RNA and protein levels in the LPS and ATP-stimulated THP-1 macrophages by increasing pro-caspase-1 and decreasing active caspase-1 expression.

Recently an anti-allergic drug named as tranilast (TR) that has been gaining attention due to functions as anti-chemotactic factor on controlling inflammation. An earlier study by Y. Huang et al. demonstrated that the drug can directly bind to the NACHT domain of NLRP3 and prevent the assembly of NLRP3 inflammasome by blocking NLRP3 oligomerization [96]. A recent review by A. Saeedi-Boroujeni et al. demonstrated that TR can inhibit NLRP3 inflammasome formation by inhibiting the activation NF-κB pathway, thus the drug may help the improvement of the acute form of COVID-19 [97]. Meanwhile, the study further suggested that since this drug can inhibit factors involved in acute airway inflammation such as IL-33 and cytokine, which significantly induce mucosal secretions like IL-13, it can be prescribed as a potential treatment for patients affected with a severe form of COVID-19 in addition with other medication [97].

A K^+ efflux is a common event of NLRP3 activation, and it could be a potential drug target for inhibiting the NLRP3 oligomerization and downstream release of caspase-1 and inflammatory cytokines. Glyburide, a sulfonylurea drug, acts by blocking the ATP-sensitive K^+ channels (KATP) in β-cells of the pancreas, which was shown to act upstream and prevent NLRP3 inflammasome activation in cells infected with RNA viruses [98]. Moreover, Colchicine, an alkaloid drug, has been shown to inhibit activation of the NLRP3 inflammasome with subsequent blocking of the release of proinflammatory IL-1β and IL-18 cytokine production [99]. Meanwhile, Chloroquine was suggested as a potential drug for targeting NLRP3 inflammasome formation [95] since a mice model with endotoxic shock showed that the drug significantly reduced IL-1β and IL-18 production in serum, peritoneal fluid, and lung tissue with a consequent improvement of survivability, reduced the levels of the NLRP3 protein and caspases-1 (p10) in homogenates in the lungs of mice [100]. Furthermore, hydroxychloroquine, is another ion-channel inhibitor, also shown to act as an anti-inflammatory

Table 2
Clinical trials (ClinicalTrial.gov) of inhibitors targeting NLRP3 mediated inflammasome.

Target/Inhibitor	Clinical trial	Study details	Interventions and outcomes
IL-1 targeted inhibitors			
Anakinra	NCT04643678	Allocation: non-randomized Participants: 80 Clinical Phase: phase 2, phase 3	100 mg SC injection every 12 h for 3 days, then 100 mg once daily from day 4 to day 7 plus Standard of Care. Primary clinical Outcome: day 14 of administration
Anakinra	NCT04357366	Allocation: N/A Participants: 1000 Clinical phase: phase 2	Treatment with 100 mg Anakinra subcutaneously once daily for ten days. Primary clinical Outcome: day 14
Rilonacept	NCT01045772	Allocation: N/A Participants: 10 Clinical phase: phase 2	160 mg of Rilonacept once per week. Primary clinical outcome: 1 year.
Pyroptosis and Caspase 1			
Emricasan	NCT04803227	Allocation: randomized, Double-blind, Placebo-controlled Participants: 50 Clinical Phase: phase 1	Emricasan 25 mg BID (days 1−14). Oral (capsule) administration. Primary clinical outcome: 14 days
PF-06650833	NCT04933799	Allocation: randomized, Double-blind, Placebo-controlled Participants: 68 Clinical Phase: phase 2	400 mg of the MR formulation orally under fasted conditions (preferably at least 4 h after and 1.5 h before meal). Primary clinical outcome: up to 29 days of administration
Disulfiram	NCT04594343	Allocation: randomized, Double-blind, Placebo-controlled Participants: 140 Clinical Phase: phase 2	500 mg of disulfiram orally or enterally through NG tube once daily for 14 days. Primary clinical outcome: up to 28 days of enrollment
Disulfiram	NCT04485130	Allocation: randomized, Double-blind, Placebo-controlled Participants: 60 Clinical Phase: phase 2	1000 mg DSF plus 27.75 mg microcrystalline cellulose powder per day for a total of 5 consecutive days. Outcome: Change in plasma inflammatory biomarker levels (5, 15, and 31)
NLRP3 inflammasome			
DFV890	NCT04382053	Allocation: randomized, controlled Participants: 143 Clinical Phase: phase 2	DFV890 administered in addition to the SoC for 14 days. Clinical outcome: Serum C-reactive protein (CRP) levels (up to 29 days); APACHE II severity of disease score on Day 15 or on the day of discharge
Tranilast	NCT03923140	Allocation: N/A, single-arm prospective cohort Participants: 71 Clinical phase: phase 2	5 mg/kg for juvenile patients with a maximum dose of 0.3 g per day; 0.1 g each time, three times a day for adult patients. Outcome: Changes in Auto-Inflammatory Diseases Activity Index score after 6-month treatment over baseline
Dapansutrile capsules	NCT04540120	Allocation: randomized, Double-blind, Placebo-controlled Participants: 80 Clinical Phase: phase 2	4 × 250 mg Dapansutrile capsules BID for 14 days with an initial (first) dose of 8 × 250 mg (2000 mg). Primary clinical outcome: 15 days
Melatonin	NCT04784754	Allocation: randomized, Double-blind, Placebo-controlled Participants: 50 Clinical Phase: phase 2	Hypromellose capsules containing 3 mg or 30 mg of the active component, orally, three times a day for 14 days. Clinical outcome: through 42 days

