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Review Paper

A Highlight on Prevention and Protection: Cytokine Storm and Covid-19, a Chronicle of Pro-Inflammatory Cytokines

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ABSTRACT:

The outbreak of the novel SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) responsible for coronavirus disease 2019 (COVID-19) has developed into an unprecedented global pandemic. Clinical investigations in patients with COVID-19 has shown a strong upregulation of cytokine and interferon production in SARSCoV2- induced pneumonia, with an associated cytokine storm syndrome. Thus, the identification of existing approved therapies with proven safety profiles to treat hyperinflammation is a critical unmet need in order to reduce COVI-19 associated mortality. To date, no specific therapeutic drugs or vaccines are available to treat COVID-19 patients. This review evaluates several options that have been proposed to control SARS-CoV2 hyperinflammation and cytokine storm, eincluding antiviral drugs, vaccines, small-molecules, monoclonal antibodies, oligonucleotides, peptides, and interferons (IFNs). The COVID-19 disease pathology is plausibly linked to the hyperinflammatory response of the body characterized by pathological cytokine levels. The term 'cytokine storm syndrome' is perhaps one of the critical hallmarks of COVID-19 disease severity. In this review, we highlight prominent cytokine families and their potential role in COVID-19, the type I and II interferons, tumour necrosis factor and members of the Interleukin family. We address various changes in cellular components of the immune response corroborating with changes in cytokine levels while discussing cytokine sources and biological functions. Finally, we discuss in brief potential therapies attempting to modulate the cytokine storm.

Keywords: Recombinant Coronavirus COVID-19 SARS-CoV-2, cytokine storm;

1. Introduction

Global health has recently been threatened by the continuous epidemic of the respiratory sickness Coronavirus Disease 2019(Covid-19) [1]. The history of human coronaviruses began in 1965.The virus's name is derived from the crown-like spikes on its surface. Corona is derived from the Latin word "Crown." Coronaviruses are a class of viruses that infect animals and ***mammals*** such as bats and pigs. They are referred to as zoonotic by scientists because they spread from animals to people. Scientists have discovered two plausible causes of coronavirus illness through experimentation: 1. Consumption of wildlife items such as bats; 2. Animal pathogens. Coronavirus is spread by coming into contact with a virus-infected surface or object and then touching your nose, eyes, or mouth.[16]The coronavirus disease 19 was emerged in Wuhan, China in December 2019, and spread around the world.SARS-CoV-2 is phylogenetically linked to severe acute respiratory syndrome-like (SARS-like) bat

viruses, suggesting that bats might be the major reservoir. Although the intermediate source of origin and transfer to humans is unknown, quick human-to-human transmission has been proven [2].As of today, seven coronaviruses have been identified as being capable of infecting humans. Three of these viruses (SARS-CoV, MERS-CoV& SARS-CoV-2) cause serious sickness, while the others (229E, NL63, OC43 & HKU1) cause minor symptoms in people [3].In the wake of the Covid-19 outbreak, public health, research and the medical community have faced significant challenges. In a journal, Li and colleagues presented a detailed clinical and epidemiologic description of the first 425 patients reported in the epicentre of the outbreak: Wuhan, Hubei Province, China. It was found that death and morbidity were highest among the elderly (Median age 59years) and those with coexisting conditions (similar to the situation with influenza); 56% of the patients were male.[4]The second wave of COVID-19 in England coincided with the introduction of VOC 20DEC-01 (lineage B.1.1.7), which became dominant in the UK by January 2021 and subsequently in many nations across the globe as of April 2021.B.1.1.7 is defined by 17 mutations, eight of these disrupt the viral spike protein, which binds to angiotensin-converting enzyme 2 (ACE2) and allows SARS-CoV-2 to enter host cells.[5] B.1.1.7 has been predicted to be 40% to 90% more transmissible than the previous lineages [6]and an elevated risk of hospitalization and death may be seen.[7] Since COVID-19related fatalities peaked in the Spring of this year, there was hope that the worst was gone. However, following a spike in fatalities during the summer, the trend has increased even more. A million new instances of SARS-CoV-2 infection, which had been increasing monthly, started showing up weekly.[8]. South Korea. The goal of this study was to investigate the primary epidemiological features and transmission patterns of coronavirus disease 2019 (COVID-19) in children and adolescents. The Korea Disease Control and Prevention Agency carried out this investigation from January 20, 2020 to June 5, 2021. There were 14,967 patients aged 0–18 years among the total confirmed COVID-19 cases. Children and adolescents aged 16–18 years were the most affected age group (3589, 24.0%). The infection pathway through friends and family members (31.9%) was the most common across all age groups.According to the South Korean Infectious Disease Control and Prevention Act, all COVID-19 patients, except those who self-treat at home, must receive inpatient treatment at an infectious disease control institution, which is a hospital specialised in infectious diseases. The COVID-19 high risk group of patients aged 0-2 years were barred from self-treatment and were mostly hospitalised. As a result, despite the high hospitalisation rate among infants and young children, there were no severe or fatal cases in this age group.[9] In a study of 181 confirmed Covid-19 positive patients' group, it was seen that COVID-19 has a median incubation time of 5 days, and it is expected that virtually all the infected people will begin experiencing symptoms within 12 days after infection.[10] A study of more than a million patients in England revealed that seven symptoms consistently and jointly predicted SARS-CoV-2 PCR positivity. The symptoms include loss or alteration of smell, loss or alteration of taste, fever, new persistent cough, chills, loss of appetite, and muscle discomfort. In an another study, it was found that When it comes to three coronavirus-related diseases (COVID-19, SARS, and MERS), fever was found to be the most common initial symptom, followed by cough in influenza. COVID-19, SARS, and MERS all have the first two symptoms of fever and cough. COVID-19, on the other hand, appears to be on a slightly different route. With COVID-19, the upper gastrointestinal system (i.e., nausea/vomiting) appears to be impacted before the lower gastrointestinal tract (i.e. diarrhoea).[11]A new SARS-CoV-2 variant of concern (VoC), omicron, was reported on November 25, 2021, about 23 months after the first reported case of COVID-19 and after an estimated 260 million cases and 5.2 million deaths worldwide. This Omicron variant is also known as B.1.1.529 variant. Although previous VoCs appeared in a world where natural immunity to COVID-19 infections was common, this fifth VoC appears at a time when global vaccine immunity is

