Minireview *Highlight article*

Role of inflammatory markers in corona virus disease (COVID-19) patients: A review

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Impact statement

In late 2019, a novel virus called SARS-CoV-2, expanded globally from Wuhan, China and was declared a pandemic on 11 March 2020 by the WHO. The mechanism of virus entry inside the host cell depends upon the cellular proteases including cathepsins, HAT, and TMPRSS2, which splits up the spike protein and causes further penetration. MERS coronavirus uses DPP4, while coronavirus HCoV-NL63 and SARS-CoV and SARS-CoV-2 employ ACE-2 as the key receptor. Cytokine storm syndrome was analyzed in critically ill nCOVID-19 patients and it is presented with high inflammatory mediators, systemic inflammation, and multiple organ failure. Among various inflammatory mediators, the level of interleukins (IL-2, IL-7, IL-10), G-CSF, MIP1A, MCP1, and TNF-α was reported to be higher in critically ill patients. Understanding this molecular mechanism of ILs, T cells, and dendritic cells will be helpful to design immunotherapy and novel drugs for the treatment of nCOVID-19 infection.

Abstract

The whole world is locked down due to the outbreak of novel Coronavirus Disease 2019 (nCOVID-19). A novel virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus pandemic 2019. Investigating the role of inflammatory mediators and understanding the virology of nCOVID-19 virus help in designing a rational and effective therapy for this infection. This review provides an overview of the inflammatory mediators activated during nCOVID-19 infection and the pathophysiology of this viral infection. In this review, the authors have a detailed discussion about the types of viral strains of nCOVID-19, its mechanism of action, host immune response, and the dysregulation caused by the viruses in the host immune system causing disease progression. Understanding the role of inflammatory cytokines, chemokines, and clinical immunology will be the approach to find out the possible novel therapeutic interventions. Therapies involving regulation of immune responses help in inhibiting the various steps in the pathologies of infection. Also, updated knowledge regarding the dysregulation of immune system and disease outcome in critically ill patients serves as a precautionary measure in the development and evaluation of vaccine.

Keywords: Corona virus, cytokines, inflammatory markers, SARS-CoV-2, COVID-19

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Introduction

In late 2019, a novel virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), expanded globally from Wuhan, China, and was reported to cause severe acute respiratory syndrome. It was declared a pandemic on 11 March 2020 by the World Health Organization (WHO). The clinical features of this novel Coronavirus Disease 2019 (nCOVID-19) is very much similar to viral pneumonia. After the clinical diagnosis of respiratory samples, the CDC (Centre for Disease Control) experts considered this pneumonia as a severe acute respiratory syndrome caused by a coronavirus family called SARS COV-2, later named as nCOVID-19.[1] The outbreak of COVID-19 in China and its worldwide spread led to the WHO declaring it as a Public Health Emergency on January 2020.[2] As of 19 May 2020, a total of 6,799,713 confirmed cases of coronavirus and 397,388 deaths have been reported.[3] This virus

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affects humans, causing respiratory and other infections.[4,5] Acute respiratory distress syndrome develops in severely affected patients as it causes diffuse alveolar damage.

Acute inflammation in the lungs is a complex pathophysiological mechanism involving inflammatory mediators such as cytokines and chemokines, which stimulate the macrophages in the alveoli, leading to poor regulation of the immune system.[6] In humans, the clinical progression of the novel coronavirus-induced disease exists in a triphasic form.[7] The clinical features in first phase include fever, dry cough, myalgia, and other systemic infections that are likely to be increased by the replication of the virus and cell necrosis. The associated feature of the second phase is the onset of IgG immunoglobulins conversion, correlated with the decrease in viral replication. During this phase, uncontrolled viral replication occurs causing severe worsening of symptoms. The exact hypothesis behind this might be the severe damage to alveoli caused by over exuberant immune response of the host.[7–9] In nCOVID-19-infected patients, the major patient population recovered after two weeks, but one-third of the patients progressed to the third phase, which is characterized by severe lung inflammation leading to ARDS, i.e. acute respiratory distress syndrome.[10] Serious adverse health outcome of nCOVID-19 infection was found much more prevalent among children, especially those below 12 years, the elderly population, and patients with comorbid diseases.[7,8]