agent against NLRP3 either *in vivo* or *in vitro* experiments in addition to artemisinin [101]. A study showed that the drug could reduce the initial signal of the inflammasome by decreasing the expression of NF-κB signaling induced by I/R or H/R [102]. As an inhibitor of ion channels, hydroxychloroquine showed to inhibit activated K^{+} and Ca^{++} channels, which is likely to interfere with the activation of inflammasome [103]. In spite of protective efficacy in treating COVID-19, the adverse effect of quinolone drugs in modulating cellular excitability and action potential [104], particular caution should be taken into consideration for treating patients with heart diseases, including tachycardia, bradycardia, cardiomegaly and arrhythmia [105].

A significant increase in IL-6 level is associated with the hyperactivity of NLRP3 inflammasome [106]; consequently, inhibition of IL-6 signaling could inhibit the inflammasome activation. Hence, in this perspective, the selection of IL-6 inhibitors such as Sarilumab and Tocilizumab is being evaluated as two anti-IL-6 receptor monoclonal antibodies in several trials to treat COVID-19 accompanying the use of Anakinra, a recombinant human interleukin-1 (IL-1) receptor antagonist that acts similarly [107]. Moreover, several other proposed drugs such as Tocilizumab (antibody against IL-6), Emapalumab (anti-IFN-γ), protease inhibitors (Darunavir, Lopinavir, and Ritonavir) are also being studied for their anti-inflammatory roles [107].

However, though numerous drugs are getting focused for NLRP3 inhibition or subsequent NLRP3 mediated inflammation reduction, the mechanism of action of these drugs and ultimate side effects should also be investigated and evaluated in detail. The following section will briefly discuss several existing/ongoing clinical trials for some of these inhibitors:

3.1. Clinical implications of NLRP3 therapeutics

Several inhibitors that can target NLRP3 components have been clinically studied for their effectiveness in COVID-19 so to implicate a promising therapeutics to deal with the disease. To control the activation of NLRP3 inflammasome and downstream inflammatory reactions, the main strategy of such clinical trial is to evaluate a drug of interest that can − a) directly target NLRP3 inflammasome so downstream signaling cascade will block, b) targeting cytokine (especially IL-1) release, so, cytokine-mediated inflammatory response will be reduced, and c) targeting molecules involved with pore formation in cell membrane (such as GSDMD), so severe complications formed from pyroptosis can be inhibited.

For direct inhibition of NLRP3 inflammasome, recently, several drugs are under evaluation by different clinical trials. Melatonin, a neuroendocrine hormone, got much attention in this perspectives due to its anti-inflammatory activity [9]. A recent clinical trial (NCT04784754) showed that Melatonin supplementation can decrease the number of inflammatory cells, reduce the levels of the cytokines IL-4, IL-5, IL-13 and TNF-α and reduce nitric oxide and hydroxyl radical concentrations [108]. In addition to Melatonin, DFV890 (NCT04382053), Dapansutrile capsules (NCT04540120) and Tranilast (NCT03923140) are several other inhibitors that are evaluating under different clinical trials for targeting NLRP3 inflammasome formation (Table 2).

Meanwhile, to inhibit cytokine mediated inflammatory responses, IL-1 targeted inhibitors are most feasible strategies since IL-1 mediated cytokines especially IL-1β and IL-18 not only promote inflammation by itself, but also promote the release of other inflammatory cytokines such as IL-6 [9]. Currently, there are 3 available drugs that either block IL-1 binding to the IL-1 receptor (anakinra) or bind directly to IL-1 (rilonacept and canakinumab) approved by US Food and Drug Administration, FDA [109]. Among these 3 drugs clinical trial with Anakinra in patients with severe COVID-19 represent an effective treatment option that may confer clinical benefit, though some trials have mixed review [43]. However, a clinical trial of Anakinra in Italy (ClinicalTrials.gov NCT04318366) recently evaluated the efficacy of intravenous (IV) anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation [108]. The trial included 29 patients where each of them received IV infusions of high-dose anakinra (5 mg/kg twice a day), with a median treatment time of 9 days and the study found that at 21 days the survival rates were 90% in the high-dose anakinra group and 56% in the standard treatment group who received standard therapy only [110].