increasing.[12] Because of the high mutation rate in the spike glycoprotein, the Omicron variant appears to be more transmissible than earlier SARS-CoV-2 variants, but it is impossible to establish whether it is more lethal than the Delta version.[13] RNA viruses are known for their ability to quickly mutate and change in order to adapt to and survive in changing surroundings. The OMICRON variant's most worrying feature is its collection of more than 50 mutations, roughly 30 of which are in the spike protein. The 15 altered locations in the receptor-binding domain (RBD), which interacts with human cells before cell entry and may increase transmissibility, are the most concerning.[14] The first sequenced omicron case was discovered in Botswana, a country in Southern Africa, and a few days later, another sequenced case was discovered in a tourist from South Africa in Hong Kong. The main worries concerning omicron are whether it is more infectious or severe than other VoCs, or, it can bypass vaccine protection. Omicron has over 30 mutations and some deletions. Increased transmissibility, stronger viral binding affinity, and antibody escape have all been linked to these deletions and alterations. Other omicron mutations with documented consequences include those that improve transmissibility and affect binding affinity.[15] According to GISAID, as of February 22, 2022, the OMICRON variant has already appeared in 156 nations, sharing 1.5 million OMICRON genome sequences, with the majority of cases coming from the United Kingdom, the United States, and Denmark. In India, there have been 15,458 OMICRON positive cases. The first mortality associated with this novel COVID-19 mutation was reported in the United Kingdom.[16]. As of March 14, 2022, 458.95 million people are affected and 6.07 million people died worldwide in Covid-19. Countries like USA, Brazil, India, Russia, Mexico has the most deaths in covid-19. Here are 10 countries that have the most death rate. In USA 993.8k, In Brazil 655.1k, In India 515.9k, In Russia 361.32k, In Mexico 321.1k, In Peru 211.54k, In UK 162.73k, In Italy 157k, In Indonesia 152.43k, In France 140.1k people died because of Covid-19. Countries like Colombia (139.3k), Iran (130k), Argentina (116k), Germany (126.17k) are also suffering with huge death rates.[17]

Defining Inflammatory Pathways

SARS-2-CoV is a single stranded RNA virus, which binds to ACE-2 receptors in the lung epithelium and enters the cell via endocytosis . As part of viral replication and manufacture, damage associated molecular patterns (DAMPs) are released into the cytosol and are sensed by circulating macrophages via pattern recognition receptors (PRR) and RIG-I-like receptors (RLRs) [8]. Macrophage recognition of viral invasion initiates the process of chemotaxis and the recruitment of other immune cells via the secretion of acute phase response cytokines IL-6, TNF-α, IL-1β and type-1 interferons (innate immune system) [10]. Other, macrophage-bound receptors involved in the detection of foreign genetic material, include toll like receptors (TLRs), NOD like receptors (NLRs) and the interferon related STING/cGAS pathway. The latter is involved in cellular cross-talk and transcription of anti-viral proteins in neighboring cells. NLRs also act to induce autophagy of pathogens and are involved in inflammasome formation (NLR). These molecules activate the enzyme Caspase-1, leading to production of IL-1β from its precursors [11]. They also facilitate the upregulation of tumor necrosis factor receptor-associated factor (TRAF). The Janus Kinase/Signal Transducers and Activators of Transcription (JAK/STAT pathway) forms an integral part of innate immunity signaling and contributes to the differentiation of Natural Killer (NK) cells from lymphoid precursors. NK cells are activated by IL-12 and seek to destroy any host cell with altered or missing major histocompatibility complex (MHC) information. The adaptive immune response is initiated when dendritic cells present antigen fragments to naïve CD4 T-cells. Interleukins 2,4,5 & 10 all have a role in the differentiation of naïve T-cells to T-helper cells, which in addition to IFN-γ, stimulate IgM antibody secretion from B cells (plasma cells) . Memory B-cells are responsible for developing a ‘cellular memory’ of antigenic material, so

that neutralizing antibodies can be quickly manufactured, should viral invasion reoccur. A sub-group of T cells called T-regulator cells (Treg) are thought to have an important role in governing the amplitude of the humoral immune response, and it has been documented that reduction in Treg cell numbers is associated with increased susceptibility to auto-immune disorders . Treg cell depletion leads to an increase in the levels of IL-6, 17 and IFN-γ, and reduces the clearance of neutrophils via the secretion of IL-8 . This sequence of events contributes to the development of lung injury and ARDS [12]

