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

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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.

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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-kB 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 gasederm 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].
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 gasdemin 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 injection 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 inflamma- some 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 experimen- tal or natural infection [22]. These outcomes were linked with caspase-1 activation, IL-1β production, GSDMD cleavage, and dys- regulation 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 proin- flammatory 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-1β, 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 forma- tion [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-κB-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. 

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The second signal comes from cytosolic K\textsuperscript{+} 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\textsuperscript{2+} leakage from ERGIC/Golgi membrane by forming a Ca\textsuperscript{2+} permeable ion channel, thus the resulting ion imbalance may augment the pathogenesis [29,32]. Since, Ca\textsuperscript{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\textsuperscript{+}, 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-1\textbeta 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-1\textbeta gene transcription and IL-1\textbeta 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 mannann-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 in inflammasome activation in COVID-19

The major molecules regulated by NLRP3 in 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 NLRP3 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) 2. Telomere dysfunction 3. Reduced B cell maturation 4. Increased infiltration of inflammatory cells | [51,52] |
| Diabetes | 1. DAMPs (release by increased glyco-lipotoxicity and oxidative stress) 2. Delayed IFN response 3. Decreased T cell count 4. Infiltration of inflammatory cells due to increased vascular permeability | [49] |
| Obesity | 1. DAMPs (ceramides and cholesterol crystal mediated ROS formed from impaired adipocytes) 2. An elevated level of ACE2 expression due to increased mass of adipocytes 3. Exacerbat ed lipolysis due to hyperplasia induce mitochondrial damage 4. Aberrant ionic flux due to increased levels of cathepsin B and Ca2+ from lysosomal damage | [64] |
| Smoking | 1. Increased ACE2 expression induced by nicotine 2. An elevated level of inflammatory cytokine secretion due to increased ACE2 expression | [35] |
| Gender | 1. Hormonal difference 2. X-chromosome based sensor expression 3. Immune responses variations | [91] |

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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: 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 increase 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 pneumonia [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 smoke 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 ex- posers are more remarkable due to several reasons such as — 1) nicotine can induce the expression of ACE2 receptors in the respi- ratory tract of smokers that predispose that person to be signifi- cantly infected with the virus [85]; 2) highly ACE2 expressing cell infected by SARS-COV-2 induce the release of inflammatory cyto- kines 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 sus- ceptible 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 out- comes 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 dis- ease 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 hospi- talized 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 estrogen levels appear to upregulate ACE-2 production 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 alkaldoid drug, has been shown to inhibit activation of the NLRP3 inflammasome with subsequent blocking of the release of proin- flammatory 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

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 inflam- matory 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 Rubi- dosia 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 com- pound, 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.
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 Ca²⁺ 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 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.

### 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 Treatment with 100 mg Anakinra subcutaneously once daily for ten days. Primary clinical outcome: day 14 |
| Anakinra | NCT04357366 Allocation: N/A Participants: 1000 Clinical phase: phase 2 | | |
| 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 | | Hypermelose capsules containing 3 mg or 30 mg of the active component, orally, three times a day for 14 days. Clinical outcome: through 42 days |

*Note: Details of the clinical trials and their outcomes are subject to change and should be verified through official sources.*
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-a and reduce nitric oxide and hydroxyl radical concentrations [108]. In addition to Melatonin, DFP/V890 (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-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-1b, IL-18, IL-6, and TNF-a), and gsdemir 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 NLRP3 activation should be tailored completely.

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### Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| 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 |
| mROS | Mitochondrial reactive oxygen species |
| mtDNA | Mitochondrial DNA |

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