Besides targeting upstream molecules, clinical trials also ongoing to block downstream molecules such as GSDMD, the principle component for inducing pore formation and pyroptotic cell death. Disulfiram (DSF) is one of such inhibitor that have been reported to inhibit GSDMD pore formation by covalently modifying Cys191 and protects against LPS- induced sepsis and inflammatory cytokine secretion in mice [111]. Recently, a randomized, Double-blind, Placebo-controlled, clinical trial of DSF (NCT04594343) has been performed which included 140 participants where each of the subject receive 500 mg of disulfiram orally or enterally through NG tube once daily for 14 days with satisfactory outcome [108].

Table 2 summarizes some ongoing clinical trials to inhibit NLRP3 inflammasome targeted molecule at different point of signaling cascade available on ClinicalTrial.gov of national institute of health (NIH) [108].

4. Concluding remarks and future perspectives

Dysregulated immune response or over-activated immune components against SARS-COV-2 infection is the major contributing factors in the severity and worse clinical outcome of COVID-19. The formation of NLRP3 inflammasome and subsequent upregulation of downstream Caspase-1, inflammatory cytokines (IL-1β, IL-18, IL-6, and TNF-α), and gasdermin D with consequent damage to lung tissue in COVID-19 cases demonstrated the possibility that over-reactive immune response is the principal cause of such dysregulation. Due to the impact on physiologic changes and genetics, several underlying health conditions are associated with NLRP3 inflammasome-related inflammatory disorders following SARS-COV-2 infection. The immune system's ability to fight emerging infections and generate adequate vaccine responses gradually declines in people with underlying comorbidities. Because, in addition to promoting adequate proinflammatory phenotypes, such factors affect not only an individual's susceptibility to infections but also determine the disease course and clinical outcomes. Thus, this kind of inflammasome appears to be a potential drug target in the treatment of COVID-19. A detailed understanding of these factors is necessary, and determination of the strategies that will either directly target the inflammasome components or the upstream signaling pathways to block NLPR3 activation should be tailored completely.

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Abbreviations

NLRP3	NLR family pyrin domain containing 3
CAPSs	Cryopyrin-associated periodic syndromes
ARDS	Acute respiratory distress syndrome
ALI	Acute lung injury
IL-18	Interleukin 18
TNF-α	Tumor necrosis factor-alpha
PRR	pathogen recognition receptor
PAMP	pathogen-associated molecular pattern
DAMP	damage-associated molecular pattern
ASC	apoptosis-associated speck-like protein
CARD	caspase activation and recruitment domains
GSDMD	Gasdermin D
LDH	lactate dehydrogenase
HCV	hepatitis C virus
TLR8	toll-like receptor 8
TRAF	TNF receptor-associated factor
CFR	case fatality rate
CDC	center for disease control and prevention
ROS	reactive oxygen species
mtROS	mitochondrial reactive oxygen species
mtDNA	mitochondrial DNA

References

[1] Thomas PG, Dash P, Aldridge Jr JR, Ellebedy AH, Reynolds C, Funk AJ, et al. The intracellular sensor NLRP3 mediates key innate and healing responses to influenza A virus via the regulation of caspase-1. Immunity 2009;30:566–75.
[2] Kanneganti T-D, Body-Malapel M, Amer A, Park J-H, Whitfield J, Franchi L, et al. Critical role for Cryopyrin/Nalp3 in activation of caspase-1 in response to viral infection and double-stranded RNA. J Biol Chem 2006;281:36560–8.
[3] Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York city area. J Am Med Assoc 2020;323:2052–9.
[4] Cai Q, Chen F, Wang T, Luo F, Liu X, Wu Q, et al. Obesity and COVID-19 severity in a designated hospital in Shenzhen, China. Diabetes Care 2020: dc200576.