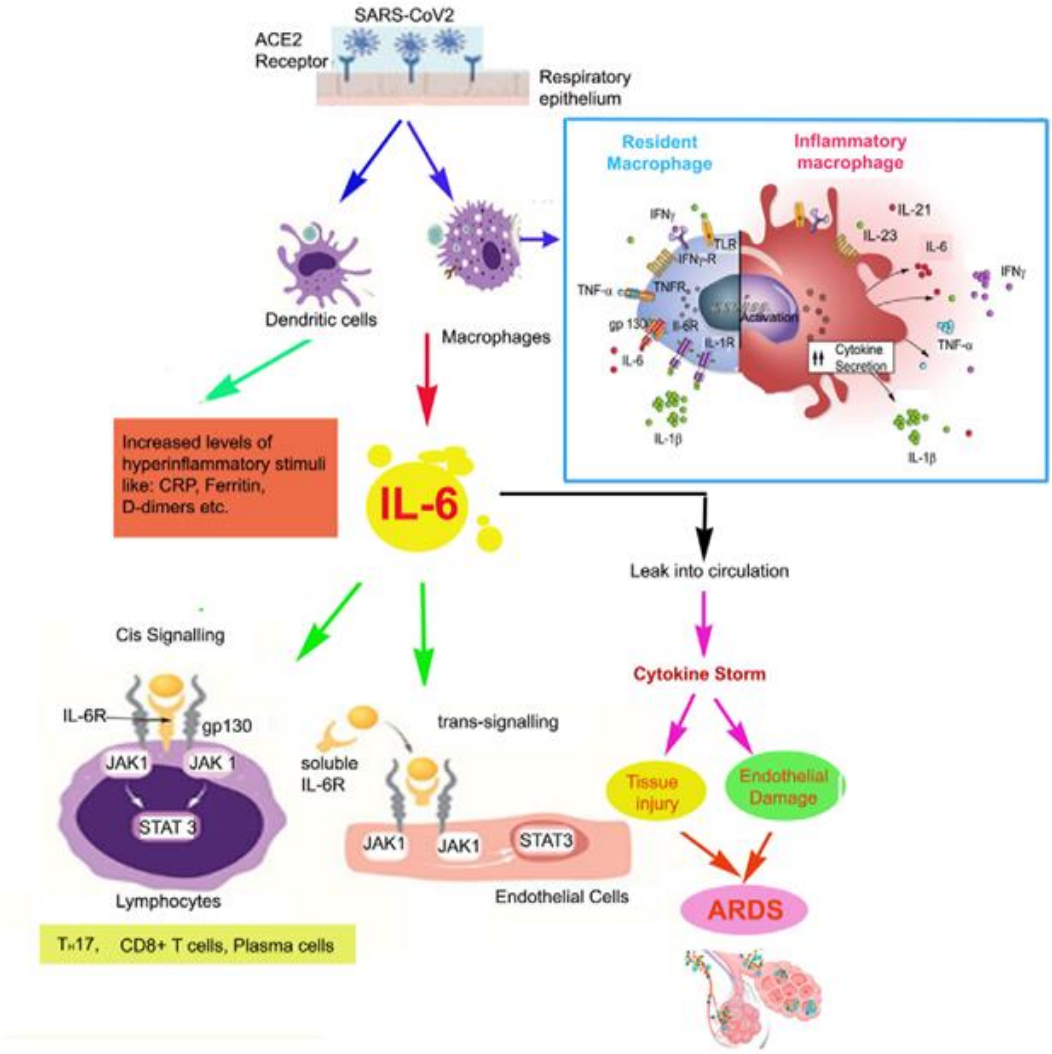
Pathogenesis of the cytokine release syndrome

The cytokine release syndrome (CRS) is a complex cascade of multiple chemokines released by the immune system in response to pathogenic material (Figure 1). The cytokines which are important in hyper-inflammation include IL-6, IL8, TNF-α, IL1-β, MCP-1, GCS-F, IP-10, CCL1-3, IL-17 and IFNγ. Delayed secretion of type-1 interferons (IFN-α/β) is also felt to accelerate the development of CRS [13]. Individual immune response may vary between different pathogens and the stage of the disease course . Consequently, researchers are faced with the dilemma of quelling immune system over activity, whilst preserving mechanisms of viral clearance and anti-body production. It is challenging to identify the cytokines which are most damaging in COVID-19, without compromising this delicate balance. The risk of impairing a beneficial adaptive immune response, from cytokine inhibition, has recently been defined. The acute-phase response cytokines, IL-6 and TNF-α, are considered by many to be the major 'culprits' in the pathogenesis of COVID-19 hyper-inflammation. It has been suggested that IL-6 promotes a macrophage activation syndrome (MAS) [14], triggering mass production of proinflammatory cytokines and inducing migration of neutrophils and fibroblasts into the pulmonary epithelium. This results in increased deposition of collagen and fibrin, leading to damage to underlining lung tissue . In humans with severe COVID-19, SARS and Ebola hemorrhagic fever (EHF), there is positive correlation between elevated IL6 levels, disease severity and mortality . In a study of patients receiving CAR-T therapy, increased IL-6 was associated with grade-4 CRS and peaked between days 2–5 of treatment [14]. IL-6 additionally causes detrimental endothelial activation, and nitrous oxide (NO) dependent increase in vascular permeability, which will be discussed below. It is possible to consider IL-6 as both 'friend and foe'; given its ability to 'class-switch' into either a pro- or anti-inflammatory form, as mediated by the protease TACE (TNF-α-converting enzyme). When IL-6 is bound to its soluble receptor (sIL-6 R), a cascade of hyper-inflammation is induced, whereas the IL6-membrane bound IL-6 complex, downregulates this response [15]. IL-6 intra-cellular signaling is complex, involving the gp130 receptor, JAK/STAT, RAS/RAF and AKT/P13 K pathways [16]. The IL-6 'classical' pathway involves the IL-6 receptor (IL-6 R) which binds directly to gp130. The 'trans' signaling pathway uses soluble sIL-6 receptors to aid gp130 binding and signal transduction in cells which do not express membrane bound IL-6 R [17]. In an animal model of influenza, IL-6 knock-out mice were found to have an increased mortality, reduced phagocytic function and increased fibroblast proliferation. Therefore, the ability of IL-6 to initiate chemotaxis and immune system polarization, may make its inhibition unfavorable in the early disease course. TNF-α has a similar role to IL-6, as an acute phase response cytokine, with pro-inflammatory actions in COVID-19. Increased serum levels of TNF-α are associated with increased CRS severity. In addition, TNF-α has a role in tissue regeneration, by activating nuclear kappa factor B (NF-kB) signaling pathways, to recruit circulating progenitor cells . Murine models of SARS virus (using recombinant S 'spike' protein) have demonstrated that TNF-α and IL-6 may be stimulated directly by the virus via the NF-kB pathway . This would explain the supra-normal levels of the acute phase response cytokines observed in response to infection by coronaviruses. In addition to stimulating the production of pro-inflammatory cytokines, COVID-19 may induce TNF-α-

mediated lymphocyte apoptosis. TNF-α induces caspase-mediated cell death in other disease states such as rheumatoid arthritis, by triggering the release of mitochondrial cytochrome c into the cytosol . Under normal circumstances, this is a carefully regulated homeostatic mechanism and TNF- α/NF-kB activation can also induce cytoprotective proteins . Cell death leads to reduced CD4/8 count (lymphopenia); a commonly reported clinical finding of severe COVID-19. Moreover, the reduction in T lymphocytes appears too great to be explained by lymphocyte sequestration in lymphoid tissue.

Endothelial activation – the eye of the cytokine storm?