Coronaviruses: Classification and structure

The virus causing nCOVID-19 belongs to a family of viruses known as Coronaviridae. Coronaviruses can be classified in four genera: alpha, beta, gamma, and delta. Human CoV belong to either alpha or beta. Gamma and delta CoV tend to infect birds. The coronaviruses affecting humans are of seven types, as depicted in Table 1. Among them, the highly pathogenic corona viruses are SARS CoV, MERS CoV, and SARS CoV-2. They cause severe pneumonia in humans by infecting the lower respiratory tract, which causes diffuse alveolar damage, resulting in increased morbidity and mortality.[11–13] Two Indian Geneticists investigated 3600 strains of nCOVID-19 collected from 55 countries, and they observed that a specific type of virus known as Type 2a now replaces the parenteral strain, which was found in Wuhan. Their research showed that Type-O was spread from China to other countries and afterwards, the virus evolved inside and outside China. The 35 viral sequences categories are grouped into four types as mentioned in Table 2.[14]

Table 1. Types of coronaviruses affecting human.

S. No.	Type
1	229E (alpha coronavirus)
2	NL63 (alpha coronavirus)
3	OC43 (beta coronavirus)
4	HKU1 (beta coronavirus)
5	MERS-CoV (beta coronavirus)
6	SARS-CoV (beta coronavirus)
7	SARS CoV-2 (novel coronavirus)

The nCOVID-19 virus has a round, enveloped structure with a diameter of approximately 80 to 120 nm and contains a positive genome of single stranded RNA of 31 kb size.[4,5] This genome, present with a basic nucleocapsid (N) protein in a complex form, forms a type of helical viral protein called spike protein (S), which is a type-1 glycoprotein forming peplomer on the surface of the virion giving the structure a crown-like appearance as shown in Figure 1.[15]

Mechanism of action and key features of human coronaviruses

Coronaviruses comprise of specific genes in open reading frames (ORF1) regions which encode proteins used for viral replication, spike formation, and the formation of nucleocapsid.[16] The spikes are made for glycoprotein functions such as virus attachment and entry in to the host cells. The coronavirus has the property of affecting multiple hosts due to the presence of receptor binding domain, which is roughly attached among viruses.[17,18] Most coronaviruses recognize key receptor carbohydrates or aminopeptidases for entry to the host cells, while MERS-CoV and SARS-CoV recognize exopeptidases. The mechanism of entering inside the host cell depends upon the cellular proteases including cathepsins, HAT (human airway trypsin like protease), and TMPRSS2 (transmembrane protease serine-2), which splits up the spike protein and causes further penetration. MERS coronavirus uses DPP4 (dipeptidyl peptidase 4), while coronavirus HCoV-NL63 and SARS-CoV and SARS-CoV-2 employ ACE-2 (angiotensin-converting enzyme-2) as the key receptor.[17,19]

The structure of nCOVID-19 consists of typical spike proteins and also other polyproteins, nucleoproteins, and membrane proteins, such as chymotrypsin-like protease, RNA polymerase, helicase, papain-like protease, glycoprotein, and accessory proteins.[19] The structural proteins in

Table 2. Different types of viral sequences (total 35).

S. No.	Types	Viral sequence
1	The ancestral type-O	5
2	Type A2a	16
3	Type A3	13
4	Type B	1

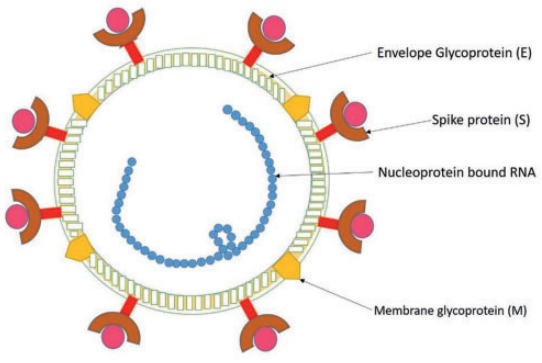

Figure 1. Structure of novel coronavirus, SARS-CoV-2. (A color version of this figure is available in the online journal.)

nCOVID-19 are encoded by the four structural genes envelope (E), membrane (M), nucleocapsid (N), and spike (S). The largest gene in the nCOVID-19 *orf1ab* encodes for proteins 15nsp and pp1ab.[20,21] Some studies report that the difference between SARS-CoV and nCOVID-19 is the fluctuation in the amino acid sequence of 3c, 8b protein, and absence of 8a protein in nCOVID-19.[22] The single mutation in N501 in nCOVID-19's spike protein has increased its binding affinity with the ACE-2 receptor.[21]