[5] Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. Nat Rev Immunol 2020;20:355–62.
[6] Campbell GR, To RK, Hanna J, Spector SA. SARS-CoV-2, SARS-CoV-1, and HIV-1 derived ssRNA sequences activate the NLRP3 inflammasome in human macrophages through a non-classical pathway. iScience 2021;24:102295.
[7] Rodrigues TS, de Sá KSG, Ishimoto AY, Becerra A, Oliveira S, Almeida L, et al. Inflammasomes are activated in response to SARS-CoV-2 infection and are associated with COVID-19 severity in patients. J Exp Med 2021;218:e20201707.
[8] Lara PC, Macías-Verde D, Burgos-Burgos J. Age-induced NLRP3 inflammasome over-activation increases lethality of SARS-CoV-2 pneumonia in elderly patients. Aging Dis 2020;11:756–62.
[9] Zhao N, Di B, Xu L-L. The NLRP3 inflammasome and COVID-19: activation, pathogenesis and therapeutic strategies. Cytokine Growth Factor Rev 2021. S1359-6101(21)00054-X.
[10] Tian S, Hu W, Niu L, Liu H, Xu H, Xiao S-Y. Pulmonary pathology of early-phase 2019 novel coronavirus (COVID-19) pneumonia in two patients with lung cancer. J Thorac Oncol 2020;15:700–4.
[11] Morris G, Bortolasci CC, Puri BK, Olive L, Marx W, O'Neil A, et al. Preventing the development of severe COVID-19 by modifying immunothrombosis. Life Sci 2021;264:118617.
[12] Franchi L, Eigenbrod T, Muñoz-Planillo R, Nuñez G. The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. Nat Immunol 2009;10:241–7.
[13] Zhao C, Zhao W. NLRP3 inflammasome—a key player in antiviral responses. Front Immunol 2020:11.
[14] Coates BM, Staricha KL, Koch CM, Cheng Y, Shumaker DK, Budinger GRS, et al. Inflammatory monocytes drive influenza A virus–mediated lung injury in juvenile mice. J Immunol 2018;200:2391–404.
[15] Saylor D, Dickens AM, Sacktor N, Haughey N, Slusher B, Pletnikov M, et al. HIV-associated neurocognitive disorder–pathogenesis and prospects for treatment. Nat Rev Neurol 2016;12:234–48.
[16] Negash AA, Ramos HJ, Crochet N, Lau DTY, Doehle B, Papic N, et al. IL-1β production through the NLRP3 inflammasome by hepatic macrophages links hepatitis C virus infection with liver inflammation and disease. PLoS Pathog 2013;9:e1003330.
[17] Wu J, Sun L, Chen X, Du F, Shi H, Chen C, et al. Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. Science 2013;339:826–30.
[18] Pedersen SF, Ho Y-C. SARS-CoV-2: a storm is raging. J Clin Investig 2020;130:2202–5.
[19] Chen J, Lau YF, Lamirande EW, Paddock CD, Bartlett JH, Zaki SR, et al. Cellular immune responses to severe acute respiratory syndrome coronavirus (SARS-CoV) infection in senescent BALB/c mice: CD4+ T cells are important in control of SARS-CoV infection. J Virol 2010;84:1289–301.
[20] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506.
[21] Zheng M, Williams EP, Malireddi RKS, Karki R, Banoth B, Burton A, et al. Impaired NLRP3 inflammasome activation/pyroptosis leads to robust inflammatory cell death via caspase-8/RIPK3 during coronavirus infection. J Biol Chem 2020;295:14040–52.
[22] Ferreira AC, Soares VC, de Azevedo-Quintanilha IG, Dias SdSG, Fintelman-Rodrigues N, Sacramento CQ, et al. SARS-CoV-2 engages inflammasome and pyroptosis in human primary monocytes. Cell Death Discov 2021;7:43.
[23] Li S, Zhang Y, Guan Z, Li H, Ye M, Chen X, et al. SARS-CoV-2 triggers inflammatory responses and cell death through caspase-8 activation. Signal Transduct Target Ther 2020;5:235.