Evidence is emerging that the multi-organ injury observed in COVID-19 is a consequence of cytokine-induced endothelial dysfunction (endothelitis) [18]. IL-6 causes endothelial activation and neutrophil infiltration, which results in NO (nitric oxide)-mediated changes to vascular permeability and loss of vascular tone . This is reflected clinically by increased neutrophil to T-cell lymphocyte ratio, and the development of septic shock . Endothelial activation occurs initially within the microcirculation, in order to prevent pathogenic material from translocating to larger vessels. Unfortunately, this increases microvascular complications including microthrombi and capillary hemorrhage; pathologies which have been observed following the post-mortems of both COVID19 and SARS patients [19 20]. Clinical markers such as d-dimer, which are predictive of poor outcome in COVID-19 are products of fibrin degradation and reflect underlying endothelial-mediated activation of the clotting cascade . Multiple cases of ST-elevation Myocardial Infarction (STEMI), in the absence of major epicardial coronary obstruction, have been reported in COVID-19 [21]. In a cohort of 28 patients from the Italian district of Lombardy, 40% of COVID-19 patients presenting with typical STEMI had no flow limiting lesion [31]. Moreover, biopsies from a STEMI patient with unobstructed coronaries in COVID-19, did not demonstrate myocarditis [22]. Myocardial infarction (MI) in COVID-19 may be triggered by cytokine-induced microvascular dysfunction [28]. Troponin remains a gold standard biomarker in the context of infarction, but may also be significantly elevated in microvascular obstruction (MVO). Marked elevations are also seen in septic shock and are prognostic [31]. Thrombolysis is not deemed appropriate treatment in COVID-19 STEMI, when type-2 myocardial infarction predominates [30, 31]. The role of ACE inhibitors has proved controversial in COVID-19, however some suggest that they do not cause harm, and may be beneficial [32, 33]. ACE inhibitors are proven to be cardioprotective and help to reduce long-term endothelial activation, by controlling hypertension and encouraging LV remodellinging [32]. It is possible that this cohort of patients may be better faced to withstand CRSinduced inflammation, given that angiotensin-II is linked to vascular NO pathways [8]. In animal models, therapeutic heparin has improved survival following challenge with LPS [34]. Heparin, however, did not reduce inflammatory cytokine levels, and the development of subsequent coagulopathy made the activated partial thromboplastin time (APTT) difficult to interpret. In addition to microvascular events, COVID-19 is associated with an increase in arterial thrombosis. A recent case study of five COVID-19 positive patients under 50 years, reported ischemic stroke as a cerebrovascular manifestation of the disease [35]. An occlusive thrombus was identified in all cases and treatment consisted of anti-coagulation with clot retrieval. These phenomena may not be surprising considering that endothelial activation in non-COVID disorders such as atherosclerosis, affects the cardiovascular and neurovascular systems in a similar manner [36]. Moreover, inflammatory molecules and leukocytes are known to traverse the blood-brain barrier causing central nervous system (CNS) inflammation; a mechanism which may be further augmented when endothelial integrity is compromised [37].



Fig(1): Contribution of IL-6 in Cytokine storm and SARS-CoV2 disease severity..[38]

2. New frontiers in cytokine suppression

As the detrimental effects of COVID-19 associated CRS are recognized, there has been a rush to trial pre-existing 4 L. PEARCE ET AL. immune-modulating therapies, used in the treatment of chronic inflammatory conditions. Anti-viral agents which predominantly target the host cell (modulating endocytosis, viral replication and manufacture) such as Remdesivir, Hydroxychloroquine/chloroquine and arbidol have been investigated widely [45, 47] and are the subject of many clinical studies [46, 48]. Although these agents have shown some early promise, they may be considered less relevant as direct mediators of the CRS of COVID-19, hence this article will focus on new frontiers in targeting cytokine release. The IL-6 receptor blocker, Tocilizumab (TCZ) has generated much interest in this context [5, 8, 11, 19]. Acting directly on the IL-6 R, TCZ can inhibit both classical and trans-signaling pathways and is licensed for treatment of CRS associated with CAR-T therapy [19]. Traditionally, TCZ is used in the treatment of rheumatoid arthritis (RA) and sHLH caused by Still's Disease (Juvenile onset arthritis) [5, 11, 15, 25]. It is generally well tolerated in patients with chronic inflammatory conditions, however in a review of phase III RCT's of RA, TCZ was found to cause upper respiratory tract infection in approximately 7% of patients and hypertension in 6% of patients [49]. A retrospective study from Wuhan, China analyzed the outcomes of 15 critical care patients who received TCZ for COVID-19. TCZ was associated with mitigation of IL-6 response, however the study lacked controls, was retrospective and patients received