A research study demonstrates the homotrimer structure of spike glycoprotein of nCOVID-19 in both open (ligand bound) and closed (ligand free) conformation responsible for host cell adhesion as well as the interaction of S1 domain of spike glycoprotein of nCOVID-19 with the human immunoregulatory factor CD26 showing virulence and the hijacking nature of nCOVID-19.[22]

The life cycle of nCOVID-19 in human cells is shown in Figure 2. Spike proteins bind to the ACE-2 receptor, and after binding, conformational changes occur in the spike protein facilitating the fusion of the viral envelope with the host cell membrane. After entering the host cell, the virus releases its RNA into the cell, and the process of translation begins. Replicase polyproteins (pp1a and 1ab) of viruses form, which are then cleaved by proteinases into smaller products. The viral genome RNA and the proteins assembled in the endoplasmic reticulum and Golgi complex as virions are then carried out through the vesicles and released out from the cells.[19]

Human immune system and pathogenesis of nCOVID-19

In humans, immunity is classified into two types – natural (innate) and specific (adaptive) immunity. Both of them are correlated in their action. Innate immune response is the first line of defense against pathogens and is inborn. It shows fast response and has no antigen specificity; therefore, it does not confer long lasting immunity. The human innate immune system consists of epithelial barriers (surface of skin, mucous membrane), cellular defenses (neutrophils, macrophages, dendritic cells, natural killer cells, mast cells, lymphoid cells), soluble mediators (kinins, c-reactive proteins, mannose binding lectin, surfactant coating the respiratory passages), pattern recognition receptors (PPRs) including toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I-like receptors (RLRs), C-type lectin receptors (CLRs), etc.[23] The common features observed in critically ill nCOVID-19 patients are mentioned in Table 3.[24]

Pattern recognition receptors

Viral infection is detected by PRRs, which recognize the pathogen-associated molecular patterns. Currently, the

Table 3. The following are the common features observed in critically ill nCOVID-19 patients.

S. No.	Common Features
1	Respiratory failure
2	Sudden worsening of disease around 1–2 weeks after onset
3	High level of inflammatory mediators including CRP (C-reactive proteins) and pro-inflammatory cytokines like interleukins, tumor necrosis factor (TNFα), etc.
4	Damaged immune system presented by the atrophy of lymph nodes and spleen, also reduction in the lymphocytes level
5	Elevated levels of infiltrated immune cells like monocytes, macrophages found in lung lesions
6	Hypercoagulation, vasculitis, and multiple organ damage

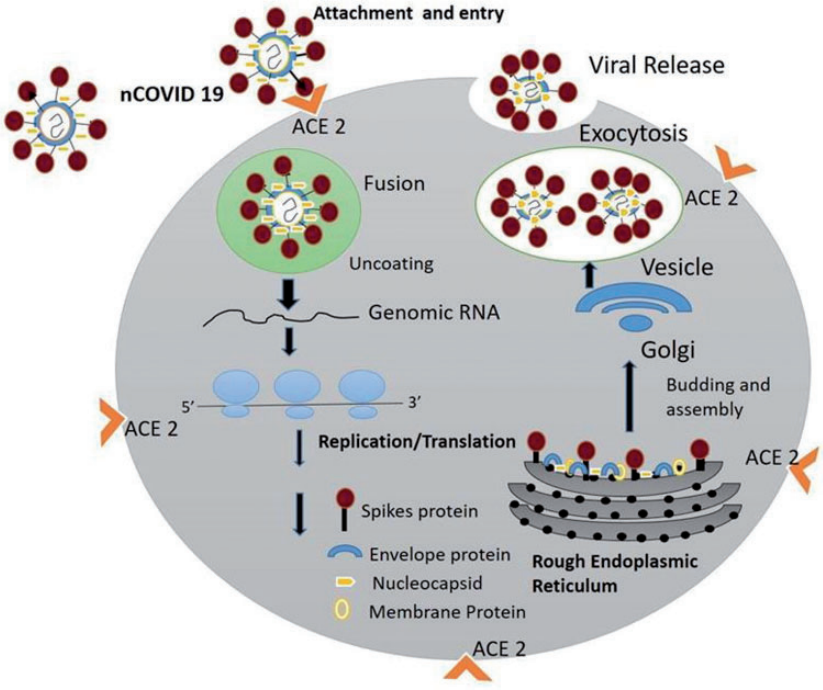

Figure 2. Life cycle of nCOVID-19 in host cell. (A color version of this figure is available in the online journal.)