[24] Toldo S, Bussani R, Nuzzi V, Bonaventura A, Mauro AG, Cannatà A, et al. Inflammasome formation in the lungs of patients with fatal COVID-19. Inflamm Res 2021;70:7–10.
[25] Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell 2006;124:783–801.
[26] Yue Y, Nabar NR, Shi C-S, Kamenyeva O, Xiao X, Hwang I-Y, et al. SARS-Coronavirus Open Reading Frame-3a drives multimodal necrotic cell death. Cell Death Dis 2018;9:904.
[27] Siu K-L, Yuen K-S, Castaño-Rodriguez C, Ye Z-W, Yeung M-L, Fung S-Y, et al. Severe acute respiratory syndrome coronavirus ORF3a protein activates the NLRP3 inflammasome by promoting TRAF3-dependent ubiquitination of ASC. FASEB J 2019;33:8865–77.
[28] Shi C-S, Nabar NR, Huang N-N, Kehrl JH. SARS-Coronavirus Open Reading Frame-8b triggers intracellular stress pathways and activates NLRP3 inflammasomes. Cell Death Discov 2019;5:101.
[29] Nieto-Torres JL, Verdiá-Báguena C, Jimenez-Guardeño JM, Regla-Nava JA, Castaño-Rodriguez C, Fernandez-Delgado R, et al. Severe acute respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome. Virology 2015;485:330–9.
[30] Magro C, Mulvey JJ, Berlin D, Nuovo G, Salvatore S, Harp J, et al. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. Transl Res 2020;220:1–13.
[31] Nieva JL, Madan V, Carrasco L. Viroporins: structure and biological functions. Nat Rev Microbiol 2012;10:563–74.
[32] Verdiá-Báguena C, Nieto-Torres JL, Alcaraz A, DeDiego ML, Torres J, Aguilella VM, et al. Coronavirus E protein forms ion channels with functionally and structurally-involved membrane lipids. Virology 2012;432:485–94.
[33] Javorsky A, Humbert PO, Kvansakul M. Structural basis of coronavirus E protein interactions with human PALS1 PDZ domain. Commun Biol 2021;4:724.
[34] Wang K, Xie S, Sun B. Viral proteins function as ion channels. Biochim Biophys Acta Biomembr 2011;1808:510–5.
[35] Chen I-Y, Moriyama M, Chang M-F, Ichinohe T. Severe acute respiratory syndrome coronavirus viroporin 3a activates the NLRP3 inflammasome. Front Microbiol 2019;10.
[36] Santos RAS, Sampaio WO, Alzamora AC, Motta-Santos D, Alenina N, Bader M, et al. The ACE2/angiotensin-(1-7)/MAS axis of the renin-angiotensin system: focus on angiotensin-(1-7). Physiol Rev 2018;98:505–53.
[37] Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;181:271–80. e8.
[38] Zhao M, Bai M, Ding G, Zhang Y, Huang S, Jia Z, et al. Angiotensin II stimulates the NLRP3 inflammasome to induce podocyte injury and mitochondrial dysfunction. Kidney Dis 2018;4:83–94.
[39] Asgari E, Le Friec G, Yamamoto H, Perucha E, Sacks SS, Köhl J, et al. C3a modulates IL-1β secretion in human monocytes by regulating ATP efflux and subsequent NLRP3 inflammasome activation. Blood 2013;122:3473–81.
[40] Ratajczak MZ, Kucia M. SARS-CoV-2 infection and overactivation of Nlrp3 inflammasome as a trigger of cytokine “storm” and risk factor for damage of hematopoietic stem cells. Leukemia 2020;34:1726–9.
[41] Bhakdi S, Tranum-Jensen J. Complement lysis: a hole is a hole. Immunol Today 1991;12:318–20.
[42] Triantafilou K, Hughes TR, Triantafilou M, Morgan BP. The complement membrane attack complex triggers intracellular Ca2+ fluxes leading to NLRP3 inflammasome activation. J Cell Sci 2013;126:2903–13.