multi immune-modulating therapies [50]. The results of randomized control trials (RCTs) are currently awaited. An important relationship may be emerging between IL-6 and androgen regulation [51]. It has been proposed that upregulation of androgens in males leads to increased expression of cellular transmembrane serine protease 2 receptors (TMPRSS2) via IL-6 signaling [51]. Animal models have demonstrated that TMPRSS2, alongside ACE-2 receptors, govern entry of COVID-19 into the host cell. Moreover, inhibition of TMPRSS2 has been found to kill the SARS-CoV-2 virus [52]. Attention has turned to established anti-androgen therapies such as spironolactone and bicalutamide as potential modulators of pro-inflammatory cytokines [53]. Similarly, inhibitors of IL-1β (AnakinraTM) and TNF- α (EtanerceptTM) have been proposed as anti-inflammatory agents in already-established severe CRS [8]. Whilst they have shown promise in animal models with LPS-induced infection [24], data in humans is limited. The JAK/STAT pathway is considered an attractive target for cytokine suppression [5, 8, 11, 16, 17]. Of note, inhibition of this pathway would disrupt the signaling of multiple cytokines and maximize anti-inflammatory effect. Caution must be exercised however, as there is a theoretical danger in blocking cytokines associated with viral clearance via (JAK 1&3) [11]. Immunosuppressants can impede viral clearance via interference with type-I IFN signaling, which upregulates NK cells and the adaptive immune response. In addition, JAK inhibitors can increase the risk of venous thrombosis, which is a significant pathology in COVID-19 [16]. The tyrosine-kinase inhibitor Ponatinib, has been found to inhibit the release of multiple cytokines in animal models of severe influenza [54]. In vivo, Ponatinib significantly down-regulated three key cytokines (IP10, IL-8 and MCP-1), and reduced overall mortality Other pharmacological agents proposed in influenza associated cytokine storms include peroxisome proliferatoractivated receptor agonists (PPARs), sphingosine-1-phosphate receptor modulator agonists (S1P1) and COX inhibitors [13]. Corticosteroids remain controversial], as they have previously increased mortality in critical care patients suffering from severe influenza and ARDS [55]. Administration of IVIG and convalescent plasma of survivors has shown early promise, however these are therapies suited to critical care and treatment of well-established grade 4 CRS [56]. A new novel target of the rapamycin (mTOR) pathway has recently been proposed [57]. This is based on the principle that tissue damage in CRS is related to antibody-dependent enhancement (ADE) and this class of drugs has been utilized previously in the prevention of CRS in transplant and H1N1 [58]. Circulatory support methods such as Extra Corporeal Membrane Oxygenation (ECMO) used on critical care, are an effective means of cytokine removal and therefore reduce multi-organ involvement [59]. It is important clinically, when considering pharmaceutical options to also consider the hemodynamic status of the patient and the consequences of shock physiology. Critical care teams have observed the importance of ensuring that the severely unwell COVID-19 patient has optimal fluid balance, so as to avoid development of ARDS, fluid overload and congestive renal injury with renal hypo-perfusion [60]. Similarly, cardio-renal syndrome related to COVID-19 can occur by this mechanism of excessive volume expansion and third space fluid accumulation, caused by IL-6 induced changes to vascular permeability. In animal models of sepsis, Remote Ischemic Conditioning (RIC) has shown significant promise in reducing mortality [61]. RIC is a widely reported cardio-protective phenomenon, evident when periods of sequential ischemia and reperfusion at a remote site to the target organ, confer protection of cardiomyocytes from reperfusion injury (RPI) [61, 62]. RIC is performed in humans by gradual inflations and deflations of a blood pressure cuff situated on the upper limb [63]. In mice challenged with LPS, RIC significantly reduced the serum concentration of pro-inflammatory cytokines (IFN-γ, IL1β, TNF- α) and improved survival. The exact mechanism by which this occurs remains unclear, however RIC may modulate adenosine receptors and downregulate the NF-kB pathway of cytokine release [62]. In humans, RIC uses vagally-mediated pathways to reduce the

inflammation from reperfusion injury, which can occur in both sepsis and acute myocardial infarction [62, 64]. RIC could therefore have a role in suppressing the inflammatory storm of COVID-19 and is both an accessible and simple intervention.

3. Role of Cytokines in Covid-19 :

Cytokines are a type of polypeptide signalling molecule that regulates a variety of biological processes by binding to cell surface receptors. Some of the most important cytokines are those involved in adaptive immunity (e.g., IL-2 and IL-4), proinflammatory cytokines and interleukins (ILs) (e.g., interferon (IFN)-I, -II, and -III; IL-1, IL-6, and IL-17; and TNF-α); and anti-inflammatory cytokines (e.g., IL-10).As a protective response to stress-producing internal processes, host cells produce cytokines that play a critical role in cell metabolic reprogramming.[41,42]Numerous investigations have identified abnormal levels of the following cytokines and chemokines in patients in the short time since the onset of COVID-19: IL-1, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-13, IL-17, M-CSF, G-CSF, GM-CSF, IP-10, IFN-γ, MCP- 1, hepatocyte growth factor (HGF), TNF-α, and Vascular Endothelial Growth Factor (VEGF)[43,44,45]. The reduction of antiviral defences related to the innate immune response, as well as an increased generation of inflammatory cytokines, may be the key point in SARS-CoV-2 infection.

Followings are the cytokines which are known to be affected by Covid-19 Virus:

1.IL-1: Activated monocytes and macrophages are the main producers of IL-1, whichplays an important role in the inflammatory response to infection [60]. SARS-CoV-2 appears to stimulate and mature IL-1β, which then activates additional proinflammatory cytokines like IL-6 and TNF-α [61][62].Hence, coronavirus infections produce a cytokine storm that includes IL-1β [63]. In 14 severe cases of COVID-19, Yang et al. discovered elevated levels of the antagonistic receptor of IL-1 (IL-1Ra), which has been linked to increased viral load, pulmonary function loss, lung damage, and mortality risk [64].In patients with severe COVID-19, Liu et al. discovered higher IL-1α levels, which were strongly linked to lung damage [65]. IL-1 levels are observed to the virulence of the process, and significantly greater serum levels have been seen in SARS-CoV-2 individuals with severe symptoms than in mild cases or in those infected with the 2003 SARS-CoV or the 2012 MERS coronavirus [66]. IL-1β, which has been linked to SARS, hypercoagulation, and disseminated intravascular coagulation, is found in the majority of COVID-19 patients with severe symptoms [67]. As a result, in order to avoid the cytokine storm, various therapeutic techniques have targeted IL-1 suppression [68]. Mesenchymal stem cells (MSCs) have been used in this manner to decrease proinflammatory cytokines such as IL-1α and TNF-α [45].

2.IL-2: IL-2 is essential for T cell proliferation as well as the development of effector and memory T cells [69]. It is implicated in adaptive immunity and promotes T, B, and NK cell proliferation and activation by increasing glucose metabolism [42]. As a result, IL-2 supports the prevention of autoimmune illnesses and is required to maintain self-tolerance and regulate immune responses [70]. This interleukin's absence has been linked to a lack of control over effector cells and, as a result, the development of autoimmunity [71]. Huang et al. discovered higher levels of IL-2 or its receptor IL-2R in COVID-19 patients, and these increases have been linked to the severity of the disease [19][45]. Patients with other forms of coronavirus have also been found to have elevated levels of this interleukin, which has been linked to disease severity [72].

3.IL-4: IL-4 is also implicated in adaptive immunity, where it regulates the immune system through activated T helper (Th) cells. It works primarily by activating, proliferating, and differentiating B lymphocytes, as well as promoting the immunoglobulin E isotype [73]. As a result, it plays an important role in the induction of humoral immunity-regulating Th2 cells [74]. It's been suggested that IL-4 has a tissue-specific anti-inflammatory activity, reflecting

the metabolic flexibility of various tissues[75]. Bot et al. discovered that its expression during infection with an influenza virus had unfavourable effects on CD8+ memory T cells in viral infections that target the respiratory system [76]. IL-4 levels have been found to be increased in COVID-19 patients in various investigations as part of the cytokine storm linked to severe respiratory symptoms [45][19].