known PRRs include TLRs, NLRs, RLRs, CLRs, and other receptors present in the cytoplasm, such as IFI 16, DAI, cGAS, STING, and so on.[23]

Toll-like receptors

The pathogen-associated molecular patterns detected by toll-like receptors involve proteins, lipids, nucleic acids, and lipoproteins of viruses, bacteria, and fungi. They also detect the pathogen-associated molecular pattern in cell membrane, lysosomes, endosomes, and other sites in the cell.[25] There are different TLRs which induce several biological immune responses subsequently by activating adapter proteins such as MyD88, TRIP, TIRAP, and TRAM. All these proteins share the TIR (Toll/Interleukin1 receptor) structure.[26] Stimulation of inflammatory mediator expression is caused by transcription factors NF-kB and MAPKs (mitogen-activated protein kinase) pathways that are activated by TLR3.[25] Binding of nCOVID-19 virus to DPP4 (dipeptidyl peptidase 4) receptor on host cell through spike protein S causes the appearance of genomic RNA in the cytoplasm. This partially generates an immune response to the double-stranded RNA during CoV replication. The dsRNA sensitizes TLR3 receptor, which causes activation of a cascade of signaling pathways such as NF-kB, IRFs, and MAPKs, which produce pro-inflammatory cytokines and type I interferons (IFNs). Type I IFNs enhances the secretion of antiviral proteins which protects the uninfected cells. In some cases, the accessory proteins of nCOVID-19 virus bind with its dsRNA during replication and interfere with TLR3 signaling, preventing its activation and suppressing the immune response. MyD88 pathway also causes activation of pro-inflammatory cytokines sensitized by another toll-like receptor, TLR4. Another adapter protein TRIF of TLR3 and TLR4 also stimulates the expression of immune responses such as type I IFNs and inflammatory mediators. The function of TIRAP and TRAM is to recruit adapter protein MyD88 to TLR2 and TLR 4 receptors and TRIF molecules to TLR 4 receptors. This is the reason TLR signaling pathway is also categorized as MyD88-dependent pathway that functions in the activation of inflammatory mediators and TRIF-activated type I IFNs.[25]

Nucleotide-binding oligomerization domain-like receptors or NOD-like receptors

These are pattern recognition receptors that detect pathogen components and possess conserved nucleotide-binding and oligomerization domain-like structure.[27] According to its functions, NLRs are categorized into three subtypes. The first type forms complexes with several proteins and are called inflammasomes like proteins. The second subtype is required in reproduction and embryo generation.[28] The third type comprises of regulatory NOD-like receptors, which are involved in positive or negative inflammatory signaling pathways.[29,30]

Retinoic acid-inducible gene-I-like receptors

These receptor proteins recognize the nucleic acids of RNA viruses. The viral nucleocapsid protein containing triphosphine RNA can be recognized by retinoic acid inducible gene-I at the 5′-end.[31]

C-type lectin-like receptor

These receptors are highly expressed in myeloid cells and because of their motif structure, they perform a wide range of functions like cell adhesion, stimulation of endocytosis, tissue repair, activation of platelets, and natural immune system. These receptors gets activated by two main mechanisms in the cells, the first one is direct activation like macrophage-induced activation of CLEC4E, Mincle, and CLEC6A receptors. Another type of activation is by HAM-like motifs in the intracellular structure of the receptor.[32,33] Both these mechanisms recruit spleen tyrosine kinases (acidified), β-cell lymphoid tissue 10 (bcl10) and forms complexes. This signaling pathway activates NF-kB and MAPKs, cellular responses, cell chemotaxis, phagocytosis, and DC cells maturation.[34]