[43] Vora SM, Lieberman J, Wu H. Inflammasome activation at the crux of severe COVID-19. Nat Rev Immunol 2021;21:694–703.
[44] Buszko M, Park J-H, Verthelyi D, Sen R, Young HA, Rosenberg AS. The dynamic changes in cytokine responses in COVID-19: a snapshot of the current state of knowledge. Nat Immunol 2020;21:1146–51.
[45] Lucas C, Wong P, Klein J, Castro TBR, Silva J, Sundaram M, et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. Nature 2020;584:463–9.
[46] Han Y, Zhang H, Mu S, Wei W, Jin C, Tong C, et al. Lactate dehydrogenase, an independent risk factor of severe COVID-19 patients: a retrospective and observational study. Aging (Albany NY) 2020;12:11245–58.
[47] Junqueira C, Crespo Â, Ranjbar S, Ingber J, Parry B, Ravid S, et al. SARS-CoV-2 infects blood monocytes to activate NLRP3 and AIM2 inflammasomes, pyroptosis and cytokine release. medRxiv 2021. 2021.03.06.21252796.
[48] Schroder K, Sagulenko V, Zamoshnikova A, Richards AA, Cridland JA, Irvine KM, et al. Acute lipopolysaccharide priming boosts inflammasome activation independently of inflammasome sensor induction. Immunobiology 2012;217:1325–9.
[49] Bajaj V, Gadi N, Spihlman AP, Wu SC, Choi CH, Moulton VR. Aging, immunity, and COVID-19: how age influences the host immune response to coronavirus infections? Front Physiol 2021;11:571416.
[50] Stout-Delgado HW, Cho SJ, Chu SG, Mitzel DN, Villalba J, El-Chemaly S, et al. Age-dependent susceptibility to pulmonary fibrosis is associated with NLRP3 inflammasome activation. Am J Respir Cell Mol Biol 2016;55:252–63.
[51] Kujoth GC, Leeuwenburgh C, Prolla TA. Mitochondrial DNA mutations and apoptosis in mammalian aging. Cancer Res 2006;66:7386–9.
[52] Oka T, Hikoso S, Yamaguchi O, Taneike M, Takeda T, Tamai T, et al. Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure. Nature 2012;485:251–5.
[53] Sahin E, Colla S, Liesa M, Moslehi J, Müller FL, Guo M, et al. Telomere dysfunction induces metabolic and mitochondrial compromise. Nature 2011;470:359–65.
[54] Albini A, Noonan DM, Pelosi G, Di Guardo G, Lombardo M. The SARS-CoV-2 receptor, ACE-2, is expressed on many different cell types: implications for ACE-inhibitor- and angiotensin II receptor blocker-based antihypertensive therapies-reply. Intern Emerg Med 2020;15:1583–4.
[55] Ciaglia E, Vecchione C, Puca AA. COVID-19 infection and circulating ACE2 levels: protective role in women and children. Front Pediatr 2020;8.
[56] Chen Y, Klein SL, Garibaldi BT, Li H, Wu C, Osevala NM, et al. Aging in COVID-19: vulnerability, immunity and intervention. Ageing Res Rev 2021;65:101205.
[57] Onder G, Rezza G, Brusaferro S. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. J Am Med Assoc 2020;323:1775–6.
[58] Lambadiari V, Kousathana F, Raptis A, Katogiannis K, Kokkinos A, Ikonomidis I. Pre-existing cytokine and NLRP3 inflammasome activation and increased vascular permeability in diabetes: a possible fatal link with worst COVID-19 infection outcomes? Front Immunol 2020;11:557235.
[59] Gregory JM, Slaughter JC, Duffus SH, Smith TJ, LeStourgeon LM, Jaser SS, et al. COVID-19 severity is tripled in the diabetes community: a prospective analysis of the pandemic's impact in type 1 and type 2 diabetes. Diabetes Care 2021;44:526.
[60] Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol 2010;11:136–40.