4.IL-6: Inflammation, the immunological response, and haematopoiesis are all affected by IL-6 [77]. This pleiotropic biomolecule is released by many different types of cells and affects a vast range of physiological functions [78]. During the early phases of inflammation, released IL-6 goes to the liver and generates a slew of acute-phase proteins, including C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen, haptoglobin, and 1-antitrypsin. Furthermore, IL-6 has been shown to reduce the formation of fibronectin, albumin, and transferrin [79]. It has been found to have regenerative and anti-inflammatory effects mediated by conventional signalling, as well as proinflammatory effects mediated via trans-signalling, as seen in viral infections [80]. IL-6 levels have been found to be increased in SARS cases and linked to the severity of symptoms, as well as in SARS-CoV, which has been linked to probable T-cell dysfunctionality [63]. SARS-CoV-induced cytokines have been found to impair T cell capacity in respect to dendritic cells, jeopardising these cells' and macrophages' ability to eradicate the infection [81]. In the case of MERS, similar observations have been made [82]. Patients with COVID-19 have elevated IL-6 levels, which are associated with a terrible prognosis. Wan et al. discovered higher IL-6 levels in one-third of patients with mild symptoms and three-quarters of those with severe symptoms, suggesting that IL-6, together with IL-10, may have predictive significance in COVID-19 patients [83]. Diao et al. discovered an inverse proportional relationship between elevated IL-6 levels and T cell counts among ICU patients [34], while another investigation of patients with severe symptoms reported elevated IL-6 and CRP serum levels [84]. In a study of 452 patients infected with SARS-CoV-2, researchers discovered that higher levels of IL-6 were associated with more severe symptoms [66]. These levels have been shown to be higher than those found in patients infected with SARS-CoV or MERS [82]. The levels of IL-6 in individuals who died from COVID-19 were similarly found to be significantly greater than in those who survived [48]. As previously stated, SARS-CoV-2 activation of IL-1β causes IL-6 and TNF-α to be activated [61]. It's also been shown that increased IL-6 expression in COVID-19 patients can hasten the inflammatory process, adding to the cytokine storm and worsening the outcome [85]. In these patients, the cytokine storm, which includes increased levels of IL-6, has also been linked to heart injury [86]. In light of the critical role of IL-6 in the SARS-CoV-2-induced cytokine storm and its evasion, it has been reported that the monoclonal antibody tocilizumab, which works by inhibiting the IL-6 receptor, can reverse cytokine hyperproduction, inflammation, and pulmonary fibrosis[87][56][88]. Bizzarri and others proposed the polyol myo-inositol as a prospective candidate medication for lowering IL-6 levels and reducing the danger of cytokine storm [89].Furthermore, the macrolide azithromycin's ability to lower nasopharyngeal levels of SARS-CoV-2 in conjunction with hydroxychloroquine has been related to its ability to suppress IL-6 and TNF-α [90]. Chloroquine can also block IL-6 and TNF-α, and it's currently being researched against COVID-19 [91]. Finally, given its efficacy in treating other disorders, blood purification therapy has been recommended to eliminate pathogenic antibodies and IL-6, among other cytokines [92].

5.IL-7: IL-7 is involved in the growth of T cells and the maintenance of peripheral homeostasis, as well as lymphocyte differentiation [73].For peripheral homeostasis, all of the key CD4 T cell subgroups (CD4+ immature, memory, and Th17 cells) rely on this cytokine. T cells are activated by IL-7, which enhances the production of proinflammatory cytokines and inhibits transforming growth factor beta (TGF-β) [73]. This biomolecule's function is dependent on IL-6 [95], and it has been proposed that viral infections can cause IL-7 release

[.As with other cytokines, it has been observed that IL-7 levels are high in COVID-19 patients and are closely connected to disease severity [18].

6.IL-10: IL-10 is a type 2 cytokine that suppresses dendritic cell maturation by blocking IL-12 and reduces the production of proinflammatory cytokines (e.g., IFNγ, TNFα, IL-1β, and IL-6) in different cell types . IL-10 can stimulate the production of IFN by CD8+ T cells, which has an immunostimulatory effect. It is also a potent stimulator of B cell, mast cell, and thymocyte growth and differentiation [98].Infection with some viruses, such as HIV, leads to a drop in IL-10 regulation, which contributes to T-cell depletion [99]. Antibody-mediated suppression of IL-10 signalling has been shown in animal experiments to increase T-cell responsiveness and helps to viral resistance elimination [100]. Hence, efficient IL-10 signalling blockade may be beneficial in the fight against resistant viral infections It has been observed that, this interleukin in patients with COVID 19 is somehow interlinked with the levels of disease severity and progression Whereas, the other cytokines have been observed to have possible prognostic value [83]. In fact, it's been discovered that IL-10 is overexpressed in anti-SARS-CoV-2 immunity, with levels higher in senior patients with regard to a "hyperinflammatory response," possibly due to the elderly's reduced T-cell receptors [102].As with other cytokines, IL-10 levels were observed to be higher in COVID-19 patients than in SARS-CoV or MERS patients .

7.IL-12:IL-12 is one of a class of heterodimeric proteins with specific properties, such as pairing versality (also seen in IL-23, IL-27, and IL-35), that are involved in molecular processes and functions that play an important part in positive and negative feedback [103]. In a positive feedback mechanism, IL-12 stimulates the production of IFN-γ by T and NK cells [104]. IL-12 gene expression is rapidly induced by viral infections, and it also acts after viral replication.It has been discovered that IL-12 improves the responsiveness of CD8 + T cells[105].Serum IL-12 levels have been found to be elevated in SARS-CoV-2 patients as well as those infected with other coronaviruses such as SARS-CoV. MSCs have been proposed as an effective COVID-19 therapy because they decrease the release of IL-12 as well as IFN-γ and TNF-α

8.IL-13: Activated Th2 cells release IL-13, which acts as a counter-regulator to the Th1 immune response. It is thought to be a key regulator of Th2-type cytokine-mediated immune responses [108]. IL-13 has a number of roles, including stimulating the creation of TGF-β, eotaxin-3, and mucin, which has been linked to the development of bronchial asthma [109][110]. There are few data on the presence of IL-13 in COVID-19 patients. There was no difference in serum IL-13 levels between individuals who needed ICU hospitalisation and those who did not, according to Huang and others [19].Liu and others discovered a directly proportionate relationship between IL-13 levels and SARS-CoV-2 virus load [65].