Cytokine regulation of inflammation in nCOVID-19 infection

Cytokines are proteins, peptides, or glycoproteins that are secreted by hematopoietic and non-hematopoietic cells in response to various stimuli. They are soluble in nature. Currently, about 200 cytokines have been investigated and they are classified into 5 broad categories on the basis of their structural similarity (Table 4).[23] Cytokines function in the regulation of growth by activating JAK (Janus family of proteins, tyrosine kinases), activates transcription and regulates gene expression. It also activates other inflammatory mediators. Cytokine storm syndrome was analyzed in critically ill nCOVID-19 patients and is presented with high inflammatory mediators, systemic inflammation, and multiple organ failure.[24] During an infection, cytokines originates from the infected area and disseminates over the body systemically. It was observed in the case of coronaviruses infection like SARS-CoV and MERS-CoV, where inflammatory cell infiltration occurs because of rapid viral replication and cytokine storm causing acute lung injury, ARDS, and death.[35,36]

The level of inflammatory mediators of 41 nCOVID-19-infected patients including non-ICU patients (28) and ICU patients (13) was measured by Huang *et al.*[1] They analyzed the elevated level of interleukins (IL-1B, 1RA, IL-7, IL-8, IL-9, and IL-10), granulocyte colony-stimulating factor (G-CSF), interferon gamma (IFN-γ), interferon gamma inducible protein (IP10), macrophages inflammatory protein one alpha (MIP1A), monocyte chemoattractant protein (MCP1), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and tumor necrosis factor (TNF-α). Among the above inflammatory mediators, the level of interleukins (IL-2, IL-7, IL-10), G-CSF, MIP1A, MCP1, and TNF-α was reported to be higher in critically ill patients.[1,37] No pronounced variation was observed in the serum level of IL-6 in the non-ICU and ICU nCOVID-19 patients.

Another study shows an elevated level of IL-6 in an nCOVID-19 patient who did not survive, compared to the group that survived. This study was a multicenter

Table 4. Classification of cytokines on the basis of their structural similarity.

S. No.	Family name	Representative members
1	Interleukin-1 family	IL-1α, IL-1β, IL-1Ra, IL-18, IL-33
2	Hematopoietin (Class-1 cytokine) family	GM-CSF (granulocyte macrophage colony-stimulating factor), M-CSF (Monocyte colony-stimulating factor), Growth hormone, erythropoietin/hematopoietin, thrombopoietin, interleukin (IL-2, IL-3 to IL-9)
3	Interferon (Class-2 cytokine) family	Interferon (IFN-α, IFN-β, IFN-γ),
4	Tumor necrosis factor family	Tumor necrosis factor (TNF-α, TNF-β), platelet-derived growth factor, transforming growth factor (TGF-β)
5	Chemokine family	IL-8, monocyte chemokine protein

retrospective cohort study conducted by Zhou *et al*.[38] Other studies also concluded and reported elevated levels of IL-6 in critically ill patients with nCOVID-19.[39–41] Xu *et al*. reported that elevated level of T-Helper-17 cells (Th-17 cells) in nCOVID-19 patients was due to the excessive release of IL-6.[42] Cytokine IL-6 is important for the production of Th-17 cells in the interaction of T cells with dendritic cells.[43] This interaction of Th-17 cells with dendritic cells leads to the pathogenesis of various autoimmune diseases. During this process, the autoreactive T cells are stimulated by autoantigens presented by the dendritic cells.[44] Hence, elevated level of IL-6 and the interaction of Th-17 cells with dendritic cells play a major role in the pathogenesis of ARDS. Understanding this molecular mechanism of ILs, T cells, and dendritic cells will be helpful to design immunotherapy and novel drugs for the treatment of nCOVID-19 infection.

Lymphocyte activation was analyzed in a study showing lymphocytopenia in 123 patients, ^and reduction in CD8+ T cells, i.e. 28.43% in mild and 47.62% in severe cases, was reported. The percentage of natural killer cells (NK-cells) was also found to be reduced in both mild and severe cases of corona infection, i.e. 34.31% and 47.62%. Deficiencies of particular subsets of lymphocytes (e.g. CD8+, T cells, NK cells) lead to increased risk of autoimmune disease and opportunistic infections.[45] In addition to this, the expression of cytotoxic particles like granolysin and perforin was profoundly expressed in CD8+ cytotoxic T cells. [46]