[61] Channappanavar R, Fehr Anthony R, Vijay R, Mack M, Zhao J, Meyerholz David K, et al. Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. Cell Host Microbe 2016;19:181–93.
[62] Secchi M, Bazzigaluppi E, Brigatti C, Marzinotto I, Tresoldi C, Rovere-Querini P, et al. COVID-19 survival associates with the immunoglobulin response to the SARS-CoV-2 spike receptor binding domain. J Clin Investig 2020;130:6366–78.
[63] Piché M-E, Tchernof A, Després J-P. Obesity phenotypes, diabetes, and cardiovascular diseases. Circ Res 2020;126:1477–500.
[64] López-Reyes A, Martinez-Armenta C, Espinosa-Velázquez R, Vázquez-Cárdenas P, Cruz-Ramos M, Palacios-Gonzalez B, et al. NLRP3 inflammasome: the stormy link between obesity and COVID-19. Front Immunol 2020:11.
[65] Russo L, Lumeng CN. Properties and functions of adipose tissue macrophages in obesity. Immunology 2018;155:407–17.
[66] Kassir R. Risk of COVID-19 for patients with obesity. Obes Rev 2020;21. e13034-e.
[67] Chu Y, Yang J, Shi J, Zhang P, Wang X. Obesity is associated with increased severity of disease in COVID-19 pneumonia: a systematic review and meta-analysis. Eur J Med Res 2020;25:64.
[68] Sun Y, Wang Q, Yang G, Lin C, Zhang Y, Yang P. Weight and prognosis for influenza A(H1N1)pdm09 infection during the pandemic period between 2009 and 2011: a systematic review of observational studies with meta-analysis. Infect Dis 2016;48:813–22.
[69] Zhang F, Xiong Y, Wei Y, Hu Y, Wang F, Li G, et al. Obesity predisposes to the risk of higher mortality in young COVID-19 patients. J Med Virol 2020;92:2536–42.
[70] Simonnet A, Chetboun M, Poissy J, Raverdy V, Noulette J, Duhamel A, et al. High prevalence of obesity in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) requiring invasive mechanical ventilation. Obesity (Silver Spring) 2020;28:1195–9.
[71] Peng Y, Meng K, Guan H, Leng L, Zhu R, Wang B, et al. Clinical characteristics and outcomes of 112 cardiovascular disease patients infected by 2019-nCoV. Zhonghua Xin Xue Guan Bing Za Zhi 2020;48:E004.
[72] a WHO Statement: Tobacco Use and COVID-19. 289, 2801–2809. [CrossRef]. b WHO Statement: Tobacco Use and COVID-19. Available online: https://www.who.int/news-room/detail/11-05-2020-who-statement-tobacco-use-and-covid-19. [Accessed 18 June 2020].
[73] Li C, Zhihong H, Wenlong L, Xiaoyan L, Qing C, Wenzhi L, et al. The nucleotide-binding oligomerization domain–like receptor family pyrin domain-containing 3 inflammasome regulates bronchial epithelial cell injury and proapoptosis after exposure to biomass fuel smoke. Am J Respir Cell Mol Biol 2016;55:815–24.
[74] Yi G, Liang M, Li M, Fang X, Liu J, Lai Y, et al. A large lung gene expression study identifying IL1B as a novel player in airway inflammation in COPD airway epithelial cells. Inflamm Res 2018;67:539–51.
[75] Buscetta M, Di Vincenzo S, Miele M, Badami E, Pace E, Cipollina C. Cigarette smoke inhibits the NLRP3 inflammasome and leads to caspase-1 activation via the TLR4-TRIF-caspase-8 axis in human macrophages. FASEB J 2020;34:1819–32.
[76] Xie J, Zhong R, Wang W, Chen O, Zou Y. COVID-19 and smoking: what evidence needs our attention? Front Physiol 2021;12:603850.
[77] Vardavas CI, Nikitara K. COVID-19 and smoking: a systematic review of the evidence. Tob Induc Dis 2020;18:20.
[78] Kashyap VK, Dhasmana A, Massey A, Kotnala S, Zafar N, Jaggi M, et al. Smoking and COVID-19: adding fuel to the flame. Int J Mol Sci 2020:21.
[79] Emami A, Javanmardi F, Pirbonyeh N, Akbari A. Prevalence of underlying diseases in hospitalized patients with COVID-19: a systematic review and meta-analysis. Arch Acad Emerg Med 2020;8. e35-e.
[80] Zheng Z, Peng F, Xu B, Zhao J, Liu H, Peng J, et al. Risk factors of critical & mortal COVID-19 cases: a systematic literature review and meta-analysis. J Infect 2020;81:e16–25.
[81] Baskaran V, Murray RL, Hunter A, Lim WS, McKeever TM. Effect of tobacco smoking on the risk of developing community acquired pneumonia: a systematic review and meta-analysis. PLoS One 2019;14. e0220204-e.
[82] Liu W, Tao Z-W, Wang L, Yuan M-L, Liu K, Zhou L, et al. Analysis of factors associated with disease outcomes in hospitalized patients with 2019 novel coronavirus disease. Chin Med J 2020:133.
[83] Chen Q, Zheng Z, Zhang C, Zhang X, Wu H, Wang J, et al. Clinical characteristics of 145 patients with corona virus disease 2019 (COVID-19) in Taizhou, Zhejiang, China. Infection 2020;48:543–51.
[84] Zheng Y, Xiong C, Liu Y, Qian X, Tang Y, Liu L, et al. Epidemiological and clinical characteristics analysis of COVID-19 in the surrounding areas of Wuhan, Hubei Province in 2020. Pharmacol Res 2020;157:104821.
[85] Oakes JM, Fuchs RM, Gardner JD, Lazartigues E, Yue X. Nicotine and the renin-angiotensin system. Am J Physiol Regul Integr Comp Physiol 2018;315:R895–906.