9.IL-17: Th17 cells produce IL-17, which is increased in inflammatory and autoimmune disorders [112]. CD8+ cells and immature lymphocytes, such as gamma-delta T cells, NK cells, and group 3 innate lymphoid cells, also produce it .Antimicrobial peptides are produced in response to the presence of IL-17[66]. Hence, IL-17 is a proinflammatory cytokine involved in tissue damage, physiological stress, and infection . As part of the cytokine storm, increased IL-17 levels have been found in SARS-CoV-2 patients [79]. Wan et al., on the other hand, discovered that IL-17 levels in COVID-19 patients were normal, with no significant variations between patients with severe and mild symptoms [83]. Patients with SARS-CoV or MERS have previously been reported to have higher IL-17 levels .The ability of Th17 cells to create IL-17, among other, has led to suggestions for a COVID-19 treatment based on Fedratinib, a Janus kinase 2 (JAK2) inhibitor. In murine studies, this JAK2 inhibitor reduces IL-17 production by Th17 cells.

4. Therapies for the cytokine storm in COVID-19

Prevention and mitigation of the cytokine storm may be the crux to saving patients with severe COVID-19. Currently, many therapies are being evaluated in clinical trials due to a lack of high-quality evidence.

I.Corticosteroids

Corticosteroids inhibit the host inflammatory response and suppress the immune response and pathogen clearance46. In a retrospective study of 401 patients infected with SARS-CoV, the rational use of corticosteroids shortened hospital stays and reduced the mortality of seriously ill patients without complications47. Given the urgent clinical demand, some experts have recommended the rational use of corticosteroids in individuals with severe COVID-1948, 49. However, the outcomes of corticosteroid use in patients with MERS, SARS, and influenza indicated an impaired clearance of viral RNA and complications (e.g., secondary infection, psychosis, diabetes, and avascular necrosis) 50. A recent meta-analysis of 15 studies found that corticosteroids were associated with significantly higher mortality rates in COVID-19 patients51. Overall, although evidence indicates a potential role for the use of corticosteroid in patients with severe COVID-19, caution should be exercised given the possibilities of viral rebound and adverse events.

II.Hydroxychloroquine (HCQ) and chloroquine (CQ)

Given their in vitro antiviral effects and anti-inflammatory properties, CQ and its analog HCQ are considered to be potential therapies for COVID-19. Considering the severe side effects of CQ, HCQ may be a better therapeutic option. CQ and HCQ are able to reduce CD154 expression in T cells52 and suppress the release of IL-6 and TNF53. A test of the pharmacological activities of CQ and HCQ in SARS-CoV-2-infected Vero cells revealed that low doses of HCQ might mitigate cytokine storm in patients with severe COVID-1954. A small French trial of COVID-19 patients who received 600 mg/day HCQ for 10 days showed significant reductions in viral load and the duration of viral infection, and these effects could be enhanced by cotreatment with azithromycin55. However, a meta-analysis of clinical trials indicated no clinical benefits of HCQ treatment in COVID-19 patients56. In fact, HCQ might actually do more harm than good given its side effects, which include retinopathy, cardiomyopathy, neuromyopathy, and myopathy57. Some clinical trials have suggested that taking high doses of HCQ or CQ may cause arrhythmia[58, 59]. The role and risks of HCQ and CQ in the treatment of COVID-19 still need more data to further verify.

III.Tocilizumab (TCZ)

TCZ, an IL- 6 receptor (IL-6R) antagonist, can inhibit cytokine storms by blocking the IL-6 signal transduction pathway60. Currently, a small-sample clinical trial in China (clinical trial registration ID: ChiCTR2000029765) has found TCZ to be effective in critically ill patients with COVID-1961. Xu et al.62 found that out of 21 patients with severe COVID-19, 90% recovered after a few days of treatment with TCZ. A retrospective case-control study of COVID-19 patients with ARDS revealed that TCZ might improve survival outcomes63. However, the risks associated with TCZ (e.g., severe infections, thrombocytopenia, neutropenia, liver damage) should also be noted64. It is unclear whether there are different effects between IL-6 antagonists (siltuximab) and IL-6R antagonists (TCZ). Siltuximab binds to sIL-6 and inhibits only cis and trans signaling. TCZ binds to both mIL-6R and sIL-6R and inhibits both cis and trans signaling and trans presentation. Of note, IL-6 inhibitors are not able to bind to IL-6 produced by viruses such as HIV and human herpesvirus-865. Currently, the application of TCZ for COVID-19 treatment is under study. The three drugs mentioned above (corticosteroids, HCQ, TCZ) are immunosuppressants. Owing to overall damage to the immune system caused by autoimmune diseases and the iatrogenic effects of immunosuppressants, the risk of infection in patients with autoimmune diseases will be increased compared to the general population Currently, rheumatology societies66-69

recommend the use of immunosuppressive drugs (except glucocorticoids) to be suspended in COVID-19 patients.

IV.Mesenchymal stem cells (MSCs)

MSCs have a wide range of immune regulatory functions and can inhibit the abnormal activation of T lymphocytes and macrophages and the secretion of pro-inflammatory cytokines70. MSC therapy was found to significantly reduce the mortality of patients with H7N9-induced ARDS and had no harmful side effects. A clinical trial of MSC therapy revealed that MSCs were able to rapidly and significantly improve the clinical symptoms of COVID-19 without any observed adverse effects 72. Although side effects of MSC treatment are rarely reported, the safety and effectiveness of this treatment require further investigation.[72]