Persistently elevated level of ESR (erythematosus sedimentation rate), CRP (C-reactive protein), and TNF-α was also reported in severe cases of corona-infected patients, and they are found to be associated with an increased risk of ARDS, disseminated intravascular coagulation (DIC), hypercoagulation manifested as thrombocytopenia, and gangrene of extremities and thrombosis. DIC is identified as the systemic stimulation of blood coagulation resulting in generation and accumulation of fibrin causing microvascular thrombi in the organ system, leading to multi organ dysfunction syndrome (MODS).[47] Extra pulmonary systemic hyperinflammation syndrome is the clinical feature manifested in the third stage, which is the most severe stage of illness in nCOVID-19 patients. This inflammation causes airflow obstruction, which has a profound effect on gas exchange processes and cardiac functions. This systemic inflammation worsens the conditions of comorbid patients suffering from diseases like hypertension, diabetes, depression, lung cancer, normocytic anemia, and osteoporosis.[48]

Chemokines regulation of inflammation in nCOVID-19 infection

Chemokines are proteins that play a major role in cell signaling in the immune system and act through seven transmembrane G-protein receptors. Previous research studies supported the evidence that differential expression of chemokine set, receptors, and adhesion particles delivers the molecular code responsible for the precise organization of leukocytes, migration under homeostatic, as well as proinflammatory conditions like infection, allergy, and autoimmune diseases. They are defined by structure and classified into four subgroups (two large and two small) depending on cysteines arrangement. They are named C, CC, CXC, and CX_3C. Depending upon their immunological functions, they are again classified into (i) homeostatic chemokines, which coordinates trafficking of leukocytes during circulation of lymphocytes and hematopoiesis; (ii) inflammatory chemokines induced by antigens, and primary cytokines, which direct the transport of immune cells at the sites of infection; (iii) angiogenesis chemokines, and (iv) angiostatic chemokines. During a viral infection, changes occur in both homeostatic and inflammatory chemokine receptors, guiding dendritic cells to the lymph nodes where they facilitate the changes from innate to adaptive immunity.[49] Initially, the virus stimulates the plasmacytoid subsets present on the dendritic cells to generate IFN-α, which induces the production of CCL3, an inflammatory chemokine.[50,51] CCL3 helps in part of the recruitment of NK cells that tries to kill the viral-infected cells directly and helps in producing a large quantity of IFN-γ. The IFN-γ activates the macrophages and causes local production of CXCL9 or MIG (monokine-induced by IFN-γ) which plays a major role in the enrollment of Tc1-polarized CD8+ T and Th1-polarized CD4+ cells, most likely caused by the activation of CXCR3 chemotactic receptor. The general cascade of immunoregulation in the occurrence of a viral infection has the concept of a cytokine to chemokines and then again to cytokines.[52]

Inflammation and hyperferritinemia

Some studies suggests that ferritin helps in protecting the host body system against active infection by restricting the availability of iron to pathogen.[53,54] Also, iron supplements

taken during infection increase the chances of mortality in human beings.[55] Elevated level of ferritin in the blood causes hypoferremia-like condition in patients who are exposed to very high pathogens.[56] Experimental studies performed in mice show effects on macrophages, uptake of iron, and recirculation and storage when they are injected with cytokines, interleukin IL-1α, or TNF-α.[57] Along with significant activation of macrophages, patients manifesting hyperferritinemic phenotype show an abnormal pattern of activation of reticuloendothelial system and multiple organ damage.[57] This condition is described as familial hemophagocytic lymphohistiocytosis (FHLH), a recessive gene disorder where excess macrophage activation caused by IFN-γ results from its failure to eliminate pathogens subsequently to inherited disorder in NK cell and CTL-mediated cytolysis.[58] The level of serum ferritin may be used as a biomarker to determine the pathological condition of nCOVID-19 infection and it can also be used as a target for various therapeutic interventions in clinical practice.

Fibrinogen regulation of inflammation in nCOVID-19 infection

Major mediators of the coagulation reaction such as fibrinogen, tissue factor, and thrombin are found to be associated with diseases as an inflammatory component. Fibrinogen plays a pro-inflammatory role in several diseases like stroke, brain trauma, multiple sclerosis, bacterial infection, and different types of cancers. Various epidemiological studies investigate the association between disease development and coagulation markers.[59] Fibrinogen has been identified as a risk factor as well as modulator of inflammatory pathways in several infectious diseases. It is a soluble glycoprotein 340-KDa synthesized in liver by hepatocytes.[60] It consists of three polypeptide chains A-alpha, B-beta, and gamma encoded by three genes.[61] The three chains form an extended molecule that binds with other similar molecules by disulfide bonds forming fibrinogen, a homodimeric molecule.[62] It is an acute phase mediator which gets broken down into two fibrinopeptides A and B during activation of coagulation reaction, thus exposing several sites for polymerization and allowing fibrin fibrils formation. Platelet plug formation takes place with the involvement of fibrinogen and platelets.[63] The C terminal of the gamma chain of fibrinogen binds to the αIIb β3 site of the integrin receptor present on platelet surface, mediating the bridge formation in between fibrinogen and platelets causing platelet aggregation. Fibrinogen and its derivative peptides play a major role in activating immune cells by ligand receptor interactions. This pro-inflammatory response is caused by non-overlapping fibrinogen signals through binding sites by binding with integrin receptor CD11b/CD18, expressed by leukocytes of innate immune system, which circulates macrophages, monocytes, and CNS residents microglia.[64] SARS corona virus 3a protein upregulates fibrinogen expression in epithelial cells in the lungs of patient by upregulating the mRNA levels of three polypeptide chains of fibrinogen. As a result, the secretion as well as intracellular level of fibrinogen was found to be elevated in nCOVID-19 patient.[65] nCOVID-19 infection may be associated with disseminated intravascular coagulopathy (DIC). It was analyzed that initially nCOVID-19-induced coagulopathy presented with highly elevated levels of D-dimer and degraded products of fibrinogen.[66]

Dysregulation of immune system in nCOVID-19 patient

The nCOVID-19 infection activates both adaptive and innate immune system. Impairment in inflammatory adaptive and innate immune responses causes damage to the tissue systemically and locally. Lymphocytopenia is the prominent clinical and diagnostic feature observed in nCOVID-19 patients. T cells as well as NK cells were also reduced, and this point of reduction was found even lower and undetectable in critically ill patients. Also T cells, both regulatory and helper T cells, were also found to be decreased in critical cases.[50] An autopsy study on nCOVID-19 patients revealed that cellular damage occurs to secondary lymphoid tissue, which is unusual from cytokine storm-related inflammatory response. Spleen autopsy studies observed that there is a reduction in the number of lymphocytes, macrophage activation, proliferation, and phagocytosis with focal hemorrhage necrosis. Decrease in the number of lymph nodes and lymph node atrophy followed by necrosis was also observed. Immunohistochemical analysis revealed the low levels of CD4+ and CD8+ T cells in spleen and lymph nodes.[51] In lungs, diffused alveolar damage (DAD) along with infiltration of cells, i.e. macrophages, monocytes, moderate multinucleated giant cells and few lymphocytes (CD4+ T cells), were observed. In some cases, virus inclusion bodies can be detected in epithelium of type II alveoli and macrophages, although PCR test was found negative in throat swab or blood.[42,52,67] These observations are consistent with the features of the "primary cytokine" storm stimulated by nCOVID-19 infection, mainly produced by epithelial cells, alveolar macrophages, and endothelial cells, whereas in the later stages of viral infections, a "secondary cytokine" storm was analyzed induced by activated T cells and causes complications of T cells involving therapies.[68]

Conclusion

This review suggested the role of inflammatory mediators in nCOVID-19-infected patients which is diverse and multifunctional. In majority of the cases, the immunopathogenesis of nCOVID-19 infection involves disturbances of inflammatory mediators that do not cause the disease but helps in the progression of the disease. Specific set of cytokines and chemokines may be therapeutically targeted, and a synergistic action may be obtained with the drugs that are used for the treatment of nCOVID-19 infection. Understanding the pathogenesis of inflammatory mediators as well as the type of corona virus helps in developing a model which may guide the therapeutic interventions. This study also reflects the presence of different types of strain of corona viruses, which has to be taken into account to understand the mechanism of pathogenesis caused by them. Also, a thorough knowledge of pattern recognition

receptors, inflammatory mediators like cytokines, chemokines, ferritin, fibrinogen, and clinical immunology are now required in order to find out the novel therapeutic interventions. Therapies involving regulation of immune responses help in inhibiting the various steps in pathologies of infection. Also understanding the dysregulation of immune system and disease outcome in critically ill patients serves as a precautionary measure in the development and evaluation of vaccine.

Authors' contributions: JU, NT and MNA had the idea for this study and designed it and took responsibility for the integrity of the data. JU and MNA contributed in the writing of the report. NT and MNA contributed to the critical revision of the manuscript. All authors reviewed and approved the final version of the manuscript.

DECLARATION OF CONFLICTING INTERESTS

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