[86] Sharma G, Volgman Annabelle S, Michos Erin D. Sex differences in mortality from COVID-19 pandemic. JACC Case Rep 2020;2:1407–10.
[87] Vahidy FS, Pan AP, Ahnstedt H, Munshi Y, Choi HA, Tiruneh Y, et al. Sex differences in susceptibility, severity, and outcomes of coronavirus disease 2019: cross-sectional analysis from a diverse US metropolitan area. PLoS One 2021;16. e0245556-e.
[88] Takahashi T, Ellingson MK, Wong P, Israelow B, Lucas C, Klein J, et al. Sex differences in immune responses that underlie COVID-19 disease outcomes. Nature 2020;588:315–20.
[89] Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y, Zuo W. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov. bioRxiv 2020. 2020.01.26.919985.
[90] Yang X, Yu Y, Xu J, Shu H, Xia Ja, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med 2020;8:475–81.
[91] Cristiani L, Mancino E, Matera L, Nenna R, Pierangeli A, Scagnolari C, et al. Will children reveal their secret? The coronavirus dilemma. Eur Respir J 2020;55:2000749.
[92] Webb K, Peckham H, Radziszewska A, Menon M, Oliveri P, Simpson F, et al. Sex and pubertal differences in the type 1 interferon pathway associate with both X chromosome number and serum sex hormone concentration. Front Immunol 2019;9:3167.
[93] Shah A. Novel coronavirus-induced NLRP3 inflammasome activation: a potential drug target in the treatment of COVID-19. Front Immunol 2020:11.
[94] Juliana C, Fernandes-Alnemri T, Wu J, Datta P, Solorzano L, Yu J-W, et al. Anti-inflammatory compounds parthenolide and Bay 11-7082 are direct inhibitors of the inflammasome. J Biol Chem 2010;285:9792–802.
[95] Saeedi-Boroujeni A, Mahmoudian-Sani M-R, Bahadoram M, Alghasi A. COVID-19: a case for inhibiting NLRP3 inflammasome, suppression of inflammation with curcumin? Basic Clin Pharmacol Toxicol 2021;128:37–45.
[96] Huang Y, Jiang H, Chen Y, Wang X, Yang Y, Tao J, et al. Tranilast directly targets NLRP3 to treat inflammasome-driven diseases. EMBO Mol Med 2018;10:e8689.
[97] Saeedi-Boroujeni A, Mahmoudian-Sani M-R, Nashibi R, Houshmandfar S, Tahmaseby Gandomkari S, Khodadadi A. Tranilast: a potential anti-Inflammatory and NLRP3 inflammasome inhibitor drug for COVID-19. Immunopharmacol Immunotoxicol 2021;43:247–58.
[98] da Costa LS, Outlioua A, Anginot A, Akarid K, Arnoult D. RNA viruses promote activation of the NLRP3 inflammasome through cytopathogenic effect-induced potassium efflux. Cell Death Dis 2019;10:346.
[99] Misawa T, Takahama M, Kozaki T, Lee H, Zou J, Saitoh T, et al. Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. Nat Immunol 2013;14:454–60.
[100] Chen X, Wang N, Zhu Y, Lu Y, Liu X, Zheng J. The antimalarial chloroquine suppresses LPS-induced NLRP3 inflammasome activation and confers protection against murine endotoxic shock. Mediators Inflamm 2017;2017:6543237.
[101] Bai L, Li J, Li H, Song J-P, Zhou Y, Lu R, et al. Renoprotective effects of artemisinin and hydroxychloroquine combination therapy on IgA nephropathy via suppressing NF-κB signaling and NLRP3 inflammasome activation by exosomes in rats. Biochem Pharmacol 2019;169.
[102] Tang T-T, Lv L-L, Pan M-M, Wen Y, Wang B, Li Z-L, et al. Hydroxychloroquine attenuates renal ischemia/reperfusion injury by inhibiting cathepsin mediated NLRP3 inflammasome activation. Cell Death Dis 2018;9:351.
[103] Eugenia Schroeder M, Russo S, Costa C, Hori J, Tiscornia I, Bollati-Fogolín M, et al. Pro-inflammatory Ca++-activated K+ channels are inhibited by hydroxychloroquine. Sci Rep 2017;7:1892.
[104] Zhan X, Dowell S, Shen Y, Lee DL. Chloroquine to fight COVID-19: a consideration of mechanisms and adverse effects? Heliyon 2020;6. e04900-e.
[105] Xiong T-Y, Redwood S, Prendergast B, Chen M. Coronaviruses and the cardiovascular system: acute and long-term implications. Eur Heart J 2020;41:1798–800.
[106] Abbate A, Toldo S, Marchetti C, Kron J, Tassell BWV, Dinarello CA. Interleukin-1 and the inflammasome as therapeutic targets in cardiovascular disease. Circ Res 2020;126:1260–80.
[107] Bahadoram M, Keikhaei B, Saeedi-Boroujeni A, Mahmoudian-Sani M-R. Chloroquine/hydroxychloroquine: an inflammasome inhibitor in severe COVID-19? Naunyn-Schmiedeberg's Arch Pharmacol 2021;394:997–1001.
[108] NIH, U.S National Library of Medicine, ClinicalTrials.gov.
[109] Kaneko N, Kurata M, Yamamoto T, Morikawa S, Masumoto J. The role of interleukin-1 in general pathology. Inflamm Regen 2019;39:12.
[110] Pontali E, Volpi S, Antonucci G, Castellaneta M, Buzzi D, Tricerri F, et al. Safety and efficacy of early high-dose IV anakinra in severe COVID-19 lung disease. J Allergy Clin Immunol 2020;146:213–5.
[111] Hu JJ, Liu X, Xia S, Zhang Z, Zhang Y, Zhao J, et al. FDA-approved disulfiram inhibits pyroptosis by blocking gasdermin D pore formation. Nat Immunol 2020;21:736–45.
[112] Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395.