V.Other therapies

Anakinra, an IL-1 receptor antagonist that blocks the activity of pro-inflammatory cytokines IL-1α and IL-1β, has been reported to improve respiratory function and increase the survival rate of COVID-19 patients73. IL-1 receptor antagonists increase the risk of bacterial infections, but this is extremely rare for anakinra65. Janus kinase (JAK) inhibitors can inhibit inflammatory cytokines and reduce the ability of viruses to infect cells74. A small, non-randomized study reported that patients treated with JAK inhibitors exhibited improved clinical symptoms and respiratory parameters. However, JAK inhibitors can also inhibit IFN-α production, which helps to fight viruses. Intravenous immunoglobulin (IVIG) can exert various immunomodulatory effects by blocking Fc receptors, which are related to the severity of the inflammatory state77. IVIG has been reportedly used to treat COVID-19 patients78. Given its uncertain effectiveness and the risk of severe lung injury and thrombosis79, IVIG treatment requires further investigation. Furthermore, convalescent plasma therapy containing coronavirus-specific antibodies from recovered patients can be directly used to obtain artificial passive immunity. This approach has demonstrated promising results in the treatment of SARS and influenza. However, a Cochrane systematic review revealed weak evidence on the effectiveness and safety of this therapy for COVID-19 patients82. Some individuals experienced moderate fever or anaphylactic shock after receiving convalescent plasma. Currently, many effective treatments, such as interferons, TNF blockers, S1P1 receptor agonists, and continuous renal replacement therapy, remain open to further study.

5. Expert opinion: consider RISK and multi-organ protection in CRS

COVID-19 is no longer just an infection confined to the pulmonary epithelium, but a multi-system inflammatory disorder causing end-organ failure . Although anti-viral agents such as Remdesivir have reduced disease severity in case studies, there is still no definitive cure for COVID-19. We must, therefore, turn our attention to other modalities of preventing end-organ destruction, whilst perfecting pharmacological options. As is evident from critical care reports, multi-organ ischemia in COVID-19 occurs as a result of cardio-renal syndrome, cytokine release and global hypoperfusion due to loss of vascular integrity [60]. It may be possible to apply the principles of organ protection, utilized in other ischemic conditions to limit cell injury and apoptosis. The aforementioned observation that RIC can influence cytokine release in animal models, provides an excellent foundation for further research in COVID-19 [61]. Cardioprotection is a well-established concept, developed to minimize damage to cardiac myocytes following myocardial infarction and re-perfusion injury (RPI) [65, 66, 67]. As the heart is central to circulatory function and multi-organ perfusion, it follows that cardiac protection may confer multi-system benefit in COVID-19. Following ST elevation myocardial infarction (STEMI), there is a significant immune response which leads to systemic inflammation [68]. Similarly, there is upregulation of pro-inflammatory cytokines, inflammasomes and immune mediated thrombosis following plaque rupture [68].

The discovery of the Reperfusion Injury Salvage Kinase (RISK) pathway was a major breakthrough in cardioprotective research [69]. RISK describes a group of pro-survival protein kinases which act to minimize cell death by reducing mitochondrial transition pore opening (MTP), when activated early in ischemia [65]. Organ protection from tissue hypoxia is most optimal when a 'multi-hit' approach is adopted [70]. The authors feel this is best achieved by the fusion of proven cardioprotective medications, and physiological interventions which minimize reperfusion injury and cytokine release, such as RIC [62]. Moreover, within cardioprotective pharmacology there is the opportunity to target multiple pathways which reduce cell death and inflammation (additive effect) [70]. Such prosurvival pathways consist of RISK, Survivor Activating Factor Enhancement (SAFE) and Nitrous Oxide/Protein Kinase (NO/ PKG) [65]. This is relevant when considering that hyperinflammation can trigger apoptosis via NF-kB and TNF- α[16]. RIC has been more difficult to translate to humans, despite the clear benefits demonstrated in animal models [61, 63, 67]. Importantly, COVID-19 provides an optimal opportunity to apply this intervention at the onset of mild disease, before the cytokine storm develops. Preserving cardiac function during severe sepsis and CRS provides global benefit by improving contractility, increasing oxygen utilization and maintaining perfusion pressure [71]. Improving cardiac function therefore has the potential to protect renal function and neurological status. The heart is especially susceptible to injury during infection by circulating active substances known as myocardial depressant factors (MDFs). These substances include the cytokines IL-1β, IL-6, TNF-α, complements and high levels of NO. MDFs may act via NF-kB pathways of inflammation when binding to toll-like receptors such as TLR4 . Therapies which have been investigated in the septic heart of animals include caspase inhibitors, hydrogen gas (H2), melatonin and independent growth factor I (GFI-I). Cytokine suppression has been attempted in this context; however, these treatments have thus far failed to show significant benefit . In other cardioprotective developments, research is ongoing to identify the exact mechanisms by which sodium-glucose cotransporter 2 (SGLT2) inhibitors are beneficial in the diabetic heart [80,81,82,83]. Treatments targeting ischemia induced microvascular dysfunction may equally be of interest in COVID-19. In addition to P2Y12 inhibitors and statins, Rho Kinase inhibitors (ROCKi) have demonstrated many novel actions in cardiovascular protection, including modulation of vascular tone, angiogenesis and apoptosis [84,85,86] . ROCK inhibition can additionally mediate endothelial barrier function and reduce leukocyte migration [89,90,91]. These protective effects have been seen in the CNS in addition to the cardiovascular system , which makes this class of drug a versatile and exciting prospect for further research.

6. Conclusion:

The aberrant release of multiple cytokines in COVID-19 produces immunopathogenic damage to tissues and organs, even while the immune response tries to overcome the evading mechanisms Cytokine Storm, COVID-19 Indian Journal of Medical Biochemistry, Volume 24 Issue 2 (May–August 2020) 61 of virus. The cytokine storm leads to deleterious clinical manifestations or even acute mortality in critically ill patients with COVD-19. Impaired acquired immune responses and uncontrolled inflammatory innate responses may be associated with the mechanism of the cytokine storm in COVID-19. Early control of the cytokine storm through therapies such as immunomodulators and cytokine antagonists is essential to improve the survival rate of COVID-19 patients. The endothelial system as an organ is a crucial mediator of the cytokine storm and its persistent Expert Opinion on Therapeutic Targets 5 activation drives a 'septic swirl'. This opinion considers the importance of cardiovascular protection in whole body ischemia during sepsis and provides insights into implications for further research. Only time will tell whether a fusion of novel

immune-modulating drugs and cardiovascular protection can influence the dire outcomes of severe COVID-19. Although many research articles are published each month, majority of the existing literature about COVID-19 comes from descriptive works. Additionally, high-quality evidence will be necessary to understand and treat the cytokine storm of COVID-